A Multicenter Phase 2 Study of Oncolytic Polio/Rhinovirus Recombinant (PVSRIPO) in Recurrent WHO Grade IV Malignant Glioma Patients

Protocol Number: Pro00077024 (Multicenter)

Version Date: 07 February 2020

Overall Study Principal Investigator:

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Protocol Version: 08

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Date: **07 February 2020**

NOTICE:

DUE TO KNOWN PVSRIPO-MEDIATED TREATMENT EFFECTS, INCREASED TUMOR SIZE ON RADIOGRAPHIC IMAGING FOLLOWING PVSRIPO ADMINISTRATION MAY OCCUR. EXCEPT IN CASES OF EMINENT LIFE-THREATENING EMERGENCY, CONSULT WITH STUDY-DESIGNATED NEURO-ONCOLOGIST(S)/INVESTIGATOR STEERING COMMITTEE PRIOR TO INITIATING NON-PROTOCOL-SPECIFIED TUMOR TREATMENTS OR PERFORMING SURGICAL RESECTION.

Per-Protocol management of cerebral edema/ pseudoprogressive/ polycystic/ expansive tumor appearance within 6 months of any PVSRIPO infusion is as follows:

- **Radiographic findings but patient asymptomatic/stable:**
  - Observe and continue to follow per protocol
- **Radiographic findings with new onset/worsening clinical symptoms:**
  - Initiation of low dose steroids (dexamethasone ≤ 4mg/day) if rapid administration is required
  - Preferred: Low dose bevacizumab (7.5mg/kg q 3 weeks; few cycles as possible to achieve symptom control)
  - Other supportive care treatments, as required (eg, antiepileptics)
- PVSRIPO's immune-mediated mechanism of action (MOA) is known to result in atypical tumor imaging responses that can manifest as polycystic pseudoprogressive tumor enlargement
- Due to PVRIPO's MOA, reliance on radiographic imaging standards (iRANO, etc.) for determining disease progression is not valid
- Time to objective radiographic anti-tumor response ranges from approximately 5 to 29 months post-infusion in the Phase 1 study (median time to response 15 months)
- In order for patients to have the best opportunity for anti-tumor response, experience suggests that patients should be managed as noted above

Summary of Protocol Amendment Changes

Version Dates	Summary of Major Changes by Section
27FEB2019 to 07FEB2020	• This version replaces/supersedes a version of the amendment dated 30JAN2020, which contained a typographical error on page 61 and was circulated inadvertently. No patients were treated under the version circulated 30JAN2020 • Throughout protocol: Primary Endpoints changed to Objective Radiographic Response and Duration of Response, while Overall and Landmark survival were changed to secondary endpoints. Disease Control Rate also added as a secondary endpoint. Exploratory endpoints updated to include description of anti-tumor responses following PVSRIPO retreatment and based on level of institutional experience using PVSRIPO. • Throughout protocol: Noted requirement for Investigators to consult with study-designated neurooncologist and/or Investigator Steering Committee (ISC) prior to removing a patient from study and/or use of non-protocol-specified therapies. • Throughout protocol: clarification that catheter placement procedure is not required to be performed under general anesthesia. • Throughout protocol: updated treatment-related adverse event collection to include non-standard of care screening procedures required for PVSRIPO infusion. • Section 5: Added a tabular protocol synopsis. • Section 5: New sites are no longer required to wait 2 weeks between each of the first 3 patients infused; hold only after the 1st subject infused after Sponsor approval to proceed to next. • Section 5 and 11.1 (Inclusion Criterion 1a) and Section 9.1.1: language regarding catheter tip placement updated to allow for placement within, or as close to, enhancing disease as possible while remaining at least 1 cm from the ventricles. • Section 5 and 11.2 (Exclusion Criterion 3f) and Section 9.1.6: History of any gadolinium allergy changed to history of anaphylactic reaction to gadolinium. • Section 5 and 11.2 (Exclusion Criterion 9): Clarified requirements for prior receipt of standard of care therapies. • Section 5 and 11.2: Added back Exclusion Criterion 16, which excluded patients enrolled prior to Amendment 7 who have history of hypersensitivity to lomustine, dacarbazine, or any of its excipients. • Section 9.1.3 and Table 1: Removed Magnevist® from PVSRIPO infusate, as well as removal of MRI 4 hours post-infusion to infusate visualization. • Section 9.1.6 Safety Considerations: Under Special Considerations, added note that it is recommended to not start bevacizumab use within 4 weeks of PVSRIPO infusion (per bevacizumab prescribing instructions); however earlier use at physician discretion, and if in line with Institutional use guidelines following biopsy was permitted. • Added Section 9.2 to allow and provide eligibility criteria for PVSRIPO retreatment in patients ≥ 12 months out from prior PVSRIPO infusion. Note: the balance of the protocol was also updated to account for PVSRIPO retreatment, including addition of PVSRIPO Retreatment Study Tests and Procedures (Table 2), located in Section 5. • Section 10.1.4: Updated to reflect current product handling, preparation, and dispensing recommendations. • Section 11.2: Inclusion/Exclusion Notes: Updated multifocal disease stabilization to equal no size increase >0.5cm in any direction on 2 consecutive MRI at least 3 months apart • Section 12.1 and Table 2: For PVSRIPO retreatment only, the acceptable maximum time of polio vaccination booster is 6 months prior to infusion. • Section 12.3, Section 12.7.5 and Table 1: updated to reflect removal of timepoints for collection of biomarkers. • Section 12.7.3: Updated to clarify current radiographic review practices. • Section 12.8.4: Serious Adverse Event (SAE) Reporting procedures (particularly procedures related cerebral edema) were updated to reflect current Sponsor policies. • Section 14 – Statistical Methods and Data Analysis: Updated to reflect the statistical approach based on changes to primary and secondary endpoints.

