DOSE-FINDING AND SAFETY STUDY OF AN ONCOLYTIC POLIO/RHINOVIRUS RECOMBINANT AGAINST RECURRENT WHO GRADE IV MALIGNANT GLIOMA

Duke Institutional Review Board #**Pro00031169**

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4. LIST OF ABBREVIATIONS

BDP; Biopharmaceutical Developmental Program
CBER; Center for Biologics Evaluation & Research
CED; convection-enhanced delivery
CNS; central nervous system
CPC; Cancer Protocol Committee
CRM; continual reassessment method
CSF; cerebrospinal fluid
CTC; Common Toxicity Criteria
DLT; dose-limiting toxicity
DMEM; Dulbecco's minimal essential medium
DNA: deoxyribonucleic acid
DVT; deep vein thrombosis
EGFRvIII; epidermal growth factor receptor (variant III)
eCTD; electronic common technical document
eIF; eukaryotic initiation factor
FDA; Food and Drug Administration
FLAIR; fluid-attenuated inversion recovery
GBM; glioblastoma
GCS; Glasgow coma scale
Gd-DTPA; Gadolinium diethylene triamine pentaacetic acid
HRV2; human rhinovirus type 2
HAS; human serum albumin
IND; investigational new drug
IRB; Institutional Review Board
IRES; internal ribosomal entry site
KPS; Karnofsky performance score
MMSE; Mini-mental Status Examination
MTD; maximally tolerated dose
MVB; master virus bank
NCI; National Cancer Institute
Necl-5; nectin-like molecule 5
NHP; non-human primate
NINDS; National Institute of Neurological Disorders and Stroke
NSICU; Neuro-Surgical Intensive Care Unit
OS; overall survival
PET; positron emission tomography
PFS; progression free survival
PKC; protein kinase C
PV1S; poliovirus serotype 1 (SABIN)
RIO; research integrity office
SAE; serious adverse event
SIADH; syndrome of inappropriate antidiuretic hormone
TCID; tissue culture infectious dose
UTR; untranslated region
WHO; World Health Organization

5. STUDY SYNOPSIS

Title:	**DOSE-FINDING AND SAFETY STUDY OF AN ONCOLYTIC POLIO/RHINOVIRUS RECOMBINANT AGAINST RECURRENT WHO GRADE IV MALIGNANT GLIOMA**
Study Agent:	PVSRIPO administered intratumorally. PVSRIPO is the live attenuated, oral (SABIN) serotype 1 poliovirus vaccine containing a heterologous internal ribosomal entry site (IRES) stemming from human rhinovirus type 2 (HRV2). PVSRIPO recognizes nectin-like molecule-5 (Necl-5), an oncofetal cell adhesion molecule and tumor antigen widely expressed ectopically in malignancy, e.g. glioblastoma (GBM), as host cell receptor. Tumor specificity is mediated by selective expression of Necl-5 in GBM, translation repression at the heterologous HRV2 IRES in the normal central nervous system (CNS) and a supportive environment for PVSRIPO translation, growth and cytotoxicity mediated by universally active protein kinase C (PKC)-Ras-Erk signaling to translation machinery in GBM.
Primary Objective:	To determine the MTD and the Phase II dose of PVSRIPO when delivered intracerebrally by convection-enhanced delivery.
Secondary Objectives:	To obtain correlative mechanistic evidence for PVSRIPO's effects on infected WHO grade IV malignant glioma tumors and to estimate PFS and OS in recurrent WHO grade IV malignant glioma patients. To estimate the efficacy of PVSRIPO administered at the optimal dose.
Exploratory Objectives:	The primary exploratory objectives focus on outcomes associated with the initial PVSRIPO treatment: • Describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO. • Evaluate parameters of general immune activation: frequency of immune cell subsets in peripheral blood and changes in serum levels of cytokines. • Evaluate CD8 and CD4 immunologic response against polio specific and GBM specific antigens to be determined. • Identify genetic predictors of response or failure of response to treatment with PVSRIPO. Additional exploratory objectives focus on retreatment with PVSRIPO: • Describe the toxicity of retreatment with PVSRIPO in combination with a single dose of lomustine. • Describe the PFS and OS of patients after retreatment with PVSRIPO and a single dose of lomustine. • Describe changes visualized on imaging after retreatment with PVSRIPO in combination with a single dose of lomustine. • Evaluate parameters of general immune activation, including frequency of immune cell subsets in peripheral blood, changes in serum levels of cytokines, and CD8 and CD4 immunologic response against polio-specific and GBM- specific antigens.
Inclusion Criteria:	**Please see Section 9 for further details regarding eligibility.** • Patients must have a recurrent supratentorial WHO grade IV malignant glioma based on imaging studies with measurable disease (≥ 1 cm and ≤ 5.5 cm of contrast-enhancing tumor). Prior histopathology consistent with a World Health Organization (WHO) grade IV malignant glioma confirmed by the study pathologist, Roger McLendon, or his designate. • Age ≥ 18 years of age at the time of entry into the study. • Karnofsky Performance Score ≥ 70%. • Prothrombin and Partial Thromboplastin Times ≤ 1.2 x normal prior to biopsy.

	• Total bilirubin, SGOT, SGPT, alkaline phosphatase ≤ 2.5 x normal prior to biopsy. • Neutrophil count ≥ 1000 prior to biopsy. • Hemoglobin ≥ 9 prior to biopsy. • Platelet count ≥ 125,000/μl prior to biopsy; Platelet count ≥ 100,000/μl prior to infusion. • Creatinine ≤ 1.2 x normal range prior to biopsy. • Positive serum anti-poliovirus titer prior to biopsy (except for retreatment). • The patient must have received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week prior to administration of the study agent. • At the time of biopsy, prior to administration of virus, the presence of recurrent tumor must be confirmed by histopathological analysis. • A signed informed consent form approved by the Duke University Institutional Review Board (IRB) will be required for patient enrollment into the study. Patients 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. • Able to undergo brain MRI with and without contrast.
Exclusion Criteria:	• Because of the unknown risk of virus administration potentially affecting a developing fetus or growing infant, females who are pregnant or breast-feeding will be excluded. Adults of reproductive potential not employing an effective method of birth control will be excluded. Sexually active women of child bearing potential, whose partner is male, must use medically accepted birth control. Sexually active men, whose partner is a female of child bearing potential, must use a medically accepted birth control. • Patients with an impending, life-threatening cerebral herniation syndrome, based on the assessment of the study neurosurgeons or their designate, will be excluded. • Because the potential toxicities from the agent being studied in this protocol may be similar to some known diseases or may be more dangerous in the context of certain known diseases, the following patients will be excluded: o Patients with an active infection requiring intravenous treatment or having an unexplained febrile illness (T_{max} > 99.5 F/37.5 C), o Patients with known immunosuppressive disease or known human immunodeficiency virus infection, o Patients with unstable or severe intercurrent medical conditions such as severe heart (New York Heart Association Class 3 or 4) or known lung (FEV_1 < 50%) disease, uncontrolled diabetes mellitus, o Patients with albumin allergy, o Patients with Gadolinium allergy. • Patients with a previous history of neurological complications due to poliovirus infection will be excluded. • Patients who have not recovered from the toxic effects of prior chemo- and/or radiation therapy will be excluded. Guidelines for this recovery period are dependent upon the specific therapeutic agent being used. • Patients may not 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.

	• Patients may not have received immunotherapy ≤ 4 weeks prior to starting the study drug unless patients have recovered from side effects of such therapy.
	• Patients may not be less than 12 weeks from radiation therapy, unless progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation.
	• Patients must have completed all standard of care treatments, including surgical procedure and radiation therapy (at least 59Gy)
	  o If the MGMT promoter in their tumor is known to be unmethylated, patients are not mandated to have received chemotherapy prior to participating in this trial.
	  o If the MGMT promoter in their tumor is known to be methylated or the MGMT promotor methylation status is unknown at the time of screening, patients must have received at least one chemotherapy regimen prior to participating in this trial.
	• Because of the potential toxicities from the agent, patients with neoplastic lesions in the brainstem, cerebellum, or spinal cord, radiological evidence of active (growing) multifocal disease, or leptomeningeal disease will be excluded. Patients with evidence of diffuse subependymal disease or tumor in the brainstem, cerebellum, spinal cord, or CSF will also be excluded.
	
	Patients with the following will be excluded:
	• Undetectable anti-tetanus toxoid IgG (except for retreatment).
	• Known history of agammaglobulinemia.
	• Patients on greater than 4mg per day of dexamethasone within the 2 weeks prior to admission for PVSRIPO infusion.
	• Patients with worsening steroid myopathy (history of gradual progression of bilateral proximal muscle weakness, and atrophy of proximal muscle groups).
	• 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.

Study Design:	This protocol is designed primarily to determine the MTD and DLT of a novel oncolytic viral recombinant with GBM-specific viral translation, replication and cytotoxicity. PVSRIPO will be delivered intratumorally by convection-enhanced delivery using an intracerebral catheter placed within the enhancing portion of the tumor. To enable tracking of the inoculum, 1 mM Gadolinium will be co-infused. The contrast agent (Gd-DTPA) will be added to the virus-containing infusate. Based on pre-clinical toxicity studies in non-human primates, the starting amount of the agent to be delivered will be 1×10^8 tissue culture infectious dose (TCID50). Due to limitations imposed by manufacture and dosing volume in *Cynomolgus* macaques, the MTD was not reached in non-human primates. Intra-thalamic inoculation of 5×10^9 TCID in *Cynomolgus* macaques did not cause any toxicity. A total of 3 mLs of the agent in physiologic saline stabilized with 0.2% human serum albumin will be delivered over approximately 6 hrs 30 minutes, corresponding to a flow-rate of 0.5 mL/hr. The agent is stable at room temperature during the intended instillation period and the intended delivery apparatus is not associated with adsorptive loss of PVSRIPO. PVSRIPO dose escalation will be accomplished by increasing agent concentration allowing flow-rate and infusion volume to remain constant. A two-step continual reassessment method design will be used for dose escalation. Starting at dose level 1, one patient will be treated at each dose level. Decisions concerning dose escalation for subsequent patients will be based upon the occurrence of DLT during the first 4 weeks after treatment administration. Initially,

each patient will be observed for ≥ 4 weeks before the next patient is treated. If no patients experience a DLT on the first 4 dose levels, all subsequent patients will be treated at dose level 5. Escalation will be interrupted if any of the patients assigned to one of the first 4 dose levels or ≥ 20% of patients treated at dose level 5 experience a DLT. In that case, further dose escalation or de-escalation will be guided by the likelihood-based implementation of the continual reassessment method. Greater detail is provided in section 11.1.5.1.

Historical Modifications to the Protocol:
As of 11/15/2013 (AMD025), 4 patients have been treated on dose level 5. One patient experienced a DLT upon removal of the treatment catheter. The remaining 3 patients have not experienced a DLT; however, all 3 have had difficulties tapering off steroids. To address this concern, the study is amended to reduce the dose for the next cohort of patients to dose level 2. Up to 6 patients will be accrued at dose level 2. If 2 of these patients experience a DLT as defined in section 11.4, accrual will be suspended.

As of 6/5/14 (AMD041), 5 subjects have been treated on dose level 2. Four subjects have completed the two-week follow-up period and have not had a DLT. One subject remains in the 14 day follow up period. Once one last patient has been treated, the study per the November 2013 amendment is complete. We are amending the study to allow continued patient accrual to garner more information about patient safety while the single-institution phase 2 study is being developed and reviewed by various regulatory bodies. We intend to continue accruing subjects for treatment at dose level 2 until such time that the phase 2 study has been activated. After each patient has been followed for a one-week observation period, during which the patient will have been evaluated in clinic on day 7 (± 2 days), a patient summary will be prepared and submitted to the DSMB chair for approval. Once approval from the DSMB chair has been obtained, treatment of the next patient will be allowed. We will treat up to four subjects within a 30-day period as allowed by DSMB chair, and there will be only one PVSRIPO treatment per patient without specific approval for a second or more treatments from the FDA and the IRB.

As of 10/06/2014 (AMD048), 7 subjects have been treated on protocol at dose level 2. One additional subject has been treated under a Single Subject Emergency Use Request at dose level 2. Of these eight subjects, six subjects had difficulty or remained unable to be tapered off steroids more than three months post infusion (three subjects required the addition of bevacizumab to facilitate the taper down or off steroids). We are amending the study to reduce the dose to dose level -1 (5.0×10^7 TCID50) with the goal of limiting the occurrence of known possible side effects of prolonged steroid use in patients who otherwise benefit from this investigational therapy. This dose reduction will allow continued patient accrual and garner more information about patient safety and optimal dosing while the single-institution phase 2 study is being developed and reviewed by various regulatory bodies. Our last amendment allowed the enrollment of up to 18 patients, one patient was enrolled under that amendment and thus, we are planning to enroll up to 17 patients on dose level -1. After each patient has been followed for a one-week observation period, during which the patient will have been evaluated in clinic on day 7 (± 2 days), a patient summary will be prepared and submitted to the DSMB chair for approval. Once approval from the DSMB chair has been obtained, treatment of the next patient will be allowed. We will treat up to four subjects within a 30-day period as allowed by

	DSMB chair, and there will be only one PVSRIPO treatment per patient without specific approval for a second or more treatments from the FDA and the IRB. Study Design Modification (1/13/2015 AMD054)). Once the optimal dose level of PVSRIPO is determined, a total of 26 patients will be treated at that dose level on a dose expansion cohort. The survival associated with these 26 patients will be compared to that of a historical cohort (see section 14.3). The study is being amended to allow accrual and treatment of a total of 26 patients at the optimal dose level of PVSRIPO and, as needed, radiation-necrosis dose levels and schedules of bevacizumab to estimate the efficacy of PVSRIPO relative to a historical cohort. Sample Size Modification (6/24/2015 AMD071). As of 6/24/15, we have treated 13 patients on dose level -1. None of the patients have had prolonged steroid needs, two patients died at 3 and 6 months respectively, and five patients total (including the two patients who died) were initiated on bevacizumab. It is now felt that dose level -1 is the optimal dose, as none of the patients have experienced undue side effects due to PVSRIPO or the management of the cerebral inflammation secondary to PVSRIPO. We are now amending the protocol to enroll a total of 50 patients on dose level -1. Study Design Modification (11/10/2015 AMD078). Imaging response criteria were previously developed based on imaging observations following chemoradiation treatment (Macdonald criteria) and subsequently revised based on new observations from anti-angiogenic trials (RANO criteria). As imaging is dramatically different following treatment with immunotherapy, new imaging criteria are being developed (iRANO). This process is likely to take several years to validate. For that reason, we are removing response rate as a secondary objective and adding, as an exploratory objective, a description of changes visualized on imaging due to intratumoral inoculation of PVSRIPO. Study Design Modification (2/1/2016 AMD081). The protocol has been re-formatted in an electronic Common Technical Document (eCTD) format for electronic submission to the FDA for the associated IND. The statistical section has been revised and an appendix (section 18.4) has been added to define and describe the historical control group being used as a statistical comparison for the subject population. We have also further described the types of subject follow-up activity that will occur in this study. Study Design Modification (6/15/2016 AMD096). As of 6/15/2016, 23 subjects have been treated on dose level -1, including 12 subjects that were treated more than 12 months ago. Three of these 12 subjects demonstrated tumor reduction without significant inflammation necessitating prolonged use of bevacizumab and/or additional chemotherapy. In contrast, the remaining 9 patients had inflammation that was burdensome and required prolonged use of bevacizumab and/or additional chemotherapy. Caregivers of some of these patients were overwhelmed by the experience. The 3 patients without significant inflammation are alive at 20.2, 15.5, and 13.1 months after PVSRIPO infusion, and 7 of the 9 patients with burdensome inflammation have died, including 5 that died within 12 months of PVSRIPO treatment. Despite the fact that subjects on dose level -1 have been able to remain off significant doses of steroids, we believe that the subjects benefiting the most from PVSRIPO have been those who have experienced minimal or easily controllable inflammation.

As such, we are amending this study to reduce the PVSRIPO dose to dose level -2 (1.0×10^7 TCID50) 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, we are confident that dose level -2 (1.0×10^7 TCID50) will be a therapeutic dose. This amendment is not due to concerns for the safety of the patients on dose level -1, but due to the observation that less inflammatory reaction is a predictor of better survival and treatment response.

We plan to treat 27 patients at dose level -2. After each patient has been followed for a one-week observation period, during which the patient will have been evaluated in clinic on day 7 (± 2 days), a patient summary will be prepared and submitted to the DSMB chair for approval. Once approval from the DSMB chair has been obtained, treatment of the next patient will be allowed. We will treat up to four subjects within a 30-day period as allowed by DSMB chair, and there will be only one PVSRIPO treatment per patient without specific approval for a second or more treatments from the FDA and the IRB.

Furthermore, patients previously treated on trial received high dose steroids prior to catheter insertion and throughout the infusion, as part of undergoing biopsy. Given concerns for negative impact of high dose steroids on the efficacy of PVSRIPO, we will limit steroid usage to the minimal dose necessary, if even needed.

Study Design Modification (6/29/2016 AMD101). This amendment includes several revisions to tests and procedures, which we believe can be safely implemented with the aim of reducing the time between consenting to participate in the study, screening to determine eligibility, and study treatment. Previously, patients had to demonstrate a serum total IgG level of ≥ 400 mg/dL to be eligible for the study. All patients who have undergone this testing thus far have met this eligibility criterion; therefore, it is no longer considered necessary to test serum IgG in patients going forward prior to their enrollment. We are also changing the timing of the boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) from between 6 months and 2 weeks prior to PVSRIPO infusion to between 6 months and 1 week prior to PVSRIPO infusion. Also, for previously enrolled patients, a patient had to be followed for a one-week observation period post-infusion, before the next patient could be treated. Now, once a subject has been observed through successful completion of the PVSRIPO infusion, the next subject can be treated. We are updating pharmacy instructions for the preparation and administration of PVSRIPO in this amendment as well. Lastly, per Duke's Research Integrity Office (RIO), a study-specific DSMB may no longer be necessary for this study given the oversight provided by the BTC's DSMB-Plus. If the FDA concurs, the study-specific DSMB may be dissolved per its charter.

Study Design Modification (9/5/2016 AMD104). This amendment includes the addition of an exploratory objective to identify genetic predictors of response or failure of response to treatment with PVSRIPO. Molecular genetic tests, such as, but not limited to, DNA sequencing, gene amplification, and gene expression, will be performed on the tissue obtained at the protocol-specified biopsy prior to PVSRIPO infusion. Tissue may be obtained for testing as either fresh, frozen, or fixed tissue, or as slides, after the diagnostic pathologist has retained all tissue needed for diagnosis. The amount of tissue required for this testing should be sufficient to yield a minimum of one microgram of DNA.

	Study Design Modification (3/1/2017 AMD124). Based upon recent pre-clinical data and clinical data collected on dose levels -1 and -2, we have concluded that there is no evidence of pre-clinically or clinically important differences between these dose levels. The initial need for treatment with the radiation necrosis dose levels and schedules of bevacizumab for cerebral inflammation secondary to PVSRIPO or tumor growth has been comparable thus far. In addition, the toxicity profiles for the two dose levels are also similar. Therefore, we have decided to proceed with the development of PVSRIPO dose level -1 (5.0×10^7 TCID50) within the phase II study that is now in development. We are amending this phase I study to treat all future patients at the -1 dose level. We intend to continue accruing patients and treating patients at this dose level while the phase II study is being finalized and reviewed by various regulatory bodies. The protocol is also being modified to allow multiple patients to be infused with study drug simultaneously if the neuro-surgical schedule allows. There are currently 3 neurosurgeons listed as sub-investigators who are trained on the surgical procedures for this study. In the study modification dated 6/29/2016, the study was modified so that the next patient could be treated immediately following the completed infusion of the previous patient. However, we would like to increase our enrollment potential by allowing multiple patients to be treated simultaneously. As of 1/20/2017, no patients have experienced complications during the infusion of PVSRIPO. Only 1 out of 51 treated patients has experienced severe complications (Grade III or higher) during catheter removal following the infusion of the study drug, and therefore we are confident that treating multiple patients at a time would be safe for the patients and would allow for increased data collection. This amendment also includes updates to the program used for planning catheter placement, the timing of catheter removal, the infusion pump information, and to clarify the history of DLT monitoring in the study. The statement related to sending MRIs to Therataxis was modified to state that the MRIs may be sent to Therataxis. The blood test for anti-epileptics has been removed. Lastly, the exclusion criterion related to completion of standard of care treatments has been clarified based on whether or not the MGMT promotor methylation status is known at the time of screening. There are other minor corrections throughout. Following the FDA's review of this amendment, an additional section (see section 11.5) describing guidelines and criteria for study pause as a result of toxicity monitoring has been added. Specifically, the study is being monitored for the occurrence of unacceptable toxicities within dose levels -1 and -2 at any time after the initiation of protocol treatment. Study Design Modification (6/22/2017 AMD142). It has been recently observed that patients who originally benefitted from the infusion of PVSRIPO can demonstrate tumor recurrence years later. Given the limited life expectancy of recurrent glioblastoma patients, it has been hypothesized that retreatment of long-term survivors previously treated with PVSRIPO could trigger an immune recall effect and thus further extend the survival of patients. As such, in the current amendment, we will allow selected patients who have previously benefited from PVSRIPO infusion to be retreated with PVSRIPO in the event of tumor recurrence. A patient is considered to have benefited from the initial PVSRIPO infusion if they survived 12 or more months after PVSRIPO treatment. All patients retreated will be treated at dose level -1 as per the same procedure previously used. Furthermore, all retreated patients will

	receive a single dose of lomustine 8 weeks post infusion of PVSRIPO. As lomustine temporarily reduces the number of regulatory T-cells, it is hypothesized that it could potentially increase the efficacy of PVSRIPO and of the immune recall. Further details regarding this retreatment plan are in Section 11.2. Study Design Modification (9/25/2017 AMD149). Based upon the Final Clinical Shedding Study for PVSRIPO (IND 14,735) dated 8/6/2017, the IBC reviewed the requirement for stool sampling for patients receiving investigational treatment with PVSRIPO. The purpose of the stool sampling was to test for presence of viral shedding in stool samples of patients receiving PVSRIPO via intracerebral delivery. The Final Clinical Shedding Report indicates no presence of PVSRIPO in stool collected from 59 subjects who received 61 treatments with PVSRIPO (2 patients were re-treated). Per the report, "we feel that the serologic evidence indicates a formidable protective shield against shedding in patients with pre-existing and boosted anti-polio immunity." On 8/17/2017, the IBC determined that the requirement for viral shedding studies may be discontinued on the basis of the report. Therefore, we have removed stool sampling from Section 12.2 and from Table 6. In addition, we have added 3 additional biopsy core samples to be taken at the time of biopsy, prior to catheter placement, if possible. The additional biopsy core samples will be used for genetic analysis, including full genome or full exome sequencing, as well as other molecular genetic testing.

6. ABSTRACT, HYPOTHESIS AND OBJECTIVES

6.1. ABSTRACT

Despite aggressive surgery, prolonged radiation therapy, and multi-agent chemotherapy, survival after diagnosis with a malignant brain tumor is usually < 16 mo. and patients with recurrent tumors usually survive < 3 mo. Current therapies for malignant brain tumors are limited by ineffective delivery beyond the blood-brain barrier, limited diffusion of regionally-delivered macromolecules, and a lack of tumor specificity, which leads to dose-limiting toxicity.

We and others demonstrated that sustained direct intracerebral infusion at slow flow rates can overcome delivery barriers. This innovative technique, called convection-enhanced delivery (CED), may induce a convective interstitial fluid current that has the potential to homogeneously distribute even large molecules great distances within the brain by displacing interstitial fluid.

PVSRIPO is a genetically recombinant, non-pathogenic poliovirus:rhinovirus chimera with a tumor-specific conditional replication phenotype. It consists of the genome of the live attenuated poliovirus serotype 1 (SABIN) vaccine (PV1S) with its cognate IRES element replaced with that of HRV2. PVSRIPO tumor tropism is mediated by the poliovirus receptor, Necl-5. Necl-5, an onco-fetal cell adhesion molecule ectopically upregulated in all 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. Poliovirus' inherent neuropathogenicity was removed by IRES exchange. This ablated the virus' ability to propagate in cells of neuronal lineage and to cause poliomyelitis in non-human primates. 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 deficient anti-viral defenses centered on nuclear factor associated with dsRNA (NFAR) proteins in tumor cells; (ii) constitutive signal transduction via Ras-Erk to translation machinery, which stimulates viral, cap-independent translation via the HRV2 IRES in cancerous cells. The anti-neoplastic effects of PVSRIPO are due to direct, virus-mediated tumor cell killing and secondary, host-mediated inflammatory effects directed against infected and/or lysed tumor.

In this protocol, we will determine the largest feasible and phase II dose of PVSRIPO given directly into intracerebral tumors.

6.2. HYPOTHESIS

Intratumoral treatment with PVSRIPO will be feasible and safe in patients with recurrent WHO grade IV malignant glioma.

6.3. OBJECTIVES

6.3.1. Primary Objective

- To determine the MTD and phase II dose of PVSRIPO when delivered intracerebrally by convection-enhanced delivery.

6.3.2. Secondary Objectives

- To obtain correlative mechanistic evidence for PVSRIPO's effects on infected WHO grade IV malignant glioma tumors and to estimate PFS and OS in recurrent WHO grade IV malignant glioma patients.
- To estimate the efficacy of PVSRIPO administered at the optimal dose.

6.3.3. Exploratory Objectives

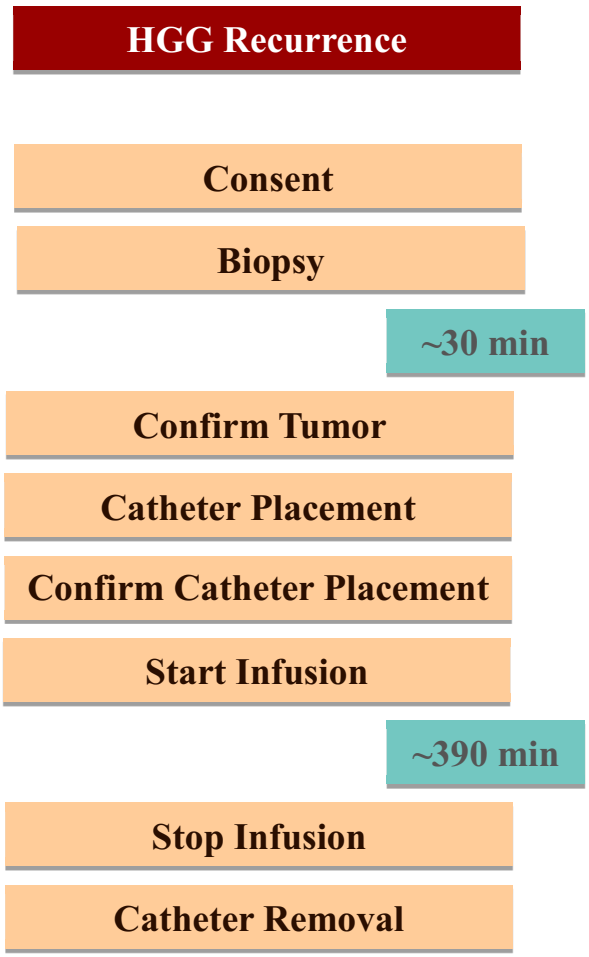
The primary exploratory objectives focus on outcomes associated with the initial PVSRIPO treatment:

- Describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO.
- Evaluate parameters of general immune activation: frequency of immune cell subsets in peripheral blood and changes in serum levels of cytokines.
- Evaluate CD8 and CD4 immunologic response against polio-specific and GBM-specific antigens to be determined.
- Identify genetic predictors of response or failure of response to treatment with PVSRIPO

Additional exploratory objectives focus on retreatment with PVSRIPO:

- Describe the toxicity of retreatment with PVSRIPO in combination with a single dose of lomustine.
- Describe the PFS and OS of patients after retreatment with PVSRIPO and a single dose of lomustine.
- Describe changes visualized on imaging after retreatment with PVSRIPO in combination with a single dose of lomustine.
- Evaluate parameters of general immune activation, including frequency of immune cell subsets in peripheral blood, changes in serum levels of cytokines, and CD8 and CD4 immunologic response against polio-specific and GBM- specific antigens

7. STUDY SCHEMA



8. BACKGROUND AND RATIONALE

8.1. DISEASE AND CURRENT THERAPY

The brain is the most frequent site of crippling and incurable disease. Brain tumors account for more than 100,000 deaths each year in the US (1). Malignant primary brain tumors are more common than Hodgkin's disease and cause more deaths than cancer of the bladder or kidney, leukemia, or melanoma. Despite aggressive surgery, prolonged radiation therapy and toxic chemotherapy, most patients with such tumors live only one year from the time of diagnosis and patients with recurrent tumors usually survive less than 12 weeks (2-6). The estimated cost of treatment for each patient with a malignant brain tumor is between $30,000 and several hundred thousand dollars annually. Therefore, the annual treatment cost alone for these patients, not mentioning the lost earning potential of afflicted individuals, is greater than the entire annual budget of the National Institute of Neurological Disorders and Stroke (NINDS). In fact, conventional therapy for a malignant brain tumor is the most expensive medical therapy per quality-adjusted life-year saved currently provided in the US (7, 8). Moreover, the non-specific nature of conventional therapy for brain tumors often results in incapacitating damage to surrounding normal brain (9, 10). The inherent selectivity of approaches based on biological agents with tumor specificity offers the prospect of more precise and effective therapy.

8.2. VIRAL ONCOLYSIS

After being recognized for their anti-neoplastic properties in the early 1900's, viruses are again being considered for use as therapeutic agents against cancer. 'Oncolytic' viruses carry the promise to efficiently target cancer cells for destruction and spread throughout tumor tissue to reach distant neoplastic loci without causing collateral damage to healthy tissues. Lytic, viral destruction of tumors elicits host immune responses directed against the infected cancer. To unfold their anti-neoplastic activity, oncolytic viruses must engage in intricate interactions with their host. Unraveling the molecular basis for specific targeting of-, translation and replication in- and killing of tumor cells is pivotal to properly support clinical investigations. PVSRIPO oncolysis is due to an unusual confluence of factors that mediate specific tropism for tumor cells and tumor-specific viral translation and cytotoxicity. The principal elements determining PVSRIPO tumor tropism, tumor-specific cell killing and safety are understood.

8.3. PVSRIPO TUMOR TROPISM

Poliovirus host cell tropism categorically depends on its sole cellular receptor, Necl-5 (11). In the normal human organism, narrowly restricted Necl-5 expression occurs at sites of poliovirus replication in the gastrointestinal tract and associated lymphatic structures and in the spinal cord. Necl-5 expression is low in normal adult tissues, but is rapidly induced upon acute injury, malignant transformation, or other events disrupting tissue architecture (12). Necl-5 is an onco-fetal cell adhesion molecule with physiological roles in morphogenesis (during embryonic development) and tissue regeneration and repair (in adult age) (12). Necl-5 is universally expressed in ectodermal/neuroectodermal malignancies, but its association with GBM is particularly compelling. Necl-5 is abundant in all high-grade GBMs, is present on GBM 'stem cell-like' cells, occurs in proliferating tumor vessels and is implicated in GBM cell dispersal and dissemination in the brain (13-16).

8.4. PSVRIPO TUMOR CELL KILLING

Infection of tumor cells with PVSRIPO results in their swift destruction. This is due to a viral strategy that relies on early biosynthesis of highly cytotoxic viral proteins engaging in the shut-off of host cell protein synthesis. PVSRIPO achieves this through an unorthodox mechanism of translation initiation (Figure 1). At eukaryotic mRNAs, protein synthesis occurs upon recruitment

of a multi-partite protein complex at the canonical 5' 'cap' structure (Figure 1A). 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 1B). eIF4G binding occurs via the IRES, a *cis*-acting genetic element located in the 5' untranslated region of the viral RNA (17).

8.5. GENERATING TUMOR SPECIFICITY OF PVSRIPO

Due to its key role in viral translation, the IRES element determines virus:host interactions and, hence, pathogenesis. We generated tumor-specificity by replacing the cognate poliovirus IRES with its counterpart of HRV2 (18). To enhance safety, the chimera was based on PV1S (19), generating PVSRIPO. PVSRIPO exhibits profound translation deficits in primate neuron-like cell lines, and lacks neuropathogenicity in mice transgenic for the human *Necl-5* gene and in non-human primates (18-20). Yet, the virus exhibits potent cytotoxic effects in malignant cells, e.g. GBM (21).

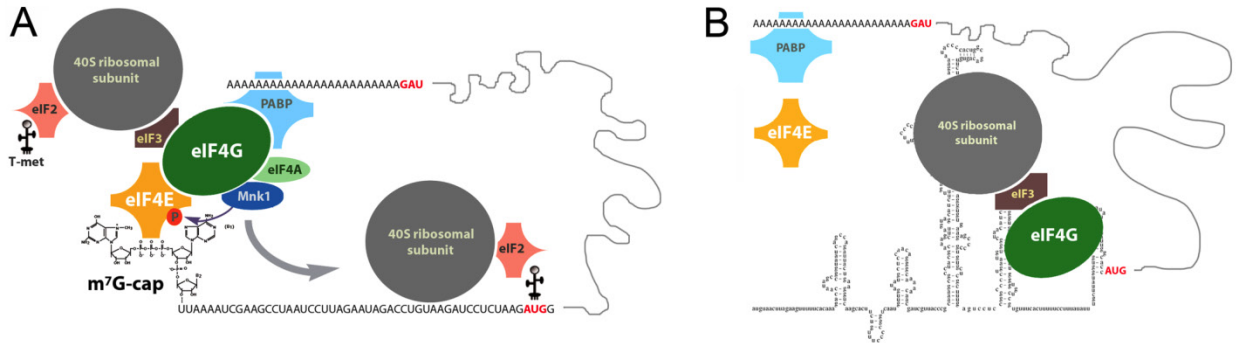


Figure 1. Translation Initiation. A. Conventional, cap-dependent initiation occurs via eIF4E, mediating assembly of the initiation complex at the m7G-cap. **B.** Cap-independent translation initiation at poliovirus RNA occurs by direct binding of eIF4G to the IRES (22).