	Section 14.2: Added clarification regarding possible use of additional relevant data sources to bolster the historical control comparison.
02NOV2018 TO 27FEB2019	• Added overall study PI, Dina Randazzo, DO, DUMC. • Throughout protocol: Removed treatment arm randomizing 50% of patients to receive a single dose of lomustine 8 weeks after PVSRIPO infusion given an interim review noting no decrease in potential prognostic indicators of long-term survival (eg, dose and duration of use of corticosteroids or bevacizumab) with addition of a single dose of lomustine, but increased hematological toxicities associated with lomustine use; added additional safety information. Endpoints and objectives have been modified accordingly, throughout. Data will still be summarized by treatment arm for the patients randomized to and receiving lomustine at week 8 post PVSRIPO infusion, relative to PVSRIPO alone. • Section 7.3 Study Purpose/Rationale: Updated that survival up to +81 months has been noted in Phase 1 study with PVSRIPO. • Section 11.1 Inclusion Criteria: Clarified inclusion criterion 1 with regard to site neurosurgical confirmation requirement that biopsy at time of catheter placement and catheter placement ≥ 1 cm from ventricle can be completed safety within protocol requirements, in addition to sponsor designated reviewer requirements regarding confirmation of patient meeting tumor size and location requirements. • Section 11.2 Exclusion Criteria: Removed EC 16 referring Patients with a known history of hypersensitivity to lomustine, dacarbazine, or any components of lomustine. • Table 1 (footnote i) and Section 12.2, Day 0 (after the infusion): Clarified that the post-PVSRIPO infusion MRI (without contrast) should occur as quickly as possible after the completion of the infusion to track the infusate; ideally, this post-infusion MRI should be obtained within 2h of completion of infusion but within 4h maximum is allowed, given scheduling constraints. • Table 1 (footnote c) and Section 12.3 Clarified that the items required for the PE must be documented/completed within 24h (±4h) of discharge. • Table 1 (footnote c) and Section 12.3 Clarified that the items required for the PE must be documented/completed within 24h (±4h) of discharge. • Table 1 and Section 12.3 Removed Week 12 visit and blood draw for immunologic analysis that was associated with lomustine use. • Section 12.4: Follow-Up for Off-Study Patients/Patients requiring retreatment with PVSRIPO: Added that for patients who initially benefitted from PVSRIPO, are at least 12 months post-infusion on this study who experience a recurrence and their clinician feels they may benefit from an additional infusion of PVSRIPO, they may be considered for a separate but related long-term follow-up rollover/re-treatment protocol.
01JUN2018 to 02NOV2018	• Sections 6 Study Schema, 9.1 Investigational Plan, 9.1.8 Randomization, 14.10 Sample Size Calculations: Prior to the activation of additional centers in the multicenter study and compilation of other center historical control data, to date (October 2018), DUMC has enrolled 48 subjects on the single center phase 2 study. As such, enrollment is being increased slightly (up to 40 additional patients; from N=62 to up to 102, distributed evenly across the study arms) in an interim fashion to allow time for continued enrollment during other site historical control data compilation, corresponding sample size calculations/adjustments and possibly additional FDA discussions. As stated previously, sample size/statistical power will be updated if necessary via a future amendment once the historical control data from other centers are available and analyzed. • Sections 9.1.4.1 Study Related Biopsy, 9.1.4.2 Archival Tissue and 9.1.4.3 Future Surgical Procedures: Clarified that only the tests outlined in the pathology manual would be sent to DUMC for analysis. • Section 14.9 Interim Analysis: Changed timing of interim analysis to occur from when 16 patients in each arm reached 6 months post-infusion to when 16 patients in each arm from the other institutions collectively reached 6 months post-infusion, in addition to when 16 patients in each arm reached this milestone at DUMC. Removed changes in immune measures (Tregs) as an interim analysis endpoint given that very few samples from the Phase 1 study have been analyzed, to date. Clarified that the interim analysis focusing on feasibility of PVSRIPO at other institutions will be based primarily on safety endpoints given that the analysis is to take place 6 months after PVSRIPO infusion, prior to when separation in survival curves occurred relative to historical

	control patients in the Phase 1 study. However, early bevacizumab use as a potential surrogate marker of long term survival as outlined, will also be investigated at other centers and DUMC. Additional details will be outlined in the statistical analysis plan and finalized prior to conducting the interim analysis. • Section 10.2 Study Agent Lomustine: In keeping with the memo to file dated 17AUG2018, the 5 mg dose of lomustine was discontinued by the manufacturer and as such, reference to the 5mg dose was deleted and the text was amended to state that the single dose of lomustine to be given at week 8 should not exceed but be within 10mg (the new lowest dose level capsule) of 110 mg/m^2 or 90 mg/m^2 if on bevacizumab since PVSRIPO infusion. • Section 11.2 Exclusion Criteria: Clarified in this quoted section of exclusion criterion #8: "Patients may not be less than 12 weeks from radiation therapy…" that this specifically refers to radiotherapy of the brain; it now reads: "8. Patients may not be less than 12 weeks from radiation therapy of the brain, unless progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation". Given recent inquires, for EC 15, added a note on how to clarify if a patient has an unrelated malignancy requiring current active treatment by stating that if patient required treated for unrelated malignancy other than exceptions noted within the past 3 years, a letter from their treating oncologist for the unrelated malignancy must be on file confirming that said unrelated malignancy does not require current active treatment (prophylactic like tamoxifen OK) and that the patient is stable with low risk of recurrence and/or death within the next 3 years (ie, is stable). If this letter is not on file, a consult with the Sponsor's medical designee is required prior to submitting the patient for enrollment in the trial. • Table 1and Section 12.3 Follow-up PVSRIPO Infusion: Added 3 ml of blood collection at Day 1 (or Day 2) post-PVSRIPO infusion to explore inflammation-associated biomarkers/for immunologic analysis. • Section 12.8.6 Definition of Unacceptable Adverse Event: In response to a site question, clarified language regarding unacceptable AE to specify relationship to protocol/test procedure, listed the tests/procedures that were applicable and defined what reversible means; an unacceptable AE is any grade 3 or grade 4 toxicity considered related (possibly, probably or definitely) to a protocol treatment (includes surgical biopsy/infusion procedure, PVSRIPO, lomustine, bevacizumab, corticosteroids) that is not reversible (ie, does not improve to ≤ grade 2 with exception of neurologic events, which must only improve from highest grade level to next lower grade level) within 2 weeks. This includes any treatment-related life-threatening event or any treatment-related death. No material changes were made to the exceptions that follow this paragraph.
18OCT2017 to 01JUN2018	• Section 0 Purpose: Under exploratory objectives, added: Obtain the quantitative gut microbiome profile via the OMNIgene®•GUT (OMR-200) kit to determine any potential influence in response to PVSRIPO or PVSRIPO/lomustine and to explore future molecular genetic analyses of the buffy coat to determine the genetic characteristics of these samples to determine any potential influence in response to PVSRIPO or PVSRIPO/lomustine. • Section 5.2 Design and Procedure: Added that as an additional precaution, sites who have never infused PVSRIPO intratumorally via an intracerebral catheter must wait at least 14 days (± 2 days) between infusing the first 3 subjects enrolled at their site until confirmation that the procedures have been conducted per protocol has been confirmed; clarified that infusion of PVSRIPO occurs over approximately 6.5hrs. • Section 6 Study Schema: Updated study schema to include overall N and n for each treatment arm; Section 9.1.1: Updated that a FDA cleared stereotactic guidance system could be utilized to assist with catheter placement from Brainlab Vector Vision system. • Section 7.2.6 PVSRIPO Clinical Experience: provided updates and clarifications around the PVSRIPO clinical experience, to date. • Section 8 Objectives and Endpoints: added that exploring any potential genetic influence on response would include the buffy coat; added exploring the quantitative gut microbiome profile via the OMNIgene®•GUT (OMR-200) kit to determine any potential influence in response to PVSRIPO or PVSRIPO/lomustine. • Section 9.1.2 PVSRIPO Infusion: Clarified that A Medfusion™ 3500 infusion pump (Smiths Medical, Minneapolis, MN) or any other comparable FDA-cleared syringe