8.6. THE MOLECULAR MECHANISMS OF PVSRIPO TUMOR SPECIFICITY

PVSRIPO exhibits tumor specific viral translation and cell killing because:

- *The foreign HRV2 IRES associates with NFAR proteins in the normal CNS*. NFAR proteins are predominantly cytoplasmic, associate with translation machinery and readily bind PVSRIPO RNA in neuronal cells. Association with NFAR proteins causes translation repression of PVSRIPO RNA in normal neuronal cells (23-25). In GBM, NFAR proteins are inactive, due to sequestration in the nucleus, expression of distinct protein isoforms and altered RNA-binding capacity (24-26). Thus, PSVRIPO translation is unimpeded in GBM;
- *Mitogenic signal transduction to translation machinery benefits PVSRIPO cap-independent translation.* Signaling via the PKC-Ras-Erk axis to the translation apparatus stimulates cap-independent viral translation (27). This may be due to enhanced affinity of eIF4G for the viral IRES element and may involve (i) direct phosphorylation of eIF4G affecting protein conformation and altering RNA-binding capacity (28); (ii) altered association of eIF4G with its binding partner, Mnk1 (29); and (iii) eIF4E phosphorylation by Mnk1, resulting in a shift from cap-dependent to IRES-mediated translation (30).

8.7. PVSRIPO ANTI-NEOPLASTIC EFFECTS IN ANIMAL TUMOR MODELS

In animal tumor models, oncolytic polioviruses elicit efficient anti-neoplastic effects resulting in tumor regression and, eventually, destruction in a dose-dependent manner. There is histologic evidence for direct, virus-mediated tumor cell killing and indirect, host-mediated inflammatory responses directed against tumor (21, 27, 31-35).

8.8. PVSRIPO NON-HUMAN PRIMATE TOXICOLOGY

PSVRIPO was subjected to dose-range finding, toxicology, biodistribution, shedding and neutralizing antibody tests with intrathalamic inoculation of up to 5 x 10e9 TCID50 of PVSRIPO in *Cynomolgus* macaques (36). These revealed:

- The absence of morbidity and mortality;
- The absence of neuropathological signs consistent with virus-induced CNS damage;
- The absence of virus dissemination form the brain or viremia;
- The absence of extraneural replication;
- The absence of shedding with saliva, urine or stool;
- The presence of a neutralizing antibody response.

9. STUDY POPULATION

9.1. INCLUSION CRITERIA

9.1.1. Disease Status

Patients must have a recurrent supratentorial WHO grade IV malignant glioma based on imaging studies with measurable disease (≥ 1 cm and ≤ 5.5 cm of contrast-enhancing tumor). Prior histopathology consistent with a World Health Organization (WHO) Grade IV malignant glioma confirmed by the study pathologist, Roger McLendon, or his designate.

9.1.2. Age

Due to the potential implications of the treatment on the developing CNS, all patients must be ≥ 18 years of age at the time of entry into the study.

9.1.3. Performance Status

The patient must have a Karnofsky Performance Score (KPS) of ≥ 70% at the time of entry.

9.1.4. Laboratory Studies

- Platelet count ≥ 125,000/μl prior to biopsy. Platelets ≧ 100,000/μl prior to infusion;
- Prothrombin and Partial Thromboplastin Times ≤ 1.2 x normal prior to biopsy;
- Positive serum anti-poliovirus titer prior to biopsy (except for retreatment);
- Creatinine ≤ 1.2 x normal prior to biopsy;
- Total bilirubin, SGOT, SGPT, alkaline phosphatase ≤ 2.5 x normal prior to biopsy;
- Neutrophil count ≥ 1000 prior to biopsy;
- Hemoglobin ≥ 9 prior to biopsy.

9.1.5. Poliovirus Immunization Booster

The patient must have received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week prior to administration of the study agent.

9.1.6. Disease Confirmation

At the time of biopsy, prior to administration of virus, the presence of recurrent tumor must be confirmed by histopathological analysis.

9.1.7. Informed Consent

A signed informed consent form approved by the Duke University Institutional Review Board (IRB) will be required for patient enrollment into the study. Patients 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.

9.1.8. Brain MRI

Able to undergo brain MRI with and without contrast.

9.2. EXCLUSION CRITERIA

9.2.1. Pregnancy

Because of the unknown risk of virus administration potentially affecting a developing fetus or growing infant, females who are pregnant or breast-feeding during the study period will be excluded. Adults of reproductive potential not employing an effective method of birth control will be excluded. Sexually active women of child bearing potential, whose partner is male, must use medically accepted birth control.

Sexually active men, whose partner is a female of child bearing potential, must use a medically accepted birth control.

9.2.2. Disease Status

Because patients will receive drug intracerebrally, patients with an impending, life-threatening cerebral herniation syndrome, based on the assessment of the study neurosurgeons, Allan Friedman, John Sampson, or Peter Fecci, or their designate, will be excluded.

9.2.3. Medical Conditions

Because the potential toxicities from the agent being studied in this protocol may be similar to some known diseases or may be more dangerous in the context of certain known diseases, if the patients meet any of the following they will be excluded:

- Active infection requiring intravenous treatment or having an unexplained febrile illness (T_{max} > 99.5 F/37.5 C).
- Known immunosuppressive disease or known human immunodeficiency virus infection.
- Unstable or severe intercurrent medical conditions such as severe heart (New York Heart Association Class 3 or 4) or known lung (FEV_1 < 50%) disease, uncontrolled diabetes mellitus.
- Albumin allergy. Albumin is added to the agent as a stabilizer. Patients with a known allergy will be excluded.
- Gadolinium allergy. Gadolinium is used as contrast for the MRI.

9.2.4. Previous Poliomyelitis

A history of neurological complications due to poliovirus infection would imply previous virus replication in the CNS. Based on animal studies, previous exposure to poliovirus administered intracerebrally can reduce subsequent virus replication in the CNS.

9.2.5. Prior Therapy

Patients who have not recovered from the toxic effects of prior chemotherapy and/or radiation therapy will be excluded. Guidelines for this recovery period are dependent upon the specific therapeutic agent being used.

- Patients may not 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.

- Patients may not have received immunotherapy ≤ 4 weeks prior to starting the study drug unless patients have recovered from side effects of such therapy.
- Patients may not be less than 12 weeks from radiation therapy, unless progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation.
- Patients must have completed all standard of care treatments, including surgical procedure and radiation therapy (at least 59Gy)
    - If the MGMT promoter in their tumor is known to be unmethylated, patients are not mandated to have received chemotherapy prior to participating in this trial.
    - If the MGMT promoter in their tumor is known to be methylated or the MGMT promoter status is unknown at the time of screening, patients must have received at least one chemotherapy regimen prior to participating in this trial.

9.2.6. Location and Extent of Tumor

Because of the potential toxicities from the agent, patients with neoplastic lesions in the brainstem, cerebellum or spinal cord, radiological evidence of active (growing) multifocal disease, or leptomeningeal disease will be excluded. Patients with evidence of diffuse subependymal disease or tumor in the brainstem, cerebellum, spinal cord, or CSF will also be excluded.

Since the study agent is a local treatment, patients with radiological evidence of active (growing) multifocal disease, tumors extending into or crossing the corpus callosum or leptomeningeal disease, will be excluded.

9.2.7. Patients must not have diagnosis of agammaglobulinemia

Patients with the following will be excluded:

- Undetectable anti-tetanus toxoid IgG (except for retreatment)
- Known history of agammaglobulinemia

9.2.8. Patients on greater than 4mg per day of dexamethasone within the 2 weeks prior to admission for PVSRIPO infusion

9.2.9. Patients with worsening steroid myopathy (history of gradual progression of bilateral proximal muscle weakness, and atrophy of proximal muscle groups)

9.2.10. 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

10. INVESTIGATIONAL PRODUCT

PVSRIPO is a version of the serotype 1 life-attenuated (Sabin) poliovirus vaccine (PV1), 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 immunogenic sequelae. The main immunogenic effect of administration of PV1S to human subjects is neutralizing immunity to poliovirus.

10.1. PREPARATION OF PVSRIPO

PVSRIPO is PV1S containing a heterologous IRES of HRV2 (Figure 2). 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 2). 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.

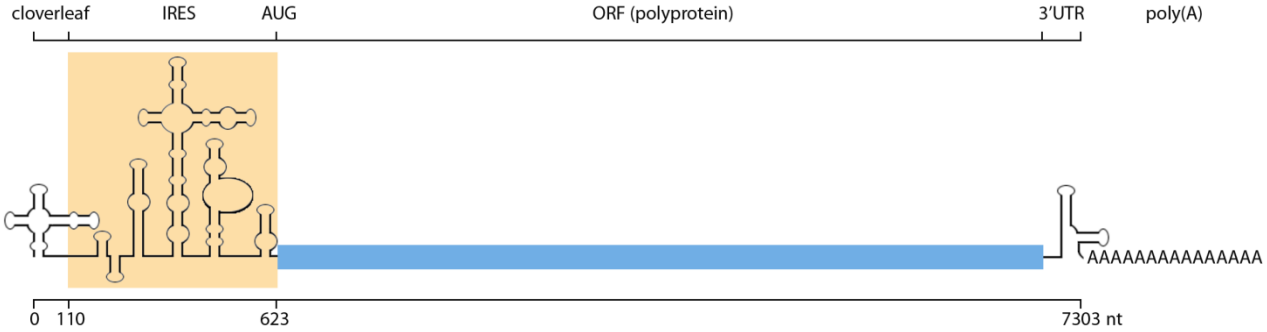


Figure 2. Genetic structure of PVSRIPO

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 must be stored at less than or equal to -70°C. Once thawed, it is a clear colorless liquid with no evidence of particulates or foreign matter. PVSRIPO was manufactured at the Biopharmaceutical Development Program/SAIC-Frederick at NCI-Frederick. For tracking intratumoral/intracerebral distribution of the inoculum, the study agent suspension will be supplemented with gadolinium-DTPA diamide (Magnevist®) at a final concentration of 1 mM. Magnevist® is stored at room temperature.

11. INVESTIGATIONAL PLAN

11.1. INITIAL PVSRIPO TREATMENT PLAN

11.1.1. 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. Poliovirus CNS invasion after intravascular administration via trans-endothelial passage is exceedingly inefficient (37). 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 below). The biopsy needle will be placed with stereotactic guidance by a Cosman-Robert-Wells, MRI-compatible, stereotactic head frame or similar frameless device. Collection of biopsy tissue for clinical 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. Molecular genetic tests, such as, but not limited to, DNA sequencing, gene amplification, and gene expression, will be performed on the tissue obtained from the three additional core biopsies. The goal of these molecular genetic tests is to identify genetic predictors of response or failure of response to treatment with PVSRIPO.

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 with monitoring of cardiac rhythm and oxygen saturation. Implantation will occur at a co-ordinate 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 and would absolutely minimize the possibility of infusates going into 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. The catheter will be pre-filled with preservative-free saline. A CT scan may be used to confirm catheter placement post-operatively.

11.1.2. Agent Infusion

The entire volume of the agent to be delivered will be pre-loaded into a syringe by the investigational pharmacist and connected to the catheter under sterile conditions in the Neuro-Surgical Intensive Care Unit (NSICU) or neuro step down unit 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 in to 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” in regards 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 Neuro-Surgical Intensive Care Unit (NSICU) or neuro step down unit so that all other emergency facilities will be available. Patients will be treated with appropriate antibiotic prior to biopsy and catheter insertion per DUHS Neuro-surgical standard practice.

Based on our own experience, previously published reports (38) and IRB- and FDA-approved trials using similar infusion techniques (IRB# 4774-03-4R0), patients will be infused at a rate of 500 μL/hr. A Medfusion 3500 or 3010 infusion pump will be pre-programmed to a delivery rate of 500 μL/hr. The agent (which will be in a total volume of 8 mL to account for ‘dead-space’ of 3.3723 mL in the infusion system) will be loaded in a 20 mL syringe into the syringe pump at the initial onset to avoid any interruptions in the infusion. The total amount of the inoculum delivered to the patient will be 3 mL. The catheter itself (30 cm length, 1 mm interior diameter) cannot be preloaded with virus suspension. The infusion pump will be programmed for delivery of 500 μL/hr per hour over approximately 6.5 hours. To account for the 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 3010 (Smiths Medical ASD, Inc, Minneapolis, MN) syringe infusion pump. The virus injection procedure will be completed within approximately 6.5 hrs. The catheter will be removed following the post-PVSRIPO infusion MRI.

The infusion catheter (PIC 030) and infusion tubing (PIT 400) will be supplied 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 manage by the Neuro-Surgical Intensive Care Unit (NSICU) or the neuro step down unit.

11.1.3. Gadolinium Distribution Quantitation

MRI imaging, within 4 hours of the completion of infusion, will be registered to define the shape and position of the contrast agent distribution relative to the patient's brain anatomy. 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 (39)]. 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 (39). 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 (39). 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 (39). The MRI obtained prior to CED (planning MRI) of the study drug and the post infusion MRI may be sent to Therataxis for analysis and simulation of the intraparenchymal infusions. If sent, these MRIs will be sent de-identified to Therataxis per Duke radiology department de-identification processes. They will be labelled with the patient study ID and will include a limited data set that is described in the Data Transfer and Analysis Agreement with Therataxis. These MRIs will be shipped via Fed-Ex to Therataxis, Attention Raghu Raghavan, 1101E 33rd Street, Suite 8305, Baltimore, MD, 21218. Standard IRB language will be placed in the informed consent form to inform study participants.

11.1.4. 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, as described in section 11.1.1, 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. This testing will include, but is not limited to, DNA sequencing, gene amplification, and gene expression. Additional pathology tests on the protocol-specified biopsy tissue may include, but are not limited to, EGFRvIII and EGFRwt status, TERT, IDH 1 and 2, MGMT IHC and MGMT promoter methylation, and anti-poliovirus receptor, if a sufficient amount of tissue remains after standard clinical pathologic testing.

Patients may undergo tumor resection or biopsy following PVSRIPO administration and outside the context of this protocol. Resection or biopsy may be medically indicated for

reasons not part of the investigative protocol. Should subjects have a resection or biopsy at a later time, we will request samples of this resected tissue for tissue analysis. If subjects 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.

11.1.5. Dose

The anti-neoplastic effects of PVSRIPO in animal tumor models are dose-dependent. PVSRIPO produced complete tumor regress in all treated animals at a 'mouse-adjusted' dose equivalent to 5 x 10^8 TCID50 [1/10^{th} of the non-toxic dose in NHPs in the dose-range finding toxicology study (Table 1)]. 'Mouse-adjusted' means that the dose was 1/70^{th} of 5 x 10^8 TCID50, since the starting tumor volume in athymic mice was 1/70^{th} of the maximum tumor size in patients. Reducing this dose 100-fold significantly diminished the anti-neoplastic efficacy of PVSRIPO in animal tumor models.

Since no prior experience with intracerebral inoculation of PVSRIPO in humans exists, consideration of dose-escalation is based on the results of neurovirulence assessments in dose-range finding and biodistribution studies in non-human primates. These assessments evaluated the clinical outcome and toxicity after intrathalamic administration of PVSRIPO in *Cynomolgus* macaques (Table 1 & Table 2).

The highest dose in the definitive, IND-directed study in NHPs (1 x 10^9 TCID50; Table 2) was limited by the highest feasible concentration in an acceptable inoculation volume (limited to 250 μL in *Cynomolgus*). In this NHP study, DLTs were not observed and the MTD was not reached. Therefore, in this trial, based on pre-clinical NHP toxicity studies, the proposed starting dose is 1 x 10^8 TCID50, which is 1/10^{th} of the highest non-toxic dose in NHPs in the definitive, IND-directed toxicology study (Table 2) and 1/50^{th} of the highest non-toxic dose in NHPs in the dose-range finding toxicology study (Table 1).

Table 1. Dose range-finding, toxicology, biodistribution and shedding study (BATTELLE Study 916-G663301) of PVSRIPO in *Cynomolgus* macaques

PVSRIPO Dose	Equiv. Human Dose	No. of Animals	Route	Day of Sacrifice	Clinical Outcome
Vehicle	N/A	1	intrathalamic	3	no toxicity/neurologic symptoms
Vehicle	N/A	1	intrathalamic	21	
1 x 10^7 TCID50	1 x 10^8 TCID50	1	intrathalamic	3	
1 x 10^7 TCID50	1 x 10^8 TCID50	1	intrathalamic	21	
1 x 10^9 TCID50	1 x 10^{10} TCID50	1	intrathalamic	3	
1 x 10^9 TCID50	1 x 10^{10} TCID50	1	intrathalamic	21	
5 x 10^9 TCID50	5 x 10^{10} TCID50	1	intrathalamic	3	
5 x 10^9 TCID50	5 x 10^{10} TCID50	2	intrathalamic	21	

Table 2. Definitive, IND-directed toxicology, biodistribution, shedding and neutralizing antibody study (BATTELLE Study 1098-G663323) of PVSRIPO in *Cynomolgus* macaques

PVSRIPO Dose	Equiv. Human Dose	No. of Animals	Route	Day of Sacrifice	Clinical Outcome
Vehicle	N/A	2	intrathalamic	3	no toxicity/neurologic symptoms
Vehicle	N/A	2	intrathalamic	10	
Vehicle	N/A	2	intrathalamic	56	
5 x 10^7 TCID50	5 x 10^8 TCID50	4	intrathalamic	3	
5 x 10^7 TCID50	5 x 10^8 TCID50	4	intrathalamic	10	
5 x 10^7 TCID50	5 x 10^8 TCID50	4	intrathalamic	56	

1 x 10^9 TCID50	1 x 10^{10} TCID50	4	intrathalamic	3	
1 x 10^9 TCID50	1 x 10^{10} TCID50	4	intrathalamic	10	
1 x 10^9 TCID50	1 x 10^{10} TCID50	4	intrathalamic	56	

11.1.5.1. Rules for Escalation

We propose adopting a phase I escalation strategy that avoids well-publicized shortcomings of standard '3+3' designs, in particular the problem of 'memory loss' with such designs (40). To minimize the number of patients treated at low-dose levels, minimize the number of patients needed to complete the study and efficiently use accumulated data to evaluate the appropriateness of dose escalation (40, 41), we plan to use a two-step continual reassessment method (CRM) design that includes an escalation step and a model-guided step (42, 43). In the escalation step, dose levels will be rapidly escalated as preclinical data suggest that dose-limiting toxicity (DLT) will not occur at any of the five dose levels that will be evaluated (44). Decisions concerning dose escalation for subsequent patients will be based upon the occurrence of DLT during the first 4 weeks after treatment administration. Initially, each patient will be observed for ≥4 weeks before the next patient is treated. Starting at dose level 1, one patient will be treated at each higher dose level (Table 3). If no DLT is observed within 4 weeks of administration, then the next patient will be treated at the next higher dose. If no DLTs are observed among patients treated on the first four dose levels, subsequent patients will be treated at dose level 5. The escalation step will be interrupted if a patient assigned to one of the first 4 dose levels or more than 20% of patients treated at dose level 5 experiences a DLT. In that case, further dose escalation or de-escalation will be guided by the likelihood-based implementation of the CRM, in which the one-parameter hyperbolic tangent model will be used to estimate the probability of DLT at each of the 5 dose levels based upon available data (45). The highest dose level for which this estimated probability is less than 20% will be identified. If this optimal dose is less than or the same as the current dose, subsequent patients will be treated at that dose level. If greater than the current dose, subsequent patients will be treated at the one dose level higher than the current dose. The optimal dose will be recomputed whenever the status of DLT has been determined for a patient.

As more patients are treated with PVSRIPO the impact of a DLT on subsequent patient treatment assignments by the model described above will decrease with the expectation that treatment assignments will stabilize over time at a particular dose level. If the outcome (i.e. DLT or not DLT) of a particular patient has no impact upon treatment assignment for the subsequent patient or patients, a 4-week observation period will not be required before accruing that additional patient or patients.

For example, consider the situation where one patient has already been treated at each of the first 4 dose levels without DLT and 4 patients have been treated at dose level 5 without DLT. Under the model described above, a 5th patient would be treated on dose level 5. If this 5th patient experiences a DLT, the treatment assignment for the subsequent patient would be dose level 5 according to the rules described above. If this 5th patient does not experience a DLT, the subsequent treatment would also be dose level 5. Hence, a 4-week observation period for the 5th patient on dose level 5 is unnecessary to determine the dose treatment assignment for the following patient. In this case, a 4-week observation period for the 5th patient will not be required as it has no impact upon treatment assignment for the subsequent patient.

The clinical team will keep the statistical team apprised of the current status of all patients. The statistical team will determine and inform clinical investigators as to whether a 4-week observation period is required for some or all patients before another patient is accrued. More specifically, based upon the rules described above, the statistical team will inform

clinical investigators as to how many patients can be accrued while other patients are within their 4-week post-treatment observation period.

Table 3. Dose escalation schedule for PVSRIPO

Dose Level	PVSRIPO dose	Anticipated No. of patients treated in original design*	Administration route
1	1.0 x 10^8 TCID50	1	intratumoral
2	3.3 x 10^8 TCID50	1	intratumoral
3	1.0 x 10^9 TCID50	1	intratumoral
4	3.3 x 10^9 TCID50	1	intratumoral
5	1.0 x 10^{10} TCID50	21	intratumoral

*Please refer to study design modifications below.

11.2. RETREATMENT WITH PVSRIPO

Selected patients who benefited from the initial infusion of PVSRIPO within this protocol will be eligible for a second infusion. The criteria for a patient to be considered for a second infusion is provided in section 11.2.1. Details about that treatment plan are provided in section 11.2.

11.2.1. Eligibility Criteria for Retreatment

- Subjects can be considered for PVSRIPO retreatment if they meet the pertinent inclusion and exclusion criteria for study retreatment. Specifically, the subject must satisfy inclusion criteria provided in Sections 9.1.2 - 9.1.4 and the exclusion criteria in Sections 9.2.1 - 9.2.10. In addition, the subject must also satisfy the following criteria: Patient must have benefited from initial treatment with PVSRIPO (i.e. be at least 12 months following their initial PVSRIPO infusion).
- Patients must have a recurrence of their supratentorial WHO grade IV malignant glioma based on imaging studies with measurable disease (≥ 1 cm of contrast-enhancing tumor).
- At the time of biopsy, prior to administration of virus, the presence of recurrent tumor must be confirmed by histopathological analysis, unless already proven within the last 3 months via tissue sampling (biopsy or resection).
- The patient must have received a new boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week and no more than 4 weeks prior to re-administration of the study agent. The boost immunization may be obtained locally, prior to signing consent, as long as the patient has been informed about this study procedure via phone script.
- A new signed informed consent form for retreatment approved by the Duke University Institutional Review Board (IRB) will be required for retreatment. Patients 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.

11.2.2. Retreatment Plan

Catheter implantation (section 11.1.1), PVSRIPO infusion at dose level -1 (section 11.1.2), gadolinium distribution quantitation (section 11.1.3), and biopsy sampling (section 11.1.4) will occur according to the procedures previously described in this protocol. In addition to the PVSRIPO retreat infusion, the patients will also receive a single dose of lomustine at approximately 8 weeks post-infusion of PVSRIPO. All procedures previously described will be followed; however, the addition of the single dose of lomustine will alter the schedule of events that was previously described. All retreated patients will follow the schedule of events shown in Table 6 and described in section 12.

11.3. HISTORICAL MODIFICATIONS TO THE PROTOCOL

11.3.1. Study Design Modifications (11/15/2013 AMD025)

As of 11/15/2013, 4 patients have been treated on dose level 5. One patient experienced a DLT upon removal of the treatment catheter. The remaining 3 patients have not experienced a DLT; however, all 3 have had difficulties tapering off steroids. To address this concern, the study is amended to reduce the dose for the next cohort of patients to dose level 2. Up to 6 patients will be accrued at dose level 2. If 2 of these patients experience a DLT as defined in section 11.4, accrual will be suspended. Following a two-week observation period, during which the patient will be evaluated in clinic on day 7 (± 2 days) and day 14 (± 2 days), a patient summary will be prepared and submitted to the DSMB chair for approval. Once approval from the DSMB chair has been obtained, treatment of the next patient will be allowed. Only one patient will be treated every 2 weeks and there will be only one PVSRIPO treatment per patient without specific approval for a second or more treatments from the FDA and the IRB.

11.3.2. Study Design Modifications (6/05/2014 AMD041)

As of 06/05/2014, 5 subjects have been treated on dose level 2. Four subjects have completed the two-week follow-up period and have not had a DLT. One subject remains in the 14 day follow up period. Once one last patient has been treated, the study per the November 2013 amendment is complete. We are amending the study to allow continued patient accrual to garner more information about patient safety while the single-institution phase 2 study is being developed and reviewed by various regulatory bodies. We intend to continue accruing subjects for treatment at dose level 2 until such time that the phase 2 study has been activated. After each patient has been followed for a one-week observation period, during which the patient will have been evaluated in clinic on day 7 (± 2 days), a patient summary will be prepared and submitted to the DSMB chair for approval. Once approval from the DSMB chair has been obtained, treatment of the next patient will be allowed. We will treat up to four subjects within a 30-day period as allowed by DSMB chair, and there will be only one PVSRIPO treatment per patient without specific approval for a second or more treatments from the FDA and the IRB.

11.3.3. Study Design Modifications (10/06/2014 AMD048)

As of 10/06/2014, 7 subjects have been treated on protocol at dose level 2. One additional subject has been treated under a Single Subject Emergency Use Request at dose level 2. Of these eight subjects, six subjects had difficulty or remained unable to be tapered off steroids more than three months post infusion (three subjects required the addition of bevacizumab to facilitate the taper down or off steroids). We are amending the study to reduce the dose to dose level -1 (5.0×10^7 TCID50) with the goal of limiting the occurrence of known possible side effects of prolonged steroid use in patients who otherwise benefit from this investigational therapy. This dose reduction will allow continued patient accrual and garner more information about patient safety and optimal dosing while the single-institution phase 2 study is being developed and reviewed by various regulatory bodies. Our last amendment allowed the enrollment of up to 18 patients, one patient was enrolled under that amendment and thus, we are planning to enroll up to 17 patients on dose level -1. After each patient has been followed for a one-week observation period, during which the patient will have been evaluated in clinic on day 7 (± 2 days), a patient summary will be prepared and submitted to the DSMB chair for approval. Once approval from the DSMB chair has been obtained, treatment of the next patient will be allowed. We will treat up to four subjects within a 30-day period as allowed by DSMB chair, and there will be only one PVSRIPO treatment per patient without specific approval for a second or more treatments from the FDA and the IRB.

11.3.4. Study Design Modifications (1/13/2015 AMD054)

Once the optimal dose level of PVSRIPO is determined, a total of 26 patients will be treated at that dose level, on a dose expansion cohort. The survival associated with these 26 patients will be compared to that of a historical cohort (see section 14.3). The study is being amended to allow accrual and treatment of a total of 26 patients at the optimal dose level of PVSRIPO and, as needed, radiation-necrosis dose levels and schedules of bevacizumab to estimate the efficacy of PVSRIPO relative to a historical cohort.

11.3.5. Sample Size Modification (6/24/2015 AMD071)

As of 6/24/15, we have treated 13 patients on dose level -1. None of the patients have had prolonged steroid needs, two patients died at 3 and 6 months respectively, and five patients total (including the two patients who died) were initiated on bevacizumab. It is now felt that dose level -1 is the optimal dose, as none of the patients have experienced undue side effects due to PVSRIPO or the management of the cerebral inflammation secondary to PVSRIPO. We are now amending the protocol to enroll a total of 50 patients on dose level -1.

11.3.6. Study Design Modification (11/10/2015 AMD078)

Imaging response criteria were previously developed based on imaging observations following chemoradiation treatment (Macdonald criteria) and subsequently revised based on new observations from anti-angiogenic trials (RANO criteria). As imaging is dramatically different following treatment with immunotherapy, new imaging criteria are being developed (iRANO). This process is likely to take several years to validate. For that reason, we are removing response rate as a secondary objective and adding, as an exploratory objective, a description of changes visualized on imaging due to intratumoral inoculation of PVSRIPO.

11.3.7. Study Design Modification (2/1/2016 AMD081)

The protocol has been re-formatted in an electronic Common Technical Document (eCTD) format for electronic submission to the FDA for the associated IND. The statistical section has been revised and an appendix (section 18.4) has been added to define and describe the historical control group being used as a statistical comparison for the subject population. We have also further described the types of subject follow-up activity that will occur in this study.

11.3.8. Study Design Modification (6/15/2016 AMD096)

As of 6/15/2016, 23 subjects have been treated on dose level -1, including 12 subjects that were treated more than 12 months ago. Three of these 12 subjects demonstrated tumor reduction without significant inflammation necessitating prolonged use of bevacizumab and/or additional chemotherapy. In contrast, the remaining 9 patients had inflammation that was burdensome and required prolonged use of bevacizumab and/or additional chemotherapy. Caregivers of some of these patients were overwhelmed by the experience. The 3 patients without significant inflammation are alive at 20.2, 15.5, and 13.1 months after PVSRIPO infusion, and 7 of the 9 patients with burdensome inflammation have died, including 5 that died within 12 months of PVSRIPO treatment.

Despite the fact that subjects on dose level -1 have been able to remain off significant doses of steroids, we believe that the subjects benefiting the most from PVSRIPO have been those who have experienced minimal or easily controllable inflammation.

As such, we amend this study to reduce the PVSRIPO dose to dose level -2 (1.0×10^7 TCID50) 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, we are confident that dose level -2 (1.0 X 10^7 TCID50) will be a therapeutic dose. This amendment is not due to concerns for the safety of the patients on dose level -1, but due to the observation that less inflammatory reaction is a predictor of better survival and treatment response.

We plan to treat 27 patients at dose level -2. After each patient has been followed for a one-week observation period, during which the patient will have been evaluated in clinic on day 7 (± 2 days), a patient summary will be prepared and submitted to the DSMB chair for approval. Once approval from the DSMB chair has been obtained, treatment of the next patient will be allowed. We will treat up to four subjects within a 30-day period as allowed by DSMB chair, and there will be only one PVSRIPO treatment per patient without specific approval for a second or more treatments from the FDA and the IRB.

Furthermore, patients previously treated on trial received high dose steroids prior to catheter insertion and throughout the infusion, as part of undergoing biopsy. Given concerns for negative impact of high dose steroids on the efficacy of PVSRIPO, we will limit steroid usage to the minimal dose necessary, if even needed.

11.3.9. Study Design Modification (6/29/2016 AMD101)

This amendment includes several revisions to tests and procedures, which we believe can be safely implemented with the aim of reducing the time between consenting to participate in the study, screening to determine eligibility, and study treatment. Previously, patients had to demonstrate a serum total IgG level of ≥ 400 mg/dL to be eligible for the study. All patients who have undergone this testing thus far have met this eligibility criterion; therefore, it is no longer considered necessary to test serum IgG in patients going forward prior to their enrollment. We are also changing the timing of the boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) from between 6 months and 2 weeks prior to PVSRIPO infusion to between 6 months and 1 week prior to PVSRIPO infusion. Also, for previously enrolled patients, a patient had to be followed for a one-week observation period post-infusion, before the next patient could be treated. Now, once a subject has been observed through successful completion of the PVSRIPO infusion, the next subject can be treated. We are updating pharmacy instructions for the preparation and administration of PVSRIPO in this amendment as well. Lastly, per Duke's Research Integrity Office (RIO), a study-specific DSMB may no longer be necessary for this study given the oversight provided by the BTC's DSMB-Plus. If the FDA concurs, the study-specific DSMB may be dissolved per its charter.

11.3.10. Study Design Modification (9/5/2016 AMD104)

This amendment includes the addition of an exploratory objective to identify genetic predictors of response or failure of response to treatment with PVSRIPO. Molecular genetic tests, such as, but not limited to, DNA sequencing, gene amplification, and gene expression, will be performed on the tissue obtained at the protocol-specified biopsy prior to PVSRIPO infusion. Tissue may be obtained for testing as either fresh, frozen, or fixed tissue, or as slides, after the diagnostic pathologist has retained all tissue needed for diagnosis. The amount of tissue required for this testing should be sufficient to yield a minimum of one microgram of DNA.

11.3.11. Study Design Modification (3/1/2017 AMD124)

Based upon recent pre-clinical data and clinical data collected on dose levels -1 and -2, we have concluded that there is no evidence of pre-clinically or clinically important differences between these dose levels. The initial need for treatment with the radiation necrosis dose levels and schedules of bevacizumab for cerebral inflammation secondary to PVSRIPO or tumor growth has been comparable thus far. In addition, the toxicity

profiles for the two dose levels are also similar. Therefore, we have decided to proceed with the development of PVSRIPO dose level -1 (5.0 X 10^7 TCID50) within the phase II study that is now in development. We are amending this phase I study to treat all future patients at the -1 dose level. We intend to continue accruing patients and treating patients at this dose level while the phase II study is being finalized and reviewed by various regulatory bodies.

This protocol is also being modified to allow multiple patients to be infused with study drug simultaneously if the neuro-surgical schedule allows. There are currently 3 neurosurgeons listed as sub-investigators who are trained on the surgical procedures for this study. In the study modification dated 6/29/2016, the study was modified so that the next patient could be treated immediately following the completed infusion of the previous patient. However, we would like to increase our enrollment potential by allowing multiple patients to be treated simultaneously. As of 1/20/2017, no patients have experienced complications during the infusion of PVSRIPO. Only 1 out of 51 treated patients has experienced severe complications (Grade III or higher) during catheter removal following the infusion of the study drug, and therefore we are confident that treating multiple patients would be safe for the patients and would allow for increased data collection.

This amendment also includes updates to the program used for planning catheter placement, the timing of catheter removal, the infusion pump information, and to clarify the history of DLT monitoring in the study. The statement related to sending MRIs to Therataxis was modified to state that the MRIs may be sent to Therataxis. A clarification has been made regarding the blood test for anti-epileptics. Lastly, the exclusion criterion related to completion of standard of care treatments has been clarified based on whether or not the MGMT promotor methylation status is known at the time of screening. There are other minor corrections throughout.

Following the FDA's review of this amendment, an additional section (see section 11.5) describing guidelines and criteria for study pause as a result of toxicity monitoring has been added. Specifically, the study is being monitored for the occurrence of unacceptable toxicities within dose levels -1 and -2 at any time after the initiation of protocol treatment.

11.3.12. Study Design Modification (6/22/2017 AMD142)

It has been recently observed that patients who originally benefitted from the infusion of PVSRIPO can demonstrate tumor recurrence years later. Given the limited life expectancy of recurrent glioblastoma patients, it has been hypothesized that retreatment of long-term survivors previously treated with PVSRIPO could trigger an immune recall effect and thus further extend the survival of patients. As such, in the current amendment, we will allow selected patients who have previously benefited from PVSRIPO infusion to be retreated with PVSRIPO in the event of tumor recurrence. A patient is considered to have benefited from the initial PVSRIPO infusion if they survived 12 or more months after PVSRIPO treatment. All patients retreated will be treated at dose level -1 as per the same procedure previously used. Furthermore, all retreated patients will receive a single dose of lomustine 8 weeks post infusion of PVSRIPO. As lomustine temporarily reduces the number of regulatory T-cells, it is hypothesized that it could potentially increase the efficacy of PVSRIPO and of the immune recall. Further details regarding this retreatment plan are in Section 11.2.