	infusion pump could be utilized for infusion after obtaining approval by the sponsor or their designee. Also clarified that for the post-PVSRIPO infusion MRI, no additional contrast agent would be given, and that the post-infusion CT was also performed without contrast. • Section 9.1.3 Gadolinium Distribution Quantitation: Clarified that the scan following PVSRIPO infusion would be conducted with no additional contrast agent to track the distribution of the contrast agent given with the PVSRIPO infusion. • Section 9.5 Definition of Evaluable Subjects and On-Study Subjects: Clarified that if the treating clinician felt adjustments in routine bevacizumab dosing were warranted, a subject would come off study but that accidental increases in bevacizumab dosing (ie, single or not routine) would not result in the subject coming off study and that these occurrences would be reviewed with the site PI. • Section 9.1.4.3 Future Surgical Procedures and Section 11.6 Follow Up for off Study Patients: Added that subjects and next-of-kin would be asked to provide consent to obtain subject brains for post-mortem examination. • Section 9.1.6 Safety Considerations: under Surgical Complications, added standard risks language associated with routine stopping of anti-coagulation therapy prior to surgical procedures; clarified that if a subject required planned treatment with bevacizumab doses > 7.5 mg/kg every 3 weeks, they would be considered off study and enter the follow-up phase; under Allergic Reactions to Contrast Agents, added that given new information of possible retention of gadolinium based contrast agents, only MRIs absolutely necessary for the proper management of a subject's brain cancer will be obtained. • Section 9.1.8 Randomization: listed the overall study N and n for each of the study arms, for clarity. • Section 10.1.4 Dispensing and Preparation, clarified that thawed vials of PVSRIPO are stable at 4°C for 48 hours and that PVSRIPO contained in the clinically intended delivery apparatus (ie, ready for infusion) is stable at room temperature for 18 hours, but should be used as soon as possible. • Section 11.2 Exclusion Criteria (EC): for greater clarity, modified EC number 6 to specify time restrictions for particular treatments prior to receiving study drug; added to EC number 10 that tumors with contrast-enhancing tumor component crossing the midline (crossing the corpus callosum) are also exclusionary; also clarified language so it is clear that extensive leptomeningeal disease is excluded but tumor touching leptomeninges is allowed. • Section 12 Screening and on-study Tests and Procedures: Table 1and throughout Section12: added that 4-6ml of whole blood would be collected for isolation of buffy coat at the screening visit and that a stool sample would be obtained at screening for a baseline sample (prior to PVSRIPO infusion) and at 8 weeks post-PVSRIPO infusion for all newly enrolled patients; those already treated with PVSRIPO under a previous protocol version would not have a baseline sample but would provide a stool sample for OMNIgene analysis at their Week 8 visit or at their next clinic visit if they have already passed the Week 8 visit; clarified that the baseline MRI of the brain would be obtained both with and without gadolinium contrast but that the MRI within 4h of PVSRIPO infusion and post-infusion CT after catheter removal would be obtained without contrast; clarified that after week 52 and for subjects in the follow-up period (off-study), that MRIs should still be obtained per the institution's SOC and all images transmitted to the sponsor or their designee at least every 6 months. • Section 12.7.4 Laboratory Evaluations: Added that in addition to immunologic testing, that genetic and gut microbiome testing may be included in the tests described in Section 14.7. • Section 12.8.4 Reporting of SAEs: clarified that SAEs must be reported by the investigator to the Sponsor within 24 hours of discovery if the event occurs during the clinical study and within 30 days after subjects are taken off study), whether or not the SAE is considered to be related to the study drug(s). • Section 14.7 Exploratory Objectives: Clarified that the exploratory objective referring to genetic markers which are predictors of response referred to tumor genetic markers; added obtaining the quantitative gut microbiome profile via the OMNIgene®•GUT (OMR-200) kit to determine any potential influence in response to PVSRIPO or PVSRIPO/lomustine and obtaining the future molecular genetic analyses of the buffy coat to determine the genetic characteristics of these samples to determine any

	potential influence in response to PVSRIPO or PVSRIPO/lomustine as exploratory objectives/analyses; added an exploratory objective as part of a smaller sub-study only for patients enrolled at DUMC, to explore inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence pre-PV boost, post-PV boost and 1 week post-PVSRIPO infusion in subjects randomized at this center. All of the changes noted here were also made to other relevant sections/tables throughout the protocol (eg, Section 5 (Protocol Synopsis and Research Summary, Section 12 (Screening and on-Study Tests and Procedures), Section 8 (Objectives and Endpoints), Table 1 Schedule of Study Tests and Procedures). • Section 14.9 Interim Analysis: corrected reference to NDA to BLA, given that PVSRIPO would be approved under a Biologics License Application.

Investigator Agreement Page for Pro00077024 (Protocol Version Multicenter 08)

I agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, with any future amendments, and with any other study-conduct procedures provided by Istari Oncology, Inc. or their designee.
- Not to implement this protocol, or any deviations from or changes to the protocol, without agreement from the Sponsor and prior review and written approval from the institutional review board, except where necessary to eliminate an immediate hazard to the subjects or to meet administrative requirements of the study (where permitted by all applicable regulatory requirements).
- That I am thoroughly familiar with the appropriate use of the investigational product(s), as described in this protocol, and any other information provided by the Sponsor, including but not limited to the current investigator's brochure provided by the Sponsor.
- That I am aware of, and will comply with, good clinical practices and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about their study-related duties and functions as described in the protocol.
- To periodic on-site monitoring of the electronic case report forms (eCRFs) and source documents by Sponsor or designee and to on-site inspection of eCRFs and source documents by appropriate regulatory authorities, including but not limited to the US Food and Drug Administration, local governing regulatory bodies, and institutional review board inspectors.
- That the subjects to whom the investigational product will be administered will be under my personal supervision or under the supervision of an investigator responsible to me.
- That I will not supply the investigational product to any other investigator, or to any clinic, for administration to a human being.

Investigator Name: _____

Investigator Signature: _____

Date: _____

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AE	Adverse Event
aPTT	Activated Partial Thromboplastin Time
CAP	College of American Pathologists
CBC	Complete Blood Count
CCNU	Chloroethyl-Cyclohexyl-Nitrosourea (Lomustine)
CED	Convection-Enhanced Delivery
CI	Confidence Interval
CLIA	Clinical Laboratory Improvement Amendments
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CRO	Clinical Research Organization
CT	Computed Tomography
CTC	Common Terminology Criteria
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Disease Control Rate
DOR	Duration of Response
DSMB	Data and Safety Monitoring Board
DUMC	Duke University Medical Center
DVT	Deep Vein Thrombosis
EC	Exclusion Criteria
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	Epidermal Growth Factor Receptor (variant III)
eIF	Eukaryotic Initiation Factor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in the first second of expiration
FLAIR	Fluid-Attenuated Inversion Recovery
G-CSF	Granulocyte-Colony-Stimulating Factor
GBM	Glioblastoma
GCP	Good Clinical Practice
Gd-DTPA	Gadolinium Diethylenetriamine Pentaacetic Acid
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HCG	Human Chorionic Gonadotropin
HIPAA	Health Insurance Portability and Accountability Act
HMGB1	High Mobility Group 1 Box
HRV2	Human Rhinovirus Type 2
HSA	Human Serum Albumin
IC	Inclusion Criteria
ICF	Informed Consent Form
ICH	International Council of Harmonization
IEC	Institutional Ethics Committee
IgG	Immunoglobulin G
IND	Investigational New Drug
iRANO	Immunotherapy Response Assessment in Neuro-Oncology
IRB	Institutional Review Board
IRES	Internal Ribosomal Entry Site
ISC	Investigator Steering Committee
IV	Intravenous
KPS	Karnofsky Performance Status
LAR	Legally Authorized Representative
LS	Landmark Survival
LSQ	Lymphocyte Subset Quantitation
MDSC	Myeloid-Derived Suppressor Cell

MGMT	O^6-methylguanine-DNA methyltransferase
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NDA	New Drug Application
NK	Natural Killer
NHP	Non-human Primate
NSF	Nephrogenic Systemic Fibrosis
ORR	Objective Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cell
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PET	Positron Emission Tomography
PI	Principal Investigator
PJP	Pneumocystis jiroveci pneumonia
PKC	Protein Kinase C
PRTBTC	Preston Robert Tisch Brain Tumor Center
PT	Prothrombin Time
PV	Poliovirus
PV1	Serotype 1 Live-Attenuated (Sabin) PV Vaccine
PV1S	Poliovirus Serotype 1 (Sabin)
PVSRIPO	Polio/Rhinovirus Recombinant
RANO	Response Assessment in Neuro-Oncology
SAE	Serious Adverse Event
SAM	Study Administration Manual
SD	Standard Deviation
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SIADH	Syndrome of Inappropriate Antidiuretic Hormone
SOC	Standard of Care
TCID	Tissue Culture Infectious Dose
TERT	Telomerase Reverse Transcriptase
US	United States
WHO	World Health Organization