11.3.13. Study Design Modification (9/25/2017 AMD149)

Based upon the Final Clinical Shedding Study for PVSRIPO (IND 14,735) dated 8/6/2017, the IBC reviewed the requirement for stool sampling for patients receiving investigational treatment with PVSRIPO. The purpose of the stool sampling was to test for presence of

viral shedding in stool samples of patients receiving PVSRIPO via intracerebral delivery. The Final Clinical Shedding Report indicates no presence of PVSRIPO in stool collected from 59 subjects who received 61 treatments with PVSRIPO (2 patients were re-treated). Per the report, "we feel that the serologic evidence indicates a formidable protective shield against shedding in patients with pre-existing and boosted anti-polio immunity." On 8/17/2017, the IBC determined that the requirement for viral shedding studies may be discontinued on the basis of the report. Therefore, we have removed stool sampling from Section 12.2 and from Table 6. In addition, we have added 3 additional biopsy core samples to be taken at the time of biopsy, prior to catheter placement, if possible. The additional biopsy core samples will be used for genetic analysis, including full genome or full exome sequencing, as well as other molecular genetic testing.

11.4. DOSE-LIMITING TOXICITY

Patients will be monitored for toxicity indefinitely or until confirmation of recurrent disease or the patient prematurely discontinues from the study at Duke University Medical Center. Adverse events will be categorized and graded in accordance with the NCI CTC (Version 4).

During the dose escalation phase of this study, patients were also monitored for dose-limiting toxicity (DLT). As no patient experienced a DLT on dose level 2, dose level 2 was determined to be the MTD. On 10/06/2014, the protocol was amended to reduce the dose to dose level -1 (see section 11.3.3). Since dose level 2 was determined to be the MTD, DLT monitoring was discontinued at that time.

With the removal of oversight by the study-specific DSMB, a patient summary with toxicity review will no longer need to be generated after each patient (see section 11.3.9).

11.4.1. Definition of Dose-limiting Toxicity

Dose-limiting toxicity (DLT) will be defined using the NCI Common Toxicity Criteria (CTC) (Version 4). Any Grade 3 or any Grade 4 toxicity that is not reversible within 2 weeks, or any life-threatening event, or treatment-related death will be considered a DLT. Any grade 2 or higher serious autoimmune toxicities particularly those affecting vital organs (e.g. cardiac, renal, CNS) will be considered a DLT if it occurs within 2 weeks with or without treatment. Exceptions to these DLT's 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 (Grade 3 or higher surgical complication from insertion of catheter is considered DLT).
- 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 DLT if, in the opinion of the 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 neurologic deficits: Due to the nature of the disease under investigation in this protocol, patients 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 a DLT. Similarly, late ($\geq$ 4 weeks after completion of infusion) DLTs thought to be drug or procedure related, which resolves within 2 weeks after initiation of medical therapy, will not be considered a DLT. New neurological symptoms will not be a DLT 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: Due to the high incidence of deep vein thrombosis (DVT) in this patient population, 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 DLT 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 DLT 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 Grade 4 toxicity and weight gain ≥ 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 DLT 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.
- Tumor Progression: Due to the nature of the disease under investigation in this protocol, patients may have an increase in pre-existing neurologic deficits or have an onset of new neurologic deficits due to tumor progression. Although such neurologic deficits may be defined as DLTs under NCI CTC, and will be reported as such in this protocol, these clinical changes are not an unexpected phenomenon in this disease in the setting of tumor growth. As a result, neurologic deficits will not be considered a DLT if unequivocal tumor progression can be documented radiographically or histologically.

11.4.2. Management of Toxicities

If a Grade 3 or 4 NCI CTC toxicity is observed, the patient will be monitored until the NCI CTC toxicity improves to a Grade 1. If this resolves within 4 weeks with or without treatment, these toxicities will not be considered dose limiting. If a new Grade 3 or 4 NCI CTC toxicity is seen again in the same subject after initial resolution within 4 weeks of the initial infusion, a DLT will be declared.

If Grade 2 or higher serious autoimmune NCI CTC toxicity is observed the patient will be monitored until the NCI CTC toxicity improves to a Grade 1. If resolution to Grade 1 occurs within 4 weeks with or without treatment, these toxicities will not be considered dose limiting.

11.5. TOXICITY MONITORING AFTER DOSE ESCALATION

Though DLT monitoring has been discontinued due to the declaration of dose level 2 as the MTD, toxicity monitoring continues. Specifically, the study is being monitored for the occurrence of unacceptable toxicities within dose levels -1 and -2 at any time after the initiation of protocol treatment. We will use the definition of DLT provided in section 11.4.2 as the definition of an unacceptable toxicity.

Given that both long- and short-term toxicities are of interest, it is not feasible to suspend accrual while toxicity is assessed as was done within the dose-escalation portion of this study. If 25% or more of patients have experienced an unacceptable adverse event or there are other reasons for concern about the safety of patient treatment (e.g. treatment-related toxic death), accrual will be suspended and data will be carefully reviewed to determine if accrual should be permanently terminated or the protocol modified. These guidelines have not been adjusted for differential length of follow-up of accrued patients.

Every 6 months, the toxicity experienced by patients accrued to this study will be summarized and reviewed, regardless of the number of patients accrued, to determine whether the overall toxicity profile of treatment is unacceptable or not.

11.6. TOXICITY MONITORING OF PVSRIPO RETREATMENT

Though there is growing knowledge about the long- and short-term toxicities associated with an initial PVSRIPO infusion, there is a possibility that the toxicities associated with a second infusion might differ in some manner from the initial infusion. Hence, the toxicity monitoring that has occurred with the initial PVSRIPO infusion will continue with the second PVSRIPO infusion. The guidelines provided in section 11.5 will also be implemented in the monitoring of toxicity associated with the second infusion of PVSRIPO.

11.7. DISCHARGE, FOLLOW-UP AND DURATION OF STUDY

Patients will be followed at a minimum of 2, 4, 8, 16, 24, 32, 40 and 48 week intervals after infusion as outlined in the Study Test Schema (see section 18.1).

Patients may not be treated with any other modality (other than bevacizumab per the 'special considerations' in section 15.2.7) 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. Subject's medical records will be reviewed for the remainder of their life, in order to follow survival, as will subjects' MRIs. Subjects will also be followed for progression and subsequent treatments. Follow-up activity will be at the discretion of the treating physician.

11.8. CRITERIA FOR SUBJECT DISCONTINUATION OF PROTOCOL TREATMENT

Protocol treatment for a subject can be discontinued for any of the following reasons listed below, although patients will continue to be followed as described above in section 11.7, if possible. An explanation will be recorded for each patient taken off treatment and the off treatment summary completed.

- Progressive or recurrent disease as documented by MRI or physical examination for which the patient's tumor is treated with another modality, with the exception of bevacizumab as described in section 15.2.7.
- Development of DLT during the dose escalation phase (please refer to sections 11.1.5.1 and 11.4).
- The occurrence of two or more grade 4 events or any death in the study that are possibly related to PVSRIPO.

- Upon request of the patient or the patient's legal representative.
- Administrative reasons, such as a major violation of the clinical trial protocol.
- Non-compliance of the patient.

12. DATA AND SPECIMEN COLLECTION

12.1. DATA AND SPECIMENS TO BE ACCESSIONED PRIOR TO TREATMENT

- Registered in the Velos e-Research software system.
- Complete physical and neurologic examination with KPS rating.
- MRI (with and without gadolinium enhancement) of the brain.
- PT/PTT, CBC with diff, CMP, within 14 days of treatment
- β-HCG serum pregnancy test within 48 hours of treatment for female patients.
- Receive a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) poliovirus vaccine at least 1 week prior to administration of the study agent. For retreatments, this may be obtained locally and prior to signing consent, as long as the patient has agreed via the phone script consent.
- Anti-tetanus toxoid IgG, LSQ, anti-poliovirus antibody, 76.5 mL of whole blood for tests of anti-tumor immune response, and 10 mL of whole blood for tests of immunologic assays within 6 months prior to treatment, prior to the boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur). Among patients who are retreated with PVSRIPO, the anti-tetanus toxoid IgG, LSQ, anti-poliovirus antibodies, 76.5 mL of whole blood for tests of anti-tumor immune response, and 10 mL of whole blood for tests of immunologic assays obtained prior to the boost immunization will not be collected.
- 76.5 mL of whole blood for tests of anti-tumor immune responses and 10 mL of whole blood for tests of immunologic assays within 14 days of treatment, at any time post boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur), but before PVSRIPO infusion.

Pathology Studies: (This will only be performed once the patient has been treated with PVSRIPO so that tissue is not being requested on ineligible patients). Tissue from the patient's original surgery or any other surgery will be requested in the form of either a block or enough unstained slides for the following tests: Immunohistochemistry (IHC), including but not limited to EGFRvIII, TERT, IDH 1 and 2, MGMT, anti-poliovirus receptor, and MGMT quantitative level. Any other laboratory tests that may guide treatment or determine prognosis may also be performed with this tissue. If slides are requested, they will be prepared on Fischer Plus glass or Histostix coated slides and will be unstained FFPE or otherwise appropriately prepared slides. Also, if any of this testing requires de-identified tissue, the slides will be de-identified by the study team and sent to Dr. Gromeier or his designee for additional testing.

12.2. DATA AND SPECIMENS TO BE ACCESSIONED DURING AND AFTER TREATMENT

DURING INFUSION AND AFTER TREATMENT AT VISITS AT 1, 2, 4, 8, 12 (RETREATMENT ONLY), AND 16 WEEKS AND EVERY 8 OR 9 (RETREATMENT) WEEKS THEREAFTER (STARTING WITH DAY 7 [WEEK 1] ALL TESTS AND PROCEDURES HAVE A SEVEN DAY PLUS OR MINUS WINDOW.)

- General physical examination and neurologic examination will be obtained daily after infusion until discharge from hospital and at each follow-up visit.
- CT to confirm catheter placement ≥ 1cm from the ventricles prior to beginning infusion.
- MRI (without gadolinium-enhancement) of the brain within 4 hours of completing the infusion to monitor distribution of infusion.
- MRI (with gadolinium-enhancement) at follow-up visits (MRI only at weeks 4, 8, 16 [retreatment] and every 8 or 9 [retreatment] weeks thereafter.)
- 76.5 mL of whole blood for tests of anti-tumor immune responses at Weeks 1, 4, 8, 12 (retreatment), 16, and 24 or 25 (retreatment) after treatment. At the discretion of the PI, an additional blood sample at least 2 years post study drug infusion may be obtained. 31 mL collection of whole blood at Day 14.
- 10 mL of whole blood for tests of immunologic assays and anti-poliovirus antibody titer at Weeks 1, 4, 8, 12 (retreatment), 16, 24 or 25 (retreatment), and 34 (retreatment). Five mL collection of whole blood at Day 14. At the discretion of the PI, an additional blood sample at least 2 years post study drug infusion may be obtained.
- 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 (please refer to section 11.1.4 for details).
- Tumor material obtained from biopsy or resection at any time after PVSRIPO administration will be tested for parameters of virus tropism, virus translation and replication, virus: host interaction, host response to infection and host response to viral tumor lysis. 1 sq cm of tumor. 1/3 stored as OCT block for future staining, and 1/3 saved as either dissociated tumor or snap frozen.
- CBCs and CMPs will be drawn day 1 post infusion and at each follow-up visit.
- Treatment data may be collected for the remainder of subject's lives.

Subjects will have approximately 465 mL's of blood drawn during the first 8 weeks of this study.

Immune monitoring samples may be shipped to the NIH for analysis per Materials Transfer Agreement.

13. CLINICAL RESPONSE EVALUATIONS

13.1. RADIOGRAPHIC TUMOR RESPONSE CRITERIA

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.

13.2. PROGRESSION FREE SURVIVAL (PFS)

Patients potentially eligible for this study will be followed from the time protocol treatment is initiated. The PFS is defined as the time between initiation of protocol treatment and the first occurrence of disease progression. The PFS will be defined radiologically, if possible; or alternatively defined by a significant overall change in clinical status as assessed by the study principal investigator, Dr. Dina Randazzo, or her designees.

13.3. SURVIVAL

Survival time is defined as the time between the initiation of protocol treatment and death, with the survival of live patients being censored at the last follow-up. Survival data in the context of a Phase I/II trial must be interpreted with great care. There are a few studies in the literature that may be able to be compared to our study to some degree. For example, the carmustine polymer study (47), has a set of very similar inclusion, and eligibility criteria. However, two important differences do exist. First, this study required a gross total resection prior to implantation of carmustine polymers. Second, patients were randomized to treatment. This will allow us to compare the results of the current clinical trial to previously published trials of patients with similar prognostic features.

14. STATISTICAL CONSIDERATIONS

14.1. SAFETY AND DOSE-FINDING COMPONENT

The dose-escalation criteria as outlined above in section 11.1.5 will be used to determine the MTD of PVSRIPO.

14.2. ANALYTICAL METHODS

Descriptive analyses will be conducted to assess secondary objectives. These analyses should be interpreted with caution in light of the relatively small number of patients and the pooling of data from all dose levels. A specific statistical hypothesis will not be tested. The product limit estimator of Kaplan and Meier will be used to describe the distribution of survival time and time to progression.

14.3. DOSE EXPANSION COHORTS

As described in section 11.3, the protocol amendment dated 6/24/2015 (AMD071) allowed the treatment of up to 50 subjects at dose level -1 to assess efficacy. The amendment dated 6/15/2016 (AMD096) reduced the PVSRIPO dose for treatment to dose level -2. Subsequently, amendment dated 2/9/2017 (AMD124) increased the treatment dose of PVSRIPO to dose level –1. Section 11.3 provides the rationale for all these changes.

14.3.1. Dose Expansion at Dose Level "-1"

Accrual at dose level -1 will be extended in order that a sufficient number of patients will be treated at that dose level to estimate its efficacy relative to a historical cohort.

As of 6/15/2015, 28 patients have been treated on this protocol, including 13 patients treated at dose level -1. The median survival is 15.2 months (95% confidence interval: 7, ∞). Median follow-up among patients treated at dose level -1 is 3.5 months.

Informal comparisons of survival associated with PVSRIPO (including all patients at all dose levels) relative to that observed in other recurrent WHO grade IV malignant glioma patient groups suggest a hazard ratio in the ballpark of 0.5 to 0.65.

The historical cohort that will be used for this comparison will include recurrent WHO grade IV malignant glioma patients in the PRoGREss registry (IRB# Pro00027120; Primary and Recurrent Glioma Registry) who would have been eligible to receive PVSRIPO if the treatment had been available. Details about the construction of this comparison group, as well as a summary of control group patient characteristics are provided in Appendix 18.4. Two control groups were generated, with one group having no limit to pre-treatment steroid usage, and the other group having a steroid limit of ≤ 4 mg per day of dexamethasone within the 2 weeks prior to being eligible for PVSRIPO infusion. The first control group has eligibility criteria similar to that which was in existence for dose levels 1-5 and the first 5 patients on dose level "-1". The second control group has eligibility criteria similar to those currently being used for dose level "-1". The first historical control group includes 124 patients of which 123 are dead; whereas, the second historical control group includes 104 patients of which 103 are dead.

Korn and Friedlin (48) recommend the use of an unconditional power estimator for the determination of sample size in single arm studies that compare time-to-event outcomes such as survival with that observed in a historical control group. That estimator resembles an estimator for a 2-arm randomized study. The power of such a comparison is dependent upon the total number of observed deaths (49).

The ultimate goal of this accrual expansion and survival comparison is to determine whether PVSRIPO administered at dose level -1 and, as needed, with a radiation-necrosis dose level and schedule of bevacizumab in the manner described within this protocol has merit for further evaluation in additional clinical studies. This goal is analogous to that of a randomized phase II screening study, except for the fact that the two groups are not created by randomization. The second historical control group will be considered in this comparison. Typically, with a randomized phase II study, there is a need to constrain the sample size requirements at the expense of either an increased false negative or false positive rate. It has been recommended that a false-positive rate of 0.2 be used to test a hypothesis comparing arms while maintaining a high power level (e.g. 90%).

A total of 50 subjects will be accrued and treated at dose level -1. Assuming survival is exponentially distributed and the median survival associated with the -1 dose level is 15.2 months, we anticipate that approximately 15 of the 50 patients will be deceased by the time accrual is complete in June 2016. Without additional follow-up, the power of a logrank test conducted at the 0.2 level of significance (one-tailed) to detect a hazard ratio of 0.5, 0.55, 0.6, and 0.65 are 0.95, 0.91, 0.84, and 0.76, respectively. Tabulated below is the power to detect these hazard ratios as a function of the number of additional months of follow-up after accrual completion (Table 4).

Table 4. Power of the survival comparison to detect the noted hazard ratios

Follow-up (Months)	Expected # Dead if Median OS = 15.2 months	Power of Comparison to Detect Noted Hazard Ratio			
		Hazard Ratio = 0.5	Hazard Ratio = 0.55	Hazard Ratio = 0.6	Hazard Ratio = 0.65
0	15	0.95	0.91	0.84	0.76
1	16	0.96	0.92	0.85	0.78
2	18	0.97	0.93	0.88	0.80
3	19	0.97	0.94	0.89	0.81
4	21	0.98	0.95	0.90	0.83
5	22	0.98	0.96	0.91	0.84
6	23	0.98	0.96	0.91	0.85
7	24	0.99	0.96	0.92	0.85
8	26	0.99	0.97	0.93	0.87
9	27	0.99	0.9	0.94	0.87
10	28	0.99	0.97	0.94	0.88
11	29	0.99	0.98	0.94	0.89
12	30	0.99	0.98	0.95	0.89

Given that the study is not a randomized study, there is a need to statistically adjust survival comparisons for confounding factors. Hence, survival comparisons will be conducted using the Cox model. Predictors within the Cox model will include treatment, age, extent of resection, prior bevacizumab treatment, Karnofsky performance status at the time of PVSRIPO treatment, number of prior recurrences, and an indicator of secondary GBM.

It should be noted that the calculations and analyses described above assume that survival with PVSRIPO is exponentially distribution and that the proportional hazards assumption is satisfied. Recently reported studies suggest that such properties may not exist for immunotherapeutic agents due to (1) delayed antitumor effects and hence delayed separation of survival curves, and (2) a percentage of patients who are “cured” or survive for a relatively long period of time. If that is the situation with PVSRIPO, the power calculations and planned analyses may not be appropriate. It might be more appropriate to focus on long-term survivorship at a specific time point such as 18 and 24-month survival. Within the second historical control group, the 18 and 24-month survival probabilities are 23.1% and 13.5%, respectively. A two-tailed chi-square test (α=0.2) conducted to compare the proportion of patients alive at 18 or 24 months at the 0.2 level has >84% power to detect an increase of 15% in survivorship. Logistic regression will be used to adjust for confounding factors.

14.3.2. Dose Expansion at Dose Level -2

As described in section 11.3.8, this protocol is amended to reduce the PVSRIPO dose to dose level -2 (1.0 X 10^7 TCID50) 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, we are confident that dose level -2 (1.0 X 10^7 TCID50) will be a therapeutic dose. This amendment is not due to concerns for the safety of the patients on dose level -1, but due to the observation that less inflammatory reaction is a predictor of better survival and treatment response.

Twenty-seven (27) patients will be accrued to the dose expansion at dose level -2. The ultimate goal of this accrual expansion is to determine whether PVSRIPO administered at dose level -2 has merit for further evaluation in additional clinical studies. The second historical control group as described in Appendix 18.4 and section 14.3.1 will be to make this assessment.

A false-positive rate of 0.2 will be used to test a hypothesis comparing dose level -2 with the historical control group while maintaining a high power level (e.g. 90%). The rationale is provided in section 14.3.1.

Given that recently reported studies of effective immunotherapeutic agents show (1) delayed antitumor effects and hence delayed separation of survival curves, and (2) a percentage of patients who are "cured" or survive for a relatively long period of time, we will focus on long-term survivorship at a specific time point (i.e. 24 months). Within the second historical control group, the 24-month survival probability is 13.5%. Assuming 27 patients in the historical group, simulation studies show that a two-tailed chi-square test (α=0.2) conducted to compare the proportion of patients alive at 24 months at the 0.2 level has 88.7% power to detect an increase to 35% in 24-month survival. Logistic regression will be used to adjust for confounding factors.

14.4. RETREATMENT WITH PVSRIPO

The analysis of data collected in conjunction with PVSRIPO retreatment will be descriptive as the sample size is expected to be limited. The rigor and approach to all the associated exploratory analyses will be dependent upon the number of patients who are retreated.

The toxicity associated with the second infusion of PVSRIPO will be summarized separately from those observed with the first infusion, but in a similar manner. A landmark survival analysis will describe PFS and OS starting at the time of the second infusion. Changes over time after the first infusion of PVSRIPO in various immune function/response parameters will be described. Among patients who receive a second PVSRIPO treatment, changes observed in immune function/response associated with the second infusion will be compared to those observed with the first infusion.

15. POTENTIAL BENEFITS AND RISKS

15.1. POTENTIAL BENEFITS

The potential benefits may include reduction and/or remission of the subject's brain cancer. Because this procedure is experimental, it cannot be guaranteed that subjects will receive any benefit as a result of participating in this research study. The information collected in this research may help scientists better understand the mechanisms involved in viral oncolysis. If such an understanding emerges from this research, it may benefit society by furthering the development of improved treatment methods for WHO grade IV malignant glioma in the future.

15.2. POTENTIAL RISKS

15.2.1. Surgical Complications

The stereotactic catheter implantation procedure carries a risk for 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.

15.2.2. 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.

15.2.3. Poliomyelitis

PVSRIPO has been tested in NHPs 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 NHPs after intracerebral inoculation of virus. However, PVSRIPO are replication-competent viral agents that, in principle, retain the potential to cause motor neuron damage. Possible complications include transient or permanent mono- or paraparesis, paralysis, and life-threatening respiratory insufficiency.

15.2.4. 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.

15.2.5. Long-Term Sequelae of Virus Injection

PVSRIPO does not encode foreign genetic material, polioviruses are unable to insert viral genetic material in the host genome and polioviruses 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 of virus replication. 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.

Prolonged gastrointestinal propagation of polioviruses, associated with prolonged virus shedding with stool, has been reported in select individuals with acquired or rare inherited immunodeficiency disorders. Primate toxicology results indicate that extraneural dissemination and shedding of PVSRIPO with stool do not occur after intracerebral inoculation. However, if PVSRIPO shedding is detected in stool samples scheduled to be collected from study subjects, virus excretion will be analyzed and documented in study subjects at monthly intervals as long as it persists.

15.2.6. Gastrointestinal Infection and Virus Excretion

After oral uptake, poliovirus replicates in the gastrointestinal tract and is excreted by infected individuals with stool. Gastrointestinal viral replication usually is asymptomatic, but may cause mild symptoms of gastrointestinal discomfort. Tests of PVSRIPO in NHPs suggest that excretion of virus does not occur after intracerebral administration, implying the absence of gastrointestinal replication. However, PVSRIPO excretion with stool cannot be categorically excluded in patients enrolled in this study.

15.2.7. 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 a patient demonstrates neurologic or radiographic signs suggestive of an inflammatory reaction secondary to the immune response expected from PVSRIPO for which it would be considered to increase 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 initiated on bevacizumab 7.5 mg/kg IV every 3 weeks and imaged with an MRI after 3 doses (every 9 weeks +/- 2 weeks) to assess if continued utilization of bevacizumab to control the cerebral inflammation is needed. 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.

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.

15.2.8. 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.

15.2.9. Risks 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).

15.2.10. Risks of MRI

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.

15.2.11. 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 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) (50). 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 (51, 52). 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 any patients in one year follow-up of series of 85 patients (50) and 95 patients (53), or in any of 51 patients after over 4 years of mean follow-up in another (54). 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.

15.2.12. 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 poliovirus 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. Study subjects will be advised of the risks of exposure to unvaccinated household contacts.

15.2.13. Unknown Risks

The overall risk classification of this research is unknown. Clinical trials using PVSRIPO in brain tumor patients have not been performed.

15.3. TREATMENT ALTERNATIVES AND FINANCIAL REIMBURSEMENT

Alternative treatments for recurrent malignant brain tumors include additional surgery, radiation, bevacizumab and/or chemotherapy. If a patient chooses not to participate in this trial, they may seek alternative treatment. If the patient fails treatment through this trial, these alternatives may still be available. There will be no financial reimbursement to subjects for study participation.

16. ETHICAL AND REGULATORY ISSUES

This study will be conducted in accordance with current US FDA regulations, ICH guidelines, GCP standards, the Declaration of Helsinki, and local ethical and legal requirements.

16.1. INSTITUTIONAL REVIEW BOARD (IRB)

Prior to patient accrual, this protocol and the protocol informed consent must be approved in writing by the Cancer Protocol Committee of the Duke Comprehensive Cancer Center (CPC) and the Duke University Medical Center IRB. The term of study approval will not exceed one year. A progress report will be submitted annually to the IRB and CPC and re-approval obtained to continue the study. The IRB will also approve any significant changes to the protocol as well as a change of PI. Records of all study review and approval documents will be kept on file by the PI and are subject to FDA inspection during or after completion of the study. Adverse events will be reported to the IRB. The IRB will receive notification of the completion of the study and final report within three months of study completion or termination. The PI will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents. The IND number assigned will be reported to the IRB. Study activities will not commence until there is full resolution of any question or concerns raised during the process of IND review by the FDA. Any changes to the protocol or informed consent will be submitted for approval to the FDA and will not be enacted until that approval is obtained. Finally, due to use of an infectious agent in this study, this protocol will also be reviewed and approved by the Duke University Medical Center Institutional Biosafety Committee (IBC) prior to patient accrual.

16.2. RECRUITMENT AND INFORMED CONSENT

Upon determination that a patient's tumor histology and radiographic findings are compatible with the eligibility criteria of this protocol, the clinical study will be briefly explained to the patient by the principal investigator (PI) or colleague. If the patient indicates interest in study participation, patient education sheets and possibly the protocol consent form will be provided to the patient as these provide the most comprehensive explanation of the study in lay terms. If the patient shows continued interest, the PI or designee will thoroughly explain the required elements of informed consent and all aspects of the study to the subject including inclusion/exclusion criteria, risks, benefits, and alternatives to study participation.

The PI or designee will fully explain the purpose and potential risks and benefits of the study to the subject prior to enrollment and address any questions posed by the subject. In accordance with guidelines in the 21CFR50.27, all subjects will sign a statement of informed consent, which has been approved by the IRB. The subject will receive a copy of the executed consent document. The signed consent will be retained at the investigative site for each subject. The informed consent document serves as authorization as defined under HIPPA and contains the appropriate statements regarding privacy and confidentiality of protected health information as well as information on withdrawal from the study. This phase I study involves research that presents risk, but holds the prospect of direct benefit to the individual subject. The PI will report to the IRB and FDA changes in the research protocol and all unanticipated problems involving risks to human subjects and others, and no changes will be made in the research activity without IRB approval.

16.3. REGISTRATION OF SUBJECTS

All subjects enrolled into this clinical study must be registered by the PRTBTC staff into eResearch per Duke Oncology CRU SOP's.

16.4. DATA COLLECTION AND MAINTENANCE

The PI will be responsible for accurate, consistent, timely, complete and reliable data collection.

The study coordinator and PI are responsible for ensuring that the following forms are completed in a legible and timely manner for every patient enrolled on study. These forms are an integral part of the study data and will be maintained in the patient's clinical chart at the Preston Robert Tisch Brain Tumor Center at Duke (BTC). Errors on these forms and any source documents should be lined through, but not obliterated with the correction inserted, initialed and dated by the study coordinator or PI. These forms and the source documents will be available at all times for inspection by the FDA and the DCI SOC.

Eligibility Checklist
Electronic DUHS Adverse Event Form
Mandatory MedWatch Form (Form 3500A) when appropriate

In addition to the patient data maintained on the forms listed above, patient data will also be entered primarily into an Oracle Clinical (OC) database. Ancillary information will be stored in Excel spreadsheets maintained on a secure DUHS server. The data are backed up daily and stored on a secure medical center server. The PI, study coordinators, and data coordinators for the study are the only individuals who will have access to the password-protected OC data file. Regulatory coordinators may have access to data stored on the Excel spreadsheets.

16.5. DOCUMENTATION AND REPORTING OF ADVERSE EVENTS

An 'Adverse Event' will be defined as any adverse change from the subject's pre-treatment baseline condition, including any clinical or laboratory test abnormality that occurs during the course of research after treatment has started. Adverse events will be categorized and graded in accordance with the NCI CTC (Version 4).

A 'Serious Adverse Event' will be defined as an undesirable sign, symptom or medical condition which 1) is fatal or life threatening; 2) requires inpatient hospitalization or a prolongation of existing hospitalization; 3) results in persistent or significant disability/incapacity; 4) constitutes a congenital anomaly or a birth defect and/or; 5) medically significant impairment such that it may jeopardize the subject, and may require medical or surgical intervention to prevent one of the outcomes listed above.

A summary of all adverse events (not just those considered related to the study drug) will be maintained in Oracle Clinical. The event will be categorized by organ system, relationship to treatment, its grade of severity, and resolution. The PI 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.

All suspected adverse events that are both serious and unexpected should be reported immediately to both the PI, Dr. Dina Randazzo (pager 970-9692) or her designee (919-684-8111), and to the FDA. Unexpected fatal or life-threatening suspected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the sponsor followed by as complete a report as possible within 8 additional calendar days. Unexpected adverse events that are both serious and suspected, but

not fatal or life-threatening, will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor.

All adverse events that are considered serious, unanticipated or unexpected, and related or possibly related to the research (as defined by 21CRF312.32[a]) will be reported to the Duke University Medical Center IRB using the appropriate SAE report form within 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 adverse event;
- Report within 10 business day of learning about any other unanticipated problem or event that was more likely related to the research than unrelated.

At the time of the annual progress report to the Duke University Medical Center IRB, a summary of the overall toxicity experience will be provided.

16.6. DATA SAFETY AND MONITORING

This clinical research study will be monitored by the PI and the Duke Cancer Institute (DCI) Protocol Review and Monitoring system in accord with the NCI-approved "Institutional Protocol Monitoring Procedures and Guidelines for NIH-sponsored Research Involving Human Subjects." In terms of internal review, the PI will continuously monitor and tabulate adverse events. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of grade III or IV events occurs, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled when applicable;
- Stopping rules for toxicity and/or response are met;
- Risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of adverse events and outcomes is done;
- Over-accrual does not occur;
- Under-accrual is addressed with appropriate amendments or actions;
- Data are being appropriately collected in a reasonably timely manner.

DCI Protocol Review and Monitoring systems review of this protocol begins with an initial review by the Cancer Protocol Committee (CPC). CPC new protocol review focuses on scientific relevance, study design, adequacy of biostatistical input, protocol prioritization, feasibility of completing the study within a reasonable time frame and risk assessment of the trial. The PI will abide by CPC assessment of the level of risk, which will determine the intensity of subsequent DCI monitoring. CPC also conducts annual scientific progress reviews on protocols that are open to enrollment and focus on protocol prioritization, accrual and scientific progress. These reviews are conducted at the time of IRB annual renewals and documentation of all CPC reviews will be maintained in eIRB systems.

A determination for the degree of monitoring conducted by the DCI monitoring team is made at the time of initial CPC approval to commensurate with the type and level of intervention, phase, endpoints, degree of risk, size and complexity of the protocol. Typically, a formal monitoring visit is conducted by the DCI monitoring team after the first 3 subjects have been enrolled, followed by annual monitoring of 1-3 subjects until closed to enrollment or subjects are no longer receiving study drug or other interventions that are more than minimal risk. In this study, beginning with the monitoring visit (MV) of September 2013, the DCI Monitoring Team has monitored the eligibility

of all subjects enrolled following completion of their 4-week follow-up. 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. Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns. Monitoring visits may also be initiated upon request by DUHS and DCI Leadership, CPC, SOC, a sponsor, an investigator, or the IRB. Following closure of enrollment to new subjects in June 2017, DCI monitoring will revert to standard monitoring practices (i.e. individual subject monitoring will be discontinued, while annual monitoring will continue while selected patients are being retreated).

The DCI monitoring team reviews the adequacy of informed consent, enrollment of appropriate patients, implementation of protocol-specified procedures and treatment, adequacy of data collection, and appropriateness of adverse event monitoring and reporting. The DCI monitoring team presents final monitoring reports to the DCI Safety Oversight Committee (SOC) highlighting safety concerns and unresolved issues. The SOC chair assigns an overall rating of satisfactory (no major deviations), marginal (1 major deviation), or unsatisfactory (2 or more major deviations or an ineligible subject) to reflect overall quality of data, regulatory, consent, eligibility, conduct and AE reporting. Corrective action plans (CAPs) are developed, implemented, and evaluated as indicated. The SOC will notify the Sponsor and DUHS IRB when significant safety concerns are identified.

The SOC, in concert with DCI monitoring team, conducts annual data and safety monitoring for DUHS sponsor-investigator phase 1 and 2, therapeutic interventional studies that do not have an independent DSMB. While this study does utilize an independent DSMB (see below), it is also being monitored as described by the DCI monitoring team. Annual safety reviews by the DCI monitoring team include review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the Sponsor. Studies are rated satisfactory when adequate accrual with lack of excessive toxicity is present.

This phase 1 study is limited to Duke University Medical Center. There will be a team meeting with the study investigator and study team members that occurs weekly, on average, during which all adverse events will be reviewed and discussed (exceptions to the weekly schedule may be made to accommodate the investigator's schedule or holidays). Monitoring of accrual, outcomes and compliance will be carried out on an ongoing basis. This will include email correspondence at each occurrence of an SAE. The principal investigator and IND holder, as well as any relevant key personnel, will participate in this correspondence. The severity, relatedness and whether or not the event is expected will all be reviewed via email. In addition, the IND sponsor and research staff meet weekly and go over all study AE's. The IND sponsor and PI sign off each week on the listing of all AE's and SAE's. If the meeting does not take place with the IND holder and Study PI, the PI may sign the AE and SAE summary as reviewed.

DSMB-Plus

The Duke School of Medicine Research Integrity Office (RIO) has issued an institutional conflict of interest management plan to the Preston Robert Tisch Brain Tumor Center requiring a DSMB-Plus for this project. Because of Duke University's potential for conflict of interest, there is a necessity for review of the study for bias in the protocol design, performance of the study, and assessment of endpoints.

In addition to the usual roles of a DSMB, this committee will also consider conflict of interest questions related to this research. As such, it will be referred to as a "DSMB-Plus." The DSMB-Plus will evaluate whether financially-linked biases have affected the design, conduct, or reporting of the research related to PVSRIPO.

Policies of the DSMB–Plus will be described in the DSMB-Plus Charter, which will be signed by the DSMB members.

16.7. SUBJECT PRIVACY AND CONFIDENTIALITY

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted below is strictly prohibited.