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

Protocol Title:	A Multicenter Phase 2 Study of Oncolytic Polio/Rhinovirus Recombinant (PVSRIPO) in Recurrent WHO Grade IV Malignant Glioma Patients
Protocol Number:	Pro00077024
Study Design:	The purpose of this single-arm multicenter Phase 2 is to investigate the safety and efficacy (anti-tumor response and survival) of PVSRIPO in recurrent World Health Organization (WHO) grade IV malignant glioma. Patients will be administered PVSRIPO intratumorally via convection enhanced delivery (CED) and followed per the Schedule of Study Tests and Procedures for Initial PVSRIPO Treatment (Table 1). Retreatment with PVSRIPO is allowed, provided the retreatment eligibility criteria are met. Patients retreated with PVSRIPO will be followed per the Schedule of Study Tests and Procedures for PVSRIPO Retreatment (Table 2).
Phase:	Phase 2
Objectives:	**Primary Objective:** • To estimate the objective anti-tumor response rate and response duration among adults with recurrent WHO grade IV malignant glioma treated with PVSRIPO **Secondary Objectives:** • To assess the safety of PVSRIPO • To assess survival outcomes of adults with recurrent WHO grade IV malignant glioma treated with PVSRIPO relative to a historical control group • To assess the disease control rate following PVSRIPO infusion **Exploratory Objectives:** • To describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO • To assess immunologic responses in peripheral blood and in serum • To identify genetic predictors of response or failure of response to treatment with PVSRIPO • To obtain the quantitative gut microbiome profile via the OMNIgene®•GUT (OMR-200) kit to determine any potential influence in response to PVSRIPO • To explore future molecular genetic analyses of the buffy coat to determine the genetic characteristics of these samples to determine any potential influence in response to PVSRIPO • As part of a smaller sub-study only at DUMC, subjects enrolled at this center may have blood drawn prior to PVSRIPO infusion (both pre- and post-PV booster) and 1 week after PVSRIPO infusion to explore inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence • Assess anti-tumor responses and outcomes following PVSRIPO retreatment(s) or treatment with any other non-protocol specified therapy

	• Assess anti-tumor responses and outcomes considering site-level experience with PVSRIPO treatment

Anticipated Number of Patients:	Up to approximately 122 participants
Anticipated Number of Study Sites:	Up to 7 sites
Follow-Up Duration:	Up to 5 years, or until death, lost to follow-up, or withdrawal of consent (whichever comes first).

Entry Criteria:	**GENERAL INCLUSION/EXCLUSION CRITERIA** **INCLUSION CRITERIA**: 1. Patients must have a recurrent (first or second recurrence only, including this recurrence; transformation from a lower grade tumor to a WHO grade IV malignant glioma will be considered a first recurrence) supratentorial WHO grade IV malignant glioma based on imaging studies with measurable disease (a minimum measurement of 1 cm and maximum of 5.5 cm of contrast-enhancing tumor) with prior histopathology consistent with a WHO grade IV malignant glioma confirmed by the site's neuropathologist or the neuropathologist's designate. a. Assuming patient meets all other criteria, site neurosurgeon must confirm placement of infusion catheter tip can occur within or as close as possible to an enhancing region of tumor that is ≥ 1cm from ventricles and at a safe distance relative to eloquent brain function. b. Tumor size and location requirements per protocol must be confirmed as qualifying and safe to proceed by the reviewer(s) designated by the Sponsor. 2. If the subject is male and sexually active, he is eligible to enter and participate in this study if his partner(s) meets the criteria outlined in 2a or if he or his partner(s) is using one of the methods of birth control outlined in 2b. If the subject is female, she is eligible to enter and participate in this study if she meets the following criteria: a. Non-childbearing potential (ie, physiologically incapable of becoming pregnant, including any female who is postmenopausal or surgically sterile). Surgically sterile females are defined as those with a documented hysterectomy and/or bilateral oophorectomy or tubal ligation. Postmenopausal for purposes of this study, is defined as 1 year without menses); or b. Childbearing potential, has a negative serum pregnancy test at screening, and agrees to use one of the following methods of birth control: approved hormonal contraceptives (eg, birth control pills, patches, implants, or infusions), an intrauterine device, or a barrier method of contraception (eg, a condom or diaphragm) used with spermicide. c. If the male has had a vasectomy or is using a condom with spermicide, the female partner does not need to use additional birth control noted in 2a and 2b. 3. Age ≥ 18 years of age at the time of entry into the study. 4. Karnofsky Performance Status (KPS) Score ≥ 70%. 5. Prothrombin and Partial Thromboplastin Times ≤ 1.2 x normal prior to biopsy. 6. Total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase ≤ 2.5 x normal prior to biopsy. 7. Neutrophil count ≥ 1000 prior to biopsy. 8. Hemoglobin ≥ 9 prior to biopsy.

9. Platelet count ≥ 100,000/μL unsupported is necessary for eligibility on study; however, because of risks of intracranial hemorrhage with catheter placement, platelet count ≥ 125,000/μL is required for the patient to undergo biopsy and catheter insertion, which can be attained with the help of platelet transfusion.
10. Creatinine ≤ 1.2 x normal range prior to biopsy.
11. Positive serum anti-PV titer prior to biopsy.
12. The patient must have received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week, but less than 6 weeks, prior to administration of the study agent (initial infusion).
13. At the time of biopsy, prior to administration of virus, the presence of recurrent tumor must be confirmed by histopathological analysis.
14. A signed informed consent form (ICF) approved by the IRB will be required for patient enrollment into the study. Patients or their legally authorized representative (LAR) 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.
15. Able to undergo brain MRI with and without contrast.

EXCLUSION CRITERIA:

1. Females who are pregnant or breast-feeding.
2. Patients with an impending, life-threatening cerebral herniation syndrome, based on the assessment of the study neurosurgeons, their designate, and the reviewer designated by the sponsor.
3. Patients with severe, active co-morbidity, defined as follow:
    a. Patients with an active infection requiring intravenous treatment or having an unexplained febrile illness (Tmax > 99.5°F/37.5°C)
    b. Patients with known immunosuppressive disease or known human immunodeficiency virus infection
    c. Patients with unstable or severe intercurrent medical conditions such as severe heart disease (New York Heart Association Class 3 or 4)
    d. Patients with known lung (forced expiratory volume in the first second of expiration [FEV1] < 50%) disease or uncontrolled diabetes mellitus
    e. Patients with albumin allergy
    f. Patients with existing or history of anaphylactic reaction to gadolinium
4. Patients with a previous history of neurological complications due to PV infection.
5. Patients who have not recovered from the toxic effects of prior chemo- and/or radiation therapy. Guidelines for this recovery period are dependent upon the specific therapeutic agent being used.

	6. Patients may not have received tumor treating fields (≤ 1 week), chemotherapy or bevacizumab ≤ 4 weeks [except for nitrosourea and lomustine (≤ 6 weeks); metronomic dosed chemotherapy, such as daily temozolomide, etoposide or cyclophosphamide (≤ 1 week)] prior to starting the study drug. 7. Patients may not have received immunotherapy ≤ 4 weeks prior to starting the study drug unless patients have recovered from side effects of such therapy. 8. Patients may not be less than 12 weeks from radiation therapy of the brain, unless progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation. 9. Prior to enrollment, has not completed all standard of care treatments, including surgical procedure and radiation therapy (at least 59Gy) a. 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 b. If the MGMT promoter in their tumor is known to be methylated or the MGMT promoter methylation status is unknown at time of screening, patients must have received at least one chemotherapy regimen prior to participating in this trial c. If enrolling at 2nd recurrence, must have failed treatments initiated at 1st recurrence 10. Patients with neoplastic lesions in the brainstem, cerebellum, or spinal cord; radiological evidence of multiple areas of active (growing) disease (active multifocal disease); tumors with contrast-enhancing tumor component crossing the midline (crossing the corpus callosum); extensive subependymal disease (tumor touching subependymal space is allowed); or extensive leptomeningeal disease (tumor touching leptomeninges is allowed). 11. Patients with undetectable anti-tetanus toxoid immunoglobulin G (IgG). 12. Patients with known history of agammaglobulinemia. 13. Patients on greater than 4 mg per day of dexamethasone within the 2 weeks prior to admission for PVSRIPO infusion. 14. Patients with worsening steroid myopathy (history of gradual progression of bilateral proximal muscle weakness, and atrophy of proximal muscle groups). 15. 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. 16. For patients randomized prior to v7, a known history of hypersensitivity to lomustine, dacarbazine, or any components of lomustine. 17. Patients with active autoimmune disease requiring systemic immunomodulatory treatment within the past 3 months.

	PVSRIPO RETREATMENT ELIGIBILITY CRITERIA: To be eligible for PVSRIPO retreatment(s), patients must continue to satisfy all general inclusion and exclusion criteria, including the following IC/EC adaptations/additions: 1. Adaptation to Inclusion Criterion (IC) 1: Histologically confirmed (≤ 3 months of planned treatment) measurable (≥ 1 cm and ≤ 5.5 cm of contrast-enhancing) supratentorial primary brain tumor that has recurred/progressed ≥ 12 months after initial PVSRIPO infusion 2. Adaptation to IC 12: New boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) ≥ 1 week, but ≤ 6 months prior to PVSRIPO retreatment 3. Addition: No treatment-limiting signs and symptoms (eg, cerebral edema) from prior PVSRIPO administration, such that PVSRIPO retreatment should be well tolerated, in the opinion of the investigator/neurosurgeon
Investigational Product:	PVSRIPO: recombinant, non-pathogenic chimera consisting of the genome from live-attenuated serotype 1 (Sabin) polio virus (PV1S) recombined with the internal ribosomal entry site (IRES) of human rhinovirus type 2 (HRV2).
Dosage:	5×10^7 tissue culture infectious dose (TCID50) in 3ml of physiologic saline stabilized with 0.2% human serum albumin (HSA)
Route:	Delivered intratumorally by convection-enhanced delivery (CED) over 6.5 hours via intracerebral catheter
Statistical Methods:	The primary endpoint of overall radiographic response (ORR) will be presented as a percentage, along with a 95% confidence interval. Duration of response (DOR), overall survival (OS) and landmark survival (LS) will each be described according to the statistical analysis plan (SAP). Descriptive statistics will be presented for all other analyses unless otherwise specified.
Interim Analysis:	An interim analysis will be conducted after approximately 50 or more PVSRIPO-treated patients have been followed for approximately 21 or more months (details described in the statistical analysis plan).

Table 1. Schedule of Study Tests and Procedures for initial PVSRIPO Treatment

Description	Screening: Within 6 weeks	Screening: Within 14 days prior to catheter placement, but as close to biopsy as possible	Screening: Within 2 days prior to PVSRIPO	Catheter placement biopsy, PVSRIPO infusion	Post-infusion (follow-up period)[a]							On-Study Subjects (required SOC visits and minimum follow-up)	Off-Study Subjects (SOC visits with follow-up)
Week					0	1	2	4	8	[WEEK 12 VISIT REMOVED IN PROTOCOL V7] 12[b]	16 + every 9 weeks until week 52	>52 (Information transmitted routinely as obtained)	From time off study while available for follow-up (Information transmitted a minimum of every 6 months [±1 month])
Day		Within 14 days prior to catheter placement	Within 2 days prior to PVSRIPO	0	1	7	14	28	56	84	112	>112	Varies
General Evaluations													
Informed Consent	X												
Clinical assessment (including medical history)		X			X[c]	X	X	X	X	X	X	X	X
Physical examination		X			X[c]	X	X	X	X	X	X	X	
Neurologic examination		X			X[c]	X	X	X	X	X	X	X	
KPS		X			X[c]	X	X	X	X	X	X	X	
Adverse events				Continuous									
Laboratory Evaluations													
PV immunization booster [d]	X												
CBC with differential		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[e]				

Description	Screening: Within 6 weeks	Screening: Within 14 days prior to catheter placement, but as close to biopsy as possible	Screening: Within 2 days prior to PVSRIPO	Catheter placement biopsy, PVSRIPO infusion	Post-infusion (follow-up period)[a]							On-Study Subjects (required SOC visits and minimum follow-up)	Off-Study Subjects (SOC visits with follow-up)
Week					**0**	**1**	**2**	**4**	**8**	**[WEEK 12 VISIT REMOVED IN PROTOCOL V7] 12[b]**	**16 + every 9 weeks until week 52**	**>52 (Information transmitted routinely as obtained)**	**From time off study while available for follow-up (Information transmitted a minimum of every 6 months [±1 month])**
Day		**Within 14 days prior to catheter placement**	**Within 2 days prior to PVSRIPO**	**0**	**1**	**7**	**14**	**28**	**56**	**84**	**112**	**>112**	**Varies**
Serum for LSQ, anti-tetanus toxoid IgG	X (pre-boost)												
PV titer[f]	X (pre-boost)												
Whole blood for immunologic/other analysis[g]	X (pre-boost)	X			X[g]	X[h]	X		X				
Whole blood for buffy coat isolation	X												
Stool sample [n]	X [n]								X[n]				
Disease evaluations													
MRI[i]		X						X	X		X	X	X
CT scan				X[j]									
Biopsy[k]				X									
Tumor samples												X[l]	X[l]
Treatment													
PVSRIPO				X (+ 1-day window)									
Lomustine									X[m]				

aPTT = activated partial thromboplastin time; CBC = complete blood count; CMP = Comprehensive Metabolic Panel; CT = computed tomography; IgG = immunoglobulin G; KPS = Karnofsky Performance Status; LSQ = lymphocyte subset quantitation; MRI = magnetic resonance imaging; PT = prothrombin time; PV = poliovirus; PVSRIPO = polio/rhinovirus recombinant; SAM = Study Administration Manual; SOC = standard of care.

[a] Tests and procedures occurring on Day 7 have a ± 3-day window. From Day 14 (Week 2) onward, all tests and procedures have a ± 7-day window.