All subject data will be identified by a subject identification number and subject initials only to protect the subject's privacy. The data will be blinded accordingly in all data analyses. However, in compliance with federal guidelines regarding the monitoring of clinical studies, the investigator will permit a representative of the FDA to review that portion of the subject's medical record that is directly related to the study. This will include all relevant study documentation including medical histories to verify eligibility, laboratory test results to verify transcription accuracy, X-ray reports, admission, discharge summaries for hospital/outpatient admissions while the subject is on-study and autopsy reports for deaths occurring during the study. As part of the required content of informed consent, the subject will be informed that his/her medical chart may be reviewed by a representative of the FDA.

Information obtained during the conduct of this study will be used by the sponsor in connection with development of the study drug. This information may be disclosed to other physicians who are conducting similar studies and to the FDA as deemed necessary by the sponsor. Patient-specific information may be provided to other appropriate medical personnel only with the patient's permission.

To ensure compliance with current Federal Regulations, data generated by this study must be available for inspection upon request by representatives of the FDA and national and local health authorities, the sponsor, and the IRB/IBC for each study site.

Should access to the medical record require a separate waiver or authorization, it is the PI's responsibility to obtain such permission from the patient in writing before the subject is entered into the study.

Any publications resulting from this study will not use any patient identifying data.

16.8. INCLUSION OF WOMEN, MINORITIES, AND CHILDREN

The subject (with no gender or minority restrictions) population for the protocol will include patients meeting the eligibility criteria. Inclusion of women and minorities is encouraged. The Brain Tumor Center at Duke typically sees more than 500 new patients per year; all patients with recurrent malignant brain tumors encountered by the investigators will be considered for the study.

17. STUDY FINANCING

Charges related to the medical care of the patient will be the responsibility of the patient or their insurance carrier as outlined in the Informed Consent document. The study agent will be made available free of charge by the NCI. Charges related to testing of tumor biopsy material will be covered by research funds administered by M. Gromeier.

18. APPENDICES

18.1. STUDY TEST SCHEMA FOR INITIAL PVSRIPO TREATMENT

Note: Patients that require the addition of bevacizumab to their treatment regimen will complete the same procedures at each visit per the schema below, however, they will follow a 9 week (+/- 2 week) schedule once bevacizumab is started. If a patient starts bevacizumab prior to the 8 week post-PVSRIPO infusion visit, the patient will bring in the stool sample at their next MRI evaluation.

Table 5. Study Test Schema

	-6 months	-14 days Screening and prior to catheter place-ment	Day -2 48 hours prior to PVSRIPO	Day 0 Catheter place-ment, biopsy, PVSRIPO infusion (infusion has a + one day window)	Day 0 After Completion of Infusion	Day 1 Post PVSRIPO	Day 7 (week 1) Post PVSRIPO	Day 14 (week 2) Post PVSRIPO	4 weeks post PVSRIPO	8 weeks post PVSRIPO	Weeks 16, 24, 32, 40, 48 post PVSRIPO
History and Physical, including KPS, neurologic evaluation		X				X [a]		X [c]	X [c]	X [c]	X [c]
MRI		X			X [b]				X [c]	X [c]	X [c]
CT				X							
CBC diff, CMP		X				X		X [c]	X [c]	X [c]	X [c]
PT, PTT		X									
Beta HCG		X	X								
Polio virus immunization booster	X (must receive booster between 6 mon and 1 wk prior to PVSRIPO)										

Serum for LSQ, anti-tetanus toxoid IgG, and poliovirus titer	**X**										
Whole Blood for immunologic analysis and poliovirus titer (10 mL except day 14. Day 14 is 5 mL)	**X**	**X (10 mL)**					**X [c] (10 mL)**	**X [c] (5 mL)**	**X [c] (10 mL)**	**X [c] (10 mL)**	**X [c,d] (at 16 and 24 weeks)**
Whole Blood for immunologic analysis (76.5 mL except day 14. Day 14 is 31 mL) (p.22)	**X [c] (76.5 mL) (whole blood must be collected before booster)**	**X (76.5 mL)**					**X [c] (76.5 mL)**	**X [c] (31 mL)**	**X [c] (76.5 mL)**	**X [c](76.5 mL)**	**X [c,d] (at 16 and 24 weeks)**
Stool for PVSRIPO titer										**X [c]**	

[a] Daily after infusion until discharged from hospital.
[b] Within 4 hours after completion of infusion.
[c] Starting with Day 7 (Week 1) all tests and procedures have a seven day plus or minus window.
[d] At the discretion of the PI, an additional blood sample at least 2 years post study drug infusion may be obtained.

18.2. STUDY TEST SCHEMA FOR RETREATMENT WITH PVSRIPO

Table 6. Schedule of Study Tests and Procedures Associated with PVSRIPO Retreatment

Description	Within 6 months	Screening: Within14 days prior to catheter placement	Screening: Within 2 days prior to PVSRIPO	Catheter Placement biopsy, PVSRIPO infusion	Post infusion (follow-up period)[1]						
Week					0	1	2	4	8	12	16 + every 9 weeks until week 61
Day		Within 14 days prior to catheter placement	Within 2 days prior to PVSRIPO	0	1	7	14	28	56	84	112
General Evaluations											
Informed Consent		X									
Physical Exam		X	X		X[2]	X	X	X	X	X	X
Neurologic Exam		X	X		X[2]	X	X	X	X	X	X
Performance Status		X	X		X[2]	X	X	X	X	X	X
Adverse Events				Continuous							
Laboratory Evaluations											
Poliovirus Immunization Booster	X[3]										
CBC w/diff		X			X	X	X	X	X	X	X
CMP		X			X	X	X	X	X	X	X
PT, aPTT		X									
Serum Pregnancy Test		X	X						X		
Whole blood for immunologic analysis and poliovirus titer (10		X				X	X	X	X	X	X (at 16, 25 and 34 weeks)[4]

[1] Starting with Day 7 (Week 1), all tests and procedures have a 7-day plus or minus window.
[2] Daily after infusion until discharged from hospital.
[3] Polio immunization booster and anti-tetanus toxoid IgG blood test may be obtained locally.
[4] At the discretion of the PI, an additional poliovirus titer blood sample may be obtained at least 2 years post study drug infusion.

mL except Day 14 will be 5 mL)											
Whole blood for immunologic analysis (76.5 mL except day 14 will be 31 mL)		X				X	X	X	X	X	X (at 16 and 25 weeks)[5]
Disease Evaluations											
MRI			X	X[6]				X	X		X
CT Scan				X							
Biopsy				X							
Treatment											
PVSRIPO				X							
Lomustine[7]									X[7]		

[5] Blood samples for immune analysis will continue to be collected at each MRI visit at the discretion of the PI.
[6] MRI should be obtained within 4 hours after completion of infusion. This MRI after the infusion is without gadolinium enhancement. All other MRIs in the study include gadolinium enhancement.
[7] One cycle of 110 mg/m2 oral lomustine at approximately 8 weeks post PVSRIPO infusion.

18.3. INVESTIGATIONAL PRODUCT HANDLING PLAN (VERSION 10.4.11)

Study Agent. The study agent, PVSRIPO, is the live-attenuated, serotype 1 poliovirus (SABIN) vaccine (PV1S) containing a heterologous human rhinovirus type 2 internal ribosomal entry site (IRES). PVSRIPO will be administered by convection-enhanced delivery to intracerebral tumors of patients with recurrent glioblastoma. The procedure consists of approximately a 6.5 hr infusion via an implanted catheter connected to an infusion pump via 4m of coiled connector tubing.

Risk assessment. The study agent PVSRIPO is based on the serotype 1 poliovirus vaccine (PV1S). The oral polio vaccine (OPV) was administered by the oral route to millions of Americans before it was replaced by the inactivated polio vaccine. OPV includes a combination of three attenuated polio strains, corresponding to serotypes 1, 2, and 3.

OPV was administered to millions of American children until 2000. Currently only the inactivated polio vaccine (IPV) is used. The primary risk associated with OPV is vaccine-associated paralytic polio (VAPP). VAPP is similar in presentation to paralytic polio produced by natural infection with the wild type virus. The rate of paralytic polio after OPV is about one case in 2.4 million vaccinations. The risk is higher for the first OPV dose (about 1 in 750,000 first-dose recipients) than for subsequent doses.

VAPP risk is increased among vaccine recipients with specific congenital and acquired immunodeficiency disease, generally those conditions associated with agammaglobulinemia (no antibodies), or hypogammaglobulinemia (low levels of antibodies). There does not appear to be an increased risk of VAPP associated with HIV infection or with the receipt of immunsuppressive drugs (e.g., steroids, cancer chemotherapy).

PVSRIPO has not been previously administered to humans, so there is no direct human safety experience. On the basis of primate toxicology studies, PVSRIPO appears to have lower neurovirulence than the PV1S vaccine strain from which it was derived. The risks of VAPP in employees from this PVSRIPO are predicted to be lower than exposure to the live attenuated polio vaccine. These risks are reduced by the following factors:

- Poliovirus is normally acquired by ingestion. This route of exposure is relatively unlikely for clinicians. Other routes of transmission such as aerosol exposure or needlestick are unlikely to lead to infection.
- PVSRIPO will be injected intracerebrally, and is unlikely to lead to viral shedding by the subjects.
- Most Americans have received a complete polio vaccine series. Childhood polio vaccination is predicted to provide a substantial level of protection from VAPP due to PVSRIPO.

PVSRIPO has been handled by hundreds of research personnel for routine research purposes in various laboratories, in NCI production facilities, laboratory animal facilities and NCI-appointed primate testing facilities, using BSL-2/ABSL-2.

Employee screening. Work practices will be designed to minimize the number of employees who come into contact with the viral preparation of materials that are in contact with the viral preparation. These employees will include personnel responsible for aliquot preparation and administration, and those who dispose of materials that in contact with the virus. Prior to work with PVSRIPO, these employees will be screened by Duke Employee Occupational Health and Wellness (EOHW) using an EOHW screening form designed to identify individuals with immunodeficiency conditions. Those who screen positive on the form will be further evaluated by EOHW to determine if they can safely handle PVSRIPO.

Most American adults have received a polio vaccine series. Inactivated polio vaccine is available for adults, but there is no recommendation for booster vaccination for employees participating in this research. Employees with uncertainty about their vaccination status or with any questions related to vaccination may contact EOHW for personalized advice.

Receipt and Storage. The study agent and vehicle will be supplied directly to the Investigational Pharmacy by the manufacturer, the National Cancer Institute, Developmental Therapeutics Program, Biological Resources Branch, Ft. Detrick, MD. The study agent will be shipped via approved methods in the appropriate packaging on dry ice. Storage of the study agent at -70°C has been empirically determined to occur without compromise in biological or biophysical properties for at least 5 years. The agent should be stored long-term at -80°C. Thawed vials should be kept at 4°C whenever possible. Thawed vials of PVSRIPO are stable at 4°C for 48 hrs.

Aliquot Preparation. 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 hrs. 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 study agent is stable at 20°C for at least 5 days, but as a precaution, the cold chain should be maintained. 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 Investigational Pharmacy. 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. pipettes, vials, etc., will be disposed of as biological waste. The final desired aliquot of the study agent will be prepared at the intended volume (8 mL) and drawn into the intended delivery device (a 20 mL syringe) using a needle. The disposable needle will be a safer hypodermic needle with a safety sheath to cover the needle using a one-handed technique after the agent is drawn into the syringe. The sheathed needle is then removed from the syringe containing the study agent aliquoted at the desired dose, and the syringe will be capped. The capped syringe will be transported to the study site in a 'ziplock' bag placed in a portable cooler while maintaining a temperature of 4°C.

For dose levels -1 and -2, the stock of PVSRIPO **must be diluted** according to the tables below respectively.

Preparation for Dose Levels -2 through 5

Dose level	PVSRIPO dose (In 3 mL)	PVSRIPO dose (in 8 mL total syringe load)	PVSRIPO	**Previously *Diluted (1:10)*** gadopentetate dimeglumine (Magnevist®)	Vehicle (phosphate-buffered saline, 0.2% human serum albumin)	Total Volume in 20 ml syringe
-2	1×10^7 TCID50	2.67×10^7 TCID50	See Preparation for Dose Level -2 table			
-1	5×10^7 TCID50	1.33×10^8 TCID50	See Preparation for Dose Level -1 table			
1	1.0×10^8 TCID50	2.67×10^8 TCID50	0.07 mL	0.16 mL	7.77 mL	8 mL
2	3.3×10^8 TCID50	8.8×10^8 TCID50	0.22 mL	0.16 mL	7.62 mL	8 mL
3	1.0×10^9 TCID50	2.67×10^9 TCID50	0.67 mL	0.16 mL	7.17 mL	8 mL
4	3.3×10^9 TCID50	8.8×10^9 TCID50	2.2 mL	0.16 mL	5.64 mL	8 mL
5	1.0×10^{10} TCID50	2.67×10^{10} TCID50	6.7 mL	0.16 mL	1.14 mL	8 mL

Preparation for Dose Level -1

PVSRIPO Dilution for Dose Level -1:

- To measure to PVSRIPO dose, we must make a 1:10 dilution.
- Dilute 0.5 ml (1 vial) PVSRIPO with 4.5 ml of Vehicle (phosphate-buffered saline, 0.2% human serum albumin) in a syringe. Refer to table 2 for remainder of steps needed for preparation.

Dose level	PVSRIPO dose (In 3 mL)	PVSRIPO (in 8 mL total syringe load)	***Diluted 1:10 (see above)*** PVSRIPO	**Previously *Diluted (1:10)*** gadopentetate dimeglumine (Magnevist®)	Vehicle (phosphate-buffered saline, 0.2% human serum albumin)	Total Volume in 20 ml syringe
-1	5×10^7 TCID50	1.33×10^8 TCID50	0.33 mL	0.16 mL	7.51 mL	8 mL

Preparation for Dose Level -2
PVSRIPO Dilution for Dose Level -2:

- To measure to PVSRIPO dose, we must make a 1:100 dilution.
- Dilute 0.5 ml (1 vial) PVSRIPO with 49.5 ml of Vehicle (phosphate-buffered saline, 0.2% human serum albumin) in a syringe. Refer to table 3 for remainder of steps needed for preparation.

Dose level	PVSRIPO dose (In 3 mL)	PVSRIPO (in 8 mL total syringe load)	***Diluted 1:100 (see above)*** PVSRIPO	**Previously *Diluted (1:10)*** gadopentetate dimeglumine (Magnevist®)	Vehicle (phosphate-buffered saline, 0.2% human serum albumin)	Total Volume in 20 ml syringe
-2	1×10^7 TCID50	2.67×10^7 TCID50	0.67 mL	0.16 mL	7.17 mL	8 mL

Administration. The study agent will be administered to patients via intracerebral delivery using a previously implanted tube radiographically determined to be placed correctly within the tumor bed. The syringe containing the study agent (upon delivery from the Investigational Pharmacy) will be connected to the tube equipped with the appropriate adaptor. This occurs by removing the cap and fastening the syringe to the Luer-Lok adaptor and does not involve needles. The infusion procedure is driven by an infusion pump at a set pace measured to last ~6 hrs. The infusion procedure will take place in dedicated space in the Neurosurgical ICU or the Neuro Step-Down Unit, where patients will remain overnight for observation.

The infusion procedure in the Neurosurgical ICU or the Neuro Step-Down Unit follows established principles, e.g. those previously used for delivery of radiochemicals by a similar infusion method and under similar containment precautions. All procedures are restricted to a single room where the patient remains throughout the duration of the infusion. Access to the room is restricted to staff caring for the patient.

For reasons pointed out above, persons involved with the care of patients enrolled in the study and who received the standard recommended poliovirus vaccine schedule as a child, are not required to be re-vaccinated.

All materials coming in contact with the study agent, the syringe delivered from the Investigational Pharmacy, tubing, dressings or coverings used to protect the trepanation site, will be disposed of as biological waste in the treatment room. All personnel caring for the patient will be wearing appropriate safety devices, e.g. disposable gloves, gown, and face protection, which will be disposed of as biological waste in the treatment room. Appropriate hand hygiene is required before and after any handling of the study agent

Disposal. 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.

List of locations.

- Investigational Pharmacy (receipt, storage, aliquot preparation)
- Closed transport container; at 4°C (for transport of the sealed delivery device containing the study agent)
- Study site in the Neurosurgical ICU or the Neuro Step-Down Unit (administration)

- Investigational Pharmacy (disposal of unused or partially used study agent)

Spill procedure. PVSRIPO can all be easily and completely inactivated with household bleach. In the event of a spill, the liquid will be absorbed with gauze (then treated as biohazardous waste as above). The spill area will then be liberally wiped down with a 20% household bleach solution for chemical disinfection. The disinfectant will be left in contact with contaminated area for at least 30 minutes, then wiped away with wet towels, which will be disposed as biohazard waste as well.

Exposure follow-up. Any virus exposures will be reported to the Employee Health Exposure Hotline (115), followed by submission of the Report of Occupational Injury or Illness form. There is no specific preventative treatment. Based on the circumstances of exposure, employee health may choose to monitor for infection by submitting stool for enterovirus culture at 7, 14, and 21 days after an exposure event.

18.4. SELECTION AND DESCRIPTION OF THE HISTORICAL CONTROL COHORT

The historical cohort that will be used for the evaluation of PVSRIPO efficacy among WHO grade IV malignant glioma patients was derived from the PRoGREss registry (IRB# Pro00027120; Primary and Recurrent Glioma Registry).