[b] The week 12 clinic visit (± 1 week) and whole blood collection for immunologic/other analysis was removed in protocol v7, as it was associated with follow-up after lomustine use. Patients enrolled under protocol version 7 and after will not have a protocol mandated week 12 visit.
[c] Daily after infusion until discharged from hospital; a PE must be documented within 24h (±4h) of discharge.
[d] PV booster must occur ≥ 1 week (but ≤ 6 weeks) prior to the initial PVSRIPO infusion.
[e] Only for women of childbearing potential who are randomized to receive lomustine prior to protocol V7.
[f] For screening prior to initial PVSRIPO infusion, PV titers are to be drawn after informed consent and prior to PV booster administration. No additional tubes need to be drawn for the PV titer. This analysis can be done from the whole blood drawn for immunologic analysis.
[g] Whole blood for immunologic analysis (3mL at Day 1 **OR** Day 2 post-PVSRIPO infusion; thereafter, ~96.5 mL; tube and sample processing and shipping details outlined in laboratory manual portion of the SAM). For patients only at DUMC, an additional 6ml (~102.5 mL total) will be drawn at both the pre-PV boost and post-PV screening visits (before PVSRIPO infusion) and again at the Week 1 visit as part of a sub-study at DUMC to explore inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence. After Week 8, only draw 76.5 mL of whole blood for all patients/all centers.
[h] Blood sample (6mL) collected on Day 7 (Week 1) post-infusion **only** for participants enrolled at DUMC consented to sub-study for immune marker analyses.
[i] All MRIs in the study include intravenous administration of gadolinium. After week 52, MRIs are to be conducted per the institution's SOC and all images are to be transmitted to the sponsor or their designee no less than every 6 months (± 1 month) after the last images were transmitted.
[j] There is a post-catheter placement CT scan and a post-catheter removal CT scan.
[k] Biopsy results should be transmitted, or blocks/slides should be shipped to the sponsor or designee per instructions in the laboratory manual portion of the SAM.
[l] If any additional samples become available or resections occur anytime during the study (make patient off study) or off-study follow-up period, materials also should be transmitted to the sponsor or their designee, as outlined in the SAM, if possible.
[m] For subjects randomized to the lomustine cohort prior to protocol v7 received 110 mg/m^2 oral lomustine for one cycle at 8 weeks post-PVSRIPO infusion. Subjects who were on bevacizumab (were treated with bevacizumab in the time since PVSRIPO infusion) received 90 mg/m^2 oral lomustine for one cycle 8 weeks post-PVSRIPO infusion. The lomustine may have been taken the day of or 1 day after the week 8 visit.
[n] Stool Sample for OMNIgene Kit (OMNIgene®•GUT (OMR-200)). For newly enrolled patients (ie, those who have not received PVSRIPO under a previous protocol version), a kit will be dispensed at the pre-PV boost screening visit and they will provide a baseline sample prior to PVSRIPO infusion and again after the week 8 visit. For patients who received PVSRIPO under a previous version of the protocol, a baseline/pre-PVSRIPO sample will not be obtained but the patient will be given a kit to provide a post-PVSRIPO infusion stool sample at their next clinic visit.

Table 2. Schedule of Study Tests and Procedures for PVSRIPO Retreatment

Study Procedure	Screening Period	PVSRIPO infusion	Post-infusion (follow-up period)						
	≤ 14 days	Day 0	Day 1	1w (+/- 3d)	2w (+/- 1w)	4w (+/-1w)	8w (+/-1w)	Q9-12w for 52w	> 52 weeks from Retreatment[b]
Informed Consent[a]	X								
Clinical assessment & medical history	X		X[d]	X	X	X	X	X	X
Physical Exam[c]	X		X[d]	X	X	X	X	X	X
Neurologic Exam	X		X[d]	X	X	X	X	X	X
KPS	X		X[d]	X	X	X	X	X	X
Record Cancer treatments & Concomitant medications[e]	Continuous from signing of ICF onward								
Adverse Events		Continuous							
PV Immunization Booster and anti-tetanus IgG[f]	X								
CBC w/diff	X		X	X	X	X	X	X	
CMP	X		X	X	X	X	X	X	
PT, aPTT	X								
Pregnancy Test[g]	X								
MRI	X					X	X	X	X
CT Scan		X[h]							
Biopsy		X							
PVSRIPO		X							

AEs = adverse events; aPTT = activated partial thromboplastin time; CBC = complete blood count; CMP = comprehensive metabolic panel; CT = computed tomography; IgG = immunoglobulin G; KPS = Karnofsky Performance Status; LSQ = lymphocyte subset quantitation; MRI = magnetic resonance imaging; PT = prothrombin time; PV = poliovirus; SAE = serious adverse events

[a]**Informed Consent** must be renewed prior to initiation of screening activities for PVSRIPO retreatment.

[b]**Follow-up beyond Week 52 post retreatment:** participants should be followed per institutional SOC for all assessments after Week 52 post PVSRIPO retreatment. Data transfers should occur no > every 3 months (including description of MRI/tumor measurements). If SOC occurs less frequently, data should be entered within 2 weeks (± 1 week) of acquisition.

[c]**Physical Examination:** The screening physical examination should be a complete physical exam of major body systems, per institutional guidelines. Following PVSRIPO infusion, physical exams should be focused and symptom-directed. Each exam should be performed in conjunction with a neurological exam and KPS assessment.

[d]**Post-PVSRIPO (re)treatment:** Clinical assessment and physical examinations (including neurologic assessment and KPS) should be performed daily until hospital discharge.
[e]**Cancer Treatments and Concomitant Medications: all** start/stop dates, dose, route, frequency, procedures and treatments for glioma or any other cancer treatment are to be recorded from baseline onward, including agents associated with managing PVSRIPO anti-tumor inflammatory response (ie, corticosteroids and bevacizumab) or for any other agents/causes.
[f]**Polio immunization booster and anti-tetanus toxoid IgG blood test:** May be obtained locally and must occur ≥ 1 week (but ≤ 6 months) prior to planned additional PVSRIPO infusion(s).
[g]**Pregnancy Test:** A serum-based pregnancy test must be performed for all female participants during screening (≤ 2 days of PVSRIPO infusion).
[h]**CT Scan:** Includes a CT to confirm catheter placement prior to PVSRIPO infusion (preferably intraoperative) and second CT upon catheter removal to confirm no bleed.

5.1 Background and Significance

Primary brain tumors represent around 1% of all diagnosed cancers[1]. Most high grade primary central nervous system (CNS) tumors are highly resistant to current available therapies. The standard of care for newly diagnosed high grade gliomas involves maximal safe surgical resection, followed by standard radiation therapy with concurrent temozolomide, and subsequent adjuvant temozolomide. There is an unmet clinical need for the therapy of malignant gliomas with a median survival of <20 months despite available therapies.

Treatment failure for subjects with recurrent malignant glioma is partly due to the presence of a blood-brain barrier resulting in poor penetration of cytotoxic drugs into areas where this barrier is intact. Moreover, the non-specific nature of conventional therapy for brain tumors often results in incapacitating damage to surrounding normal brain[2,3]. The inherent selectivity of approaches based on biological agents with tumor specificity offers the prospect of more precise and effective therapy. Various approaches have been used successfully to circumvent the blood-brain barrier, including convection-enhanced delivery (CED), a process by which large molecules (>400 daltons) are directly infused under pressure into a tumor through a catheter. CED results in adequate distribution of such molecules into the tumor over large areas via inherent interstitial fluid pathways.

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

PVSRIPO is a genetically recombinant, non-pathogenic poliovirus (PV): rhinovirus chimera with a tumor-specific conditional replication phenotype. PVSRIPO consists of the genome of the live-attenuated PV serotype 1 (Sabin) vaccine (PV1S) with its cognate internal ribosomal entry site (IRES) element replaced with that of human rhinovirus type 2 (HRV2). PVSRIPO tumor tropism is mediated by the PV receptor, CD155. CD155, an onco-fetal cell adhesion molecule ectopically upregulated in ectodermal/neuroectodermal cancers, is broadly expressed on cancerous cells, cancer ‘stem-cell-like cells’ and tumor-associated proliferating vasculature. Infection with PVSRIPO results in swift destruction of tumor cells. PV’s inherent neuropathogenicity was removed by IRES exchange; this ablated the virus’ ability to propagate in cells of neuronal lineage and to cause poliomyelitis. However, PVSRIPO replicates efficiently in cancerous cells and exhibits potent anti-neoplastic effects in animal tumor models. Tumor cell-specificity mediated by the foreign IRES in PVSRIPO relies (i) on a ‘non-productive’ ribonucleoprotein complex forming at the foreign HRV2 IRES in neurons; (ii) constitutive signal transduction via protein kinase C (PKC)-Ras-ERK1/2 to translation

machinery, which stimulates viral, cap-independent translation via the HRV2 IRES in cancerous cells.