Description of PRoGREss registry: The PRoGREss registry study is both a retrospective and prospective chart review study of all patients diagnosed after 12/31/2004 with a primary CNS tumor who were seen at the PRTBTC. Specifically, that database includes: (1) data from all patients that were deceased as of 11/3/2011, and (2) data collected retrospectively and prospectively from patients who were alive as of 11/3/2011 and provided registry consent. The latter group includes patients who were diagnosed after 12/31/2004 but before 11/3/2011, as well as patients diagnosed after 11/3/2011. Both demographic and clinical information was extracted from clinical and research records, which included such information as diagnosis, gender/race/ethnicity, medical history, medications, and types of treatment. Details about the data available from the PRoGREss registry can be found in the PRoGREss protocol.

Criteria Used to Select Historical Control Cohort: To create the historical cohort, data available from the PRoGREss registry as of 12/15/2014 were reviewed to determine which patients would have been eligible to participate in the PVSRIPO phase I study at the time their disease recurred in 2007 or later. All radiologic records were reviewed by Dr. Annick Desjardins without knowledge of the patient's survival outcome in making this determination.

During the conduct of the PVSRIPO WHO grade IV malignant glioma Phase I study, most modifications to the patient eligibility criteria were minor. As such, the eligibility criteria used for identifying the historical control cohort from the PRoGREss registry were standardized. The following relevant criteria were used:

1. Patients must have a diagnosis of recurrent supratentorial WHO Grade IV MG based on imaging studies with measurable disease (≥ 1 cm and ≤ 5.5 cm of contrast-enhancing tumor)
2. Age ≥ 18 years of age
3. Patients must not have taken part in the PVSRIPO study
4. KPS ≥70 at the time patient could have been enrolled on the PVSRIPO study
5. Absence of rapid clinical decline

After the first 5 patients were treated at dose level "-1," the PVSRIPO WHO grade IV malignant glioma Phase I study was amended to institute a limit on pre-treatment steroid usage to ≤ 4 mg per day of dexamethasone within the 2 weeks prior to admission for PVSRIPO infusion. There were no steroid limitations for all patients treated on dose levels 1-5 and for the first 5 patients treated on dose level "-1". To address this eligibility amendment, two control groups have been generated, with one group having no limitations on pre-treatment steroid usage, and the other group having a steroid limit of ≤ 4 mg per day of dexamethasone (or equivalent) within the 2 weeks prior to being eligible for PVSRIPO infusion.

Within these historical control cohorts, survival time was computed as the time between the date of the first MRI at which time the patient would have been eligible to receive PVSRIPO and the date of death. If the patient remained alive at the date of last follow-up, survival time was censored at that date.

Characteristics of the Patients within the Historical Control Group: A summary of selected patient characteristics in these two historical control groups is provided below.

Patient Characteristics	Group			
	Historical controls (with steroid expanded pts)		Historical controls (without steroid expanded pts)	
	N	%	N	%
All	124	100.0	104	100.0
Gender				
Female	44	35.5	39	37.5
Male	80	64.5	65	62.5
Histologic Diagnosis				
GBM	124	100.0	104	100.0
Age at GBM Diagnosis, mean (SD)	55.3 (10.9)		54.7 (11.2)	
Age at GBM Diagnosis, median (range)	55 (20 – 76)		54 (20 – 76)	
Primary or Secondary GBM				
Primary	110	88.7	92	88.5
Secondary	13	10.5	11	10.6
Unknown	1	0.8	1	1.0
Karnofsky Performance Status				
100	8	6.5	8	7.7
90	69	55.6	64	61.5
85	2	1.6	2	1.9
80	36	29.0	27	26.0
70	7	5.6	2	1.9
Undetermined but ≥70	2	1.6	1	1.0
Resection Type at diagnosis*				
GTR	73	58.9	60	57.7
STR	29	23.4	25	24.0
Biopsy	22	17.7	19	18.3
Further Surgery prior to Treatment**				
GTR	8	6.5	8	7.7
STR	6	4.8	4	3.8
None	110	88.7	92	88.5
PDs prior to being eligible	102	82.3	85	81.7

Patient Characteristics	Group			
	Historical controls (with steroid expanded pts)		Historical controls (without steroid expanded pts)	
	N	%	N	%
1				
2	14	11.3	12	11.5
3	8	6.5	7	6.7
Total Daily Dex (or other steroid) dose at MRI				
0	89	71.8	89	85.6
0.125	1	0.8	1	1.0
0.5	1	0.8	1	1.0
1	1	0.8	1	1.0
2	3	2.4	3	2.9
4	8	6.5	8	7.7
6	2	1.6	0	0
8	4	3.2	0	0
12	4	3.2	0	0
14	1	0.8	0	0
16	3	2.4	0	0
30 mg Hydrocortisone	1	0.8	1	1.0
Unknown	6	4.8	0	0
Avastin prior to eligible PD				
No	61	49.2	49	47.1
Yes	63	50.8	55	52.9
Avastin Failure prior to eligible PD				
No	75	60.5	61	58.7
Yes	49	39.5	43	41.3
Vital Status				
Alive	1	0.8	1	1.0
Dead	123	99.2	103	99.0

*In both control groups, this includes 1 patient with a questionable STR/GTR, and 1 patient with a GTR on the primary tumor and a STR on a second tumor.
**In both control groups, this includes 1 patient with a questionable STR/GTR.

19. REFERENCES

1. **Bruner J, Tien R.** 1998. Secondary tumors, p 419-450. *In* Bigner D, McLendon R, Bruner J (ed), Russell and Rubinstein's Pathology of Tumors of the Nervous System, vol 1. Edward Arnold, London.
2. **Dinapoli RP, Brown LD, Arusell RM, Earle JD, O'Fallon JR, Buckner JC, Scheithauer BW, Krook JE, Tschetter LK, Maier JA, et al.** 1993. Phase III comparative evaluation of PCNU and carmustine combined with radiation therapy for high-grade glioma. J Clin Oncol **11:**1316-1321.
3. **Salford LG, Brun A, Nirfalk S.** 1988. Ten-year survival among patients with supratentorial astrocytomas grade III and IV. J Neurosurg **69:**506-509.
4. **Shapiro WR, Shapiro JR.** 1986. Principles of brain tumor chemotherapy. Semin Oncol **13:**56-69.
5. **Walker MD, Alexander E, Jr., Hunt WE, MacCarty CS, Mahaley MS, Jr., Mealey J, Jr., Norrell HA, Owens G, Ransohoff J, Wilson CB, Gehan EA, Strike TA.** 1978. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. J Neurosurg **49:**333-343.
6. **Walker MD, Green SB, Byar DP, Alexander E, Jr., Batzdorf U, Brooks WH, Hunt WE, MacCarty CS, Mahaley MS, Jr., Mealey J, Jr., Owens G, Ransohoff J, 2nd, Robertson JT, Shapiro WR, Smith KR, Jr., Wilson CB, Strike TA.** 1980. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med **303:**1323-1329.
7. **Pickard JD, Bailey S, Sanderson H, Rees M, Garfield JS.** 1990. Steps towards cost-benefit analysis of regional neurosurgical care. Bmj **301:**629-635.
8. **Penar PL, Wilson JT.** 1994. Cost and survival analysis of metastatic cerebral tumors treated by resection and radiation. Neurosurgery **34:**888-893; discussion 893-884.
9. **Hall WA, Fodstad O.** 1992. Immunotoxins and central nervous system neoplasia. J Neurosurg **76:**1-12.
10. **Imperato JP, Paleologos NA, Vick NA.** 1990. Effects of treatment on long-term survivors with malignant astrocytomas. Ann Neurol **28:**818-822.
11. **Mendelsohn CL, Wimmer E, Racaniello VR.** 1989. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell **56:**855-865.
12. **Takai Y, Miyoshi J, Ikeda W, Ogita H.** 2008. Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. Nat Rev Mol Cell Biol **9:**603-615.
13. **Sloan KE, Eustace BK, Stewart JK, Zehetmeier C, Torella C, Simeone M, Roy JE, Unger C, Louis DN, Ilag LL, Jay DG.** 2004. CD155/PVR plays a key role in cell motility during tumor cell invasion and migration. BMC Cancer **4:**73.
14. **Sloan KE, Stewart JK, Treloar AF, Matthews RT, Jay DG.** 2005. CD155/PVR enhances glioma cell dispersal by regulating adhesion signaling and focal adhesion dynamics. Cancer Res **65:**10930-10937.
15. **Merrill MK, Bernhardt G, Sampson JH, Wikstrand CJ, Bigner DD, Gromeier M.** 2004. Poliovirus receptor CD155-targeted oncolysis of glioma. Neuro-oncol **6:**208-217.
16. **Castriconi R, Daga A, Dondero A, Zona G, Poliani PL, Melotti A, Griffero F, Marubbi D, Spaziante R, Bellora F, Moretta L, Moretta A, Corte G, Bottino C.**

2009. NK cells recognize and kill human glioblastoma cells with stem cell-like properties. J Immunol **182:**3530-3539.

17. **Pelletier J, Sonenberg N.** 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature **334:**320-325.
18. **Gromeier M, Alexander L, Wimmer E.** 1996. Internal ribosomal entry site substitution eliminates neurovirulence in intergeneric poliovirus recombinants. Proc Natl Acad Sci U S A **93:**2370-2375.
19. **Nomoto A, Omata T, Toyoda H, Kuge S, Horie H, Kataoka Y, Genba Y, Nakano Y, Imura N.** 1982. Complete nucleotide sequence of the attenuated poliovirus Sabin 1 strain genome. Proc Natl Acad Sci U S A **79:**5793-5797.
20. **Gromeier M, Bossert B, Arita M, Nomoto A, Wimmer E.** 1999. Dual stem loops within the poliovirus internal ribosomal entry site control neurovirulence. J Virol **73:**958-964.
21. **Gromeier M, Lachmann S, Rosenfeld MR, Gutin PH, Wimmer E.** 2000. Intergeneric poliovirus recombinants for the treatment of malignant glioma. Proc Natl Acad Sci U S A **97:**6803-6808.
22. **de Breyne S, Yu Y, Unbehaun A, Pestova TV, Hellen CU.** 2009. Direct functional interaction of initiation factor eIF4G with type 1 internal ribosomal entry sites. Proc Natl Acad Sci U S A **106:**9197-9202.
23. **Harashima A, Guettouche T, Barber GN.** Phosphorylation of the NFAR proteins by the dsRNA-dependent protein kinase PKR constitutes a novel mechanism of translational regulation and cellular defense. Genes Dev **24:**2640-2653.
24. **Merrill MK, Gromeier M.** 2006. The Double-Stranded RNA Binding Protein 76:NF45 Heterodimer Inhibits Translation Initiation at the Rhinovirus Type 2 Internal Ribosome Entry Site. J Virol **80:**6936-6942.
25. **Merrill MK, Dobrikova EY, Gromeier M.** 2006. Cell-type-specific repression of internal ribosome entry site activity by double-stranded RNA-binding protein 76. J Virol **80:**3147-3156.
26. **Neplioueva V, Dobrikova EY, Mukherjee N, Keene JD, Gromeier M.** Tissue type-specific expression of the dsRNA-binding protein 76 and genome-wide elucidation of its target mRNAs. PLoS One **5:**e11710.
27. **Goetz C, Everson RG, Zhang LC, Gromeier M.** MAPK signal-integrating kinase controls cap-independent translation and cell type-specific cytotoxicity of an oncolytic poliovirus. Mol Ther **18:**1937-1946.
28. **Dobrikov M, Dobrikova E, Shveygert M, Gromeier M.** 2010. Phosphorylation of eIF4G1 by PKCa regulates eIF4G1 binding to Mnk1. Mol Cell Biol **in press**.
29. **Shveygert M, Kaiser C, Bradrick SS, Gromeier M.** Regulation of eukaryotic initiation factor 4E (eIF4E) phosphorylation by mitogen-activated protein kinase occurs through modulation of Mnk1-eIF4G interaction. Mol Cell Biol **30:**5160-5167.
30. **Goetz C, Brown M, Gromeier M.** 2011. eIF4E phosphorylation modulates cap-independent translation at eukaryotic mRNAs. Mol Cell **submitted**.
31. **Dobrikova EY, Broadt T, Poiley-Nelson J, Yang X, Soman G, Giardina S, Harris R, Gromeier M.** 2008. Recombinant oncolytic poliovirus eliminates glioma in vivo without genetic adaptation to a pathogenic phenotype. Mol Ther **16:**1865-1872.
32. **Ochiai H, Moore SA, Archer GE, Okamura T, Chewning TA, Marks JR, Sampson JH, Gromeier M.** 2004. Treatment of intracerebral neoplasia and

neoplastic meningitis with regional delivery of oncolytic recombinant poliovirus. Clin Cancer Res **10:**4831-4838.

33. **Ochiai H, Campbell SA, Archer GE, Chewning TA, Dragunsky E, Ivanov A, Gromeier M, Sampson JH.** 2006. Targeted therapy for glioblastoma multiforme neoplastic meningitis with intrathecal delivery of an oncolytic recombinant poliovirus. Clin Cancer Res **12:**1349-1354.
34. **Toyoda H, Yin J, Mueller S, Wimmer E, Cello J.** 2007. Oncolytic treatment and cure of neuroblastoma by a novel attenuated poliovirus in a novel poliovirus-susceptible animal model. Cancer Res **67:**2857-2864.
35. **Toyoda H, Wimmer E, Cello J.** Oncolytic poliovirus therapy and immunization with poliovirus-infected cell lysate induces potent antitumor immunity against neuroblastoma in vivo. Int J Oncol **38:**81-87.
36. **Dobrikova E, Gromeier M.** 2011. Attenuation of Neurovirulence, Biodistribution, and Shedding of a Poliovirus: Rhinovirus Chimera after Intrathalamic Inoculation in Cynomolgus Macaques. J Virol **submitted**.
37. **Arita M, Ohka S, Sasaki Y, Nomoto A.** 1999. Multiple pathways for establishment of poliovirus infection. Virus Res **62:**97-105.
38. **Laske DW, Youle RJ, Oldfield EH.** 1997. Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. Nat Med **3:**1362-1368.
39. **Sampson JH, Brady M, Raghavan R, Mehta AI, Friedman AH, Reardon DA, Petry NA, Barboriak DP, Wong TZ, Zalutsky MR, Lally-Goss D, Bigner DD.** 2011. Colocalization of gadolinium-diethylene triamine pentaacetic acid with high-molecular-weight molecules after intracerebral convection-enhanced delivery in humans. Neurosurgery **69:**668-676.
40. **O'Quigley J, Zohar S.** 2006. Experimental designs for phase I and phase I/II dose-finding studies. Br J Cancer **94:**609-613.
41. **O'Quigley J, Pepe M, Fisher L.** 1990. Continual reassessment method: a practical design for phase 1 clinical trials in cancer. Biometrics **46:**33-48.
42. **Moller S.** 1995. An extension of the continual reassessment methods using a preliminary up-and-down design in a dose finding study in cancer patients, in order to investigate a greater range of doses. Stat Med **14:**911-922; discussion 923.
43. **Paoletti X, Baron B, Schoffski P, Fumoleau P, Lacombe D, Marreaud S, Sylvester R.** 2006. Using the continual reassessment method: lessons learned from an EORTC phase I dose finding study. Eur J Cancer **42:**1362-1368.
44. **Simon R, Freidlin B, Rubinstein L, Arbuck SG, Collins J, Christian MC.** 1997. Accelerated titration designs for phase I clinical trials in oncology. J Natl Cancer Inst **89:**1138-1147.
45. **O'Quigley J, Shen LZ.** 1996. Continual reassessment method: a likelihood approach. Biometrics **52:**673-684.
46. **Dunn G, Begg NT, Cammack N, Minor PD.** 1990. Virus excretion and mutation by infants following primary vaccination with live oral poliovaccine from two sources. Journal of medical virology **32:**92-95.
47. **Brem H, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, Black, K, Sisti M, Brem S, Mohr G, Muller P, Morawetz R, Shold SC.** 1995. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. Lancet **345:**1008-1012.

48. **Korn EL.** 2006. Conditional power calculations for clinical trials with historical controls. Stat Med **25:**2922-2931.
49. **Schoenfeld D.** 1981. The Asymptotic Properties of Nonparametric Tests for Comparing Survival Distributions. Biometrika **68:**316-319.
50. **Selcuk H, Albayram S, Ozer H, Ulus S, Sanus GZ, Kaynar MY, Kocer N, Islak C.** 2010. Intrathecal gadolinium-enhanced MR cisternography in the evaluation of CSF leakage. AJNR Am J Neuroradiol **31:**71-75.
51. **Arlt S, Cepek L, Rustenbeck HH, Prange H, Reimers CD.** 2007. Gadolinium encephalopathy due to accidental intrathecal administration of gadopentetate dimeglumine. J Neurol **254:**810-812.
52. **Li L, Gao FQ, Zhang B, Luo BN, Yang ZY, Zhao J.** 2008. Overdosage of intrathecal gadolinium and neurological response. Clin Radiol **63:**1063-1068.
53. **Tali ET, Ercan N, Krumina G, Rudwan M, Mironov A, Zeng QY, Jinkins JR.** 2002. Intrathecal gadolinium (gadopentetate dimeglumine) enhanced magnetic resonance myelography and cisternography: results of a multicenter study. Invest Radiol **37:**152-159.
54. **Aydin K, Terzibasioglu E, Sencer S, Sencer A, Suoglu Y, Karasu A, Kiris T, Turantan MI.** 2008. Localization of cerebrospinal fluid leaks by gadolinium-enhanced magnetic resonance cisternography: a 5-year single-center experience. Neurosurgery **62:**584-589; discussion 584-589.