In a phase 1 dose-finding study that enrolled patients with recurrent grade IV malignant glioma, PVSRIPO was well tolerated, with dominant adverse effects being complications from localized tumor inflammation and the secondary prolonged use of steroids, particularly at higher doses of PVSRIPO. To limit these effects, the dose was de-escalated, and the trial continued as a dose expansion study. The trial closed to new accrual on 5/31/2017. There has been no evidence of propagation of infectious virus beyond the tumors in these patients nor evidence of viral encephalomyelitis, poliomyelitis, meningitis, or systemic autoimmune reactions. There is evidence that, in addition to a direct cytotoxic effect of PVSRIPO on infected tumor cells, this treatment generates an immune response against the tumor itself, which can lead to a reduction in tumor size. The localized inflammatory response is observed on imaging, and, as observed with other immunotherapies, it is difficult to distinguish between localized inflammation and tumor progression. In some subjects with possible disease progression observed by imaging after PVSRIPO infusion, the decision was made to treat with temozolomide or lomustine. A number of patients treated with temozolomide or lomustine after PVSRIPO infusion showed a rapid decrease in tumor volume after initiation of chemotherapy. However, this tumor control was lost in some patients if they remained on therapy for a prolonged period. Immune monitoring in a limited number of patients showed: 1) contraction of a specific, high-impact subset of (immunosuppressive) T-regulatory cells; and 2) beginning expansion of effector T-cells at the nadir (4 weeks post lomustine). Any such beneficial effect is likely to be lost upon standard multicycle chemotherapy, as subsequent lymphodepletions will eventually dampen the immune system's capacity to target the tumor.

Therefore, in this study, we initially proposed to evaluate a single intratumoral administration of PVSRIPO in combination with a single cycle of lomustine 8 weeks post-PVSRIPO instead of the standard 9-cycle/1-year regimen for the following reasons: a) Information stemming from patients treated with lomustine post-PVSRIPO on a phase 1 clinical protocol suggests significant clinical response, radiographic response, and the involvement of an antitumor immune component (ie, a PVSRIPO-induced antitumor memory response) occurring after the first cycle, with no evidence for further benefit with additional cycles of lomustine thereafter; b) this is in line with much empirical evidence for beneficial effects of lymphodepletion with DNA-damaging chemotherapy, eg lomustine or temozolomide, in conjunction with immunotherapy regimens. Lymphodepletion is believed to generate an 'immunologic reset' by broadly eliminating suppressive T-cell subsets and favoring preferential expansion of T-cell populations with pro-inflammatory activation phenotypes during immune recovery. While the immunologic benefits of lymphodepletive chemotherapy is well documented, it is equally well known that standard cycle-therapy (eg with one year of lomustine) produces long-term immune suppression by abrogating the expansive phase of immune recovery following the initial cycle of lomustine[4]. However, a preliminary review of safety and related information of 50 patients treated on study (24 on the PVSRIPO only arm and 26 on the PVSRIPO with lomustine arm) was conducted and there was no apparent

early (ie, prior to 24 months follow-up) observable survival advantage, need for bevacizumab at radiation necrosis dose, radiographic response or need for surgery (ie, potential early indications of long-term survival benefit) between the two arms. However, an anticipated increase in hematologic toxicity in the lomustine arm was noted with 11 out of 26 patients (46%) on the PVSRIPO plus lomustine arm experiencing grade 3 or higher hematologic adverse events versus 5 out of 24 patients (21%) on the PVSRIPO only arm experiencing grade 3 or higher hematologic adverse events (Istari Oncology, Inc. data on file). For this reason, randomization to the lomustine arm/protocol-mandated treatment with a single dose of lomustine at week 8 was discontinued for all future patients consented after Version 7 of the protocol. This change was implemented immediately in consideration of subject safety on 21FEB2019 prior to implementation of this formal amendment, given elevated safety issues of which the Sponsor became aware of on 20FEB2019. It was observed that the anticipated pancytopenia following lomustine use led to the postponement of repeat bevacizumab therapy in 4 patients who likely required it for control of cerebral edema associated with the PVSRIPO anti-tumor response.

5.2 Design and Procedure

In this study, patients with recurrent WHO grade IV malignant glioma will receive PVSRIPO intratumorally via CED to evaluate the impact of this treatment on objective radiographic response (ORR; complete or partial response) and duration of response (DOR) based on criteria noted in the central imaging charter. PVSRIPO administration can cause an inflammatory pseudoprogessive-type effect, which often manifests as a tumor enlargement with a polycystic (“swiss cheese”-like) appearance prior to an objective response. This radiographic phenomenon can persist for 6 months or more, with typical time to objective response ranging between 6 to 12 months post-infusion (median time to ORR noted in Phase 1 study was 15 months (range 5 to 29 months).

Because of this known PVSRIPO-dependent treatment effect, standard imaging criteria (ie, RANO or iRANO) are insufficient for identifying disease progression without careful consideration of other clinical factors. Therefore, patients should be strictly managed according to protocol (Section 9.1.4.3 and 9.1.6; see Special Considerations for additional information), in order to give patients the greatest opportunity for clinical benefit.

Per-Protocol management of cerebral edema/pseudoprogressive/polycystic/expansive tumor appearance within 6 months of any PVSRIPO infusion is as follows:

- Radiographic findings but patient asymptomatic/stable:
  - Observe and continue to follow per protocol
- Radiographic findings with new onset/worsening clinical symptoms:
  - Initiation of low dose steroids (dexamethasone ≤ 4mg/day)
  - Low dose bevacizumab (7.5mg/kg q 3 weeks; few cycles as possible to achieve symptom control)
  - Other supportive care treatments, as required (eg, antiepileptics)

NOTE: Except in cases of eminent life-threatening emergency, consult with study-designated neurooncologist(s)/ISC prior to initiating non-protocol-specified tumor treatments or performing surgical resection.

Patients with disease recurrence/progression ≥ 12 months from prior PVSRIPO infusion may be eligible for PVSRIPO retreatment (as described in section 9.2), provided the retreatment eligibility criteria are met.

Based on a phase 1 study of PVSRIPO in adult patients with recurrent WHO grade IV malignant glioma, the amount to be delivered will be 5 x 10^7 tissue culture infectious dose (TCID50). A total of 3 mL of the agent in physiologic saline stabilized with 0.2% human serum albumin (HSA) will be delivered over approximately 6 hours 30 minutes, corresponding to a flow-rate of 0.5 mL/hr. The agent is stable at room temperature during the instillation period and there is no adsorptive loss of PVSRIPO in the intended delivery apparatus.

As an added precaution, sites that have never infused PVSRIPO intratumorally via an intracerebral catheter must wait after infusion of their first patient (or patients, per Sponsor recommendation) until verification that the entire infusion procedure (ie, biopsy, catheter placement, infusion, catheter removal) occurred per protocol by Istari or their designee prior to infusing additional subjects. With Sponsor approval the sites will be allowed to enroll future subjects without this wait period.

5.3 Selection of Subjects

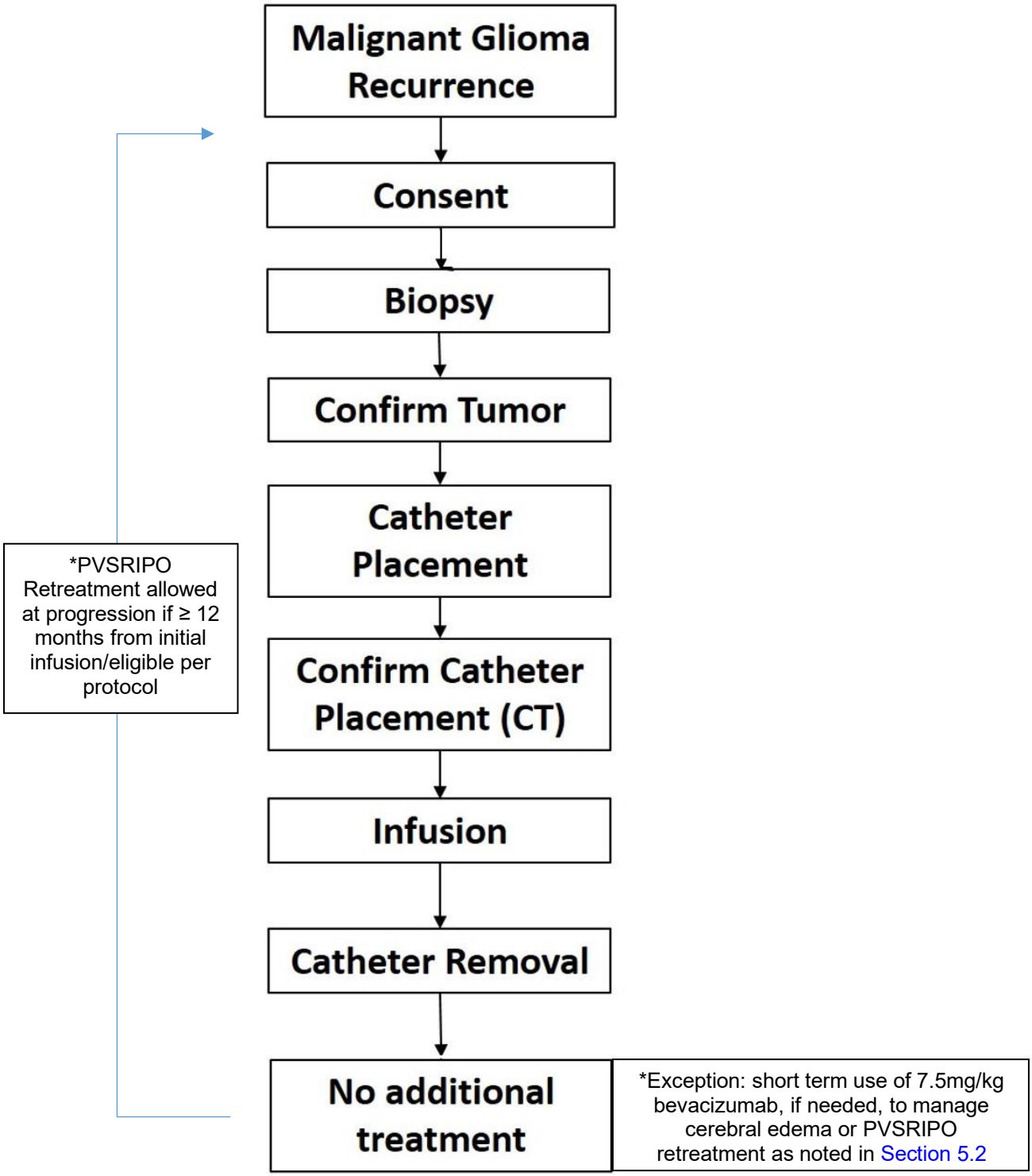
All inclusion/exclusion criteria may be found in Section 11.

5.4 Data Analysis and Statistical Considerations

Given this is a single arm trial, and on the basis of discussions with the FDA at a meeting on August 08, 2019, the primary endpoints will be ORR and DOR, which are generally accepted not to be influenced by baseline prognostic factors and salvage therapies. In addition, preliminary data show that in some patients, PVSRIPO treatment has been associated with long-term survival in patients with best response of minor response (MR) or stable disease (SD) (Istari data on file). Therefore, disease control rate (DCR) will be evaluated, considering any radiographic response.

In addition, OS will be analyzed. Immunotherapeutics are associated with durable anti-tumor effects in patients who achieve an objective response, which often results in a late, yet pronounced separation in survival curves. With PVSRIPO being an immunotherapeutic, a similar sequence of events is expected. As such, survival analysis will focus on survival at 36 months post-infusion in addition to OS. Comparisons to criteria matched external control with adjustments for known prognostic baseline factors will also be made. The SAP will present details and descriptions of these and additional analyses.

6 STUDY SCHEMA



7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

WHO grade IV malignant glioma is the most common malignant primary brain tumor[5]. The incidence is 5 per 100,000 individuals. Despite hundreds of clinical trials worldwide since the 1960s, there have been only a few treatments approved by the Food and Drug Administration (FDA). In the last decade, only temozolomide and Optune® have been approved for newly diagnosed grade IV malignant glioma and bevacizumab and Optune®[6] for recurrent WHO grade IV malignant glioma. These three agents extend median survival a few months, and less than half of treated patients show objective responses. Thus, median survival of newly diagnosed grade IV malignant glioma remains at <20 months[7-9] and for recurrent grade IV malignant glioma, only 6-12 months[10-14]. Treatment failure is due in part to the presence of a blood-brain barrier and poor penetration of cytotoxic drugs into the tumor[15]. There are very few long-term survivors, and virtually all malignant gliomas recur[16]. Clearly, new approaches are needed to improve therapy.

7.2 Study Agent: PVSRIPO

7.2.1 PVSRIPO Overview

PVSRIPO is based on the prototype neurovirulent PV serotype 1 (Mahoney) (**Figure 1A**), which is derived from a stool isolate obtained from a non-symptomatic carrier in Cleveland in the 1940s. Throughout the 1940s, PV type 1 (Mahoney) was subjected to serial passage, either in non-human primates (NHPs) or in tissue culture cells derived from various NHP tissues, which yielded the type 1 live-attenuated (Sabin) vaccine strain PV serotype 1 (Sabin) (PV1S); (**Figure 1B**). PV1S was/is the preferred agent for routine, infant vaccination in the world. PV1S vaccine was modified by exchange of its IRES with its counterpart from human HRV2, generating PVSRIPO (**Figure 1C**). PVSRIPO is characterized by loss of the inherent neurovirulence of PV[17-20].

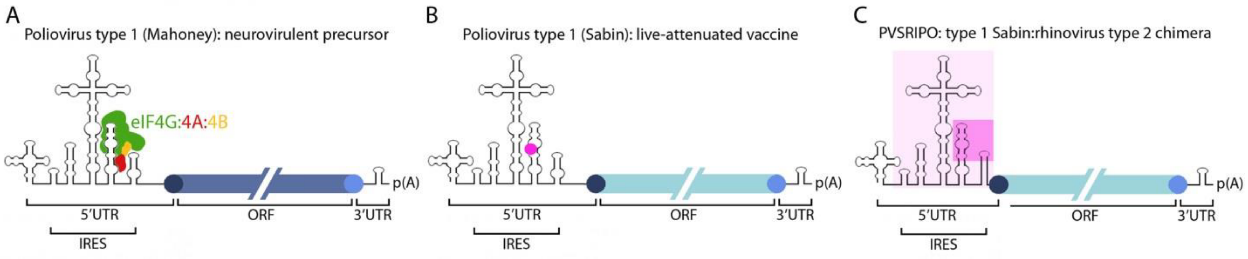


Figure 1. The genetic structure of PVSRIPO and its precursors.(A) Type 1 Mahoney precursor, (B) PV1S, (C) PVSRIPO

7.2.2 PVSRIPO Oncolysis

PVSRIPO's anti-neoplastic potential is due to a series of (i) direct lytic effects on tumor cells; (ii) presentation of tumor-associated antigens in a highly adjuvanted context; (iii) pro-inflammatory and danger signals stemming from tumor destruction and activation of an antiviral type 1 interferon response; (iv) infection and pro-inflammatory activation of dendritic cells and tumor-associated macrophages; (v) durable antitumor immunity evoked by effector cytotoxic T lymphocyte responses (**Figure 2**)[21]. The principal elements determining PVSRIPO tumor tropism, tumor-specific cell killing, neuronal incompetence/safety, and immunogenicity are well established empirically[21,22].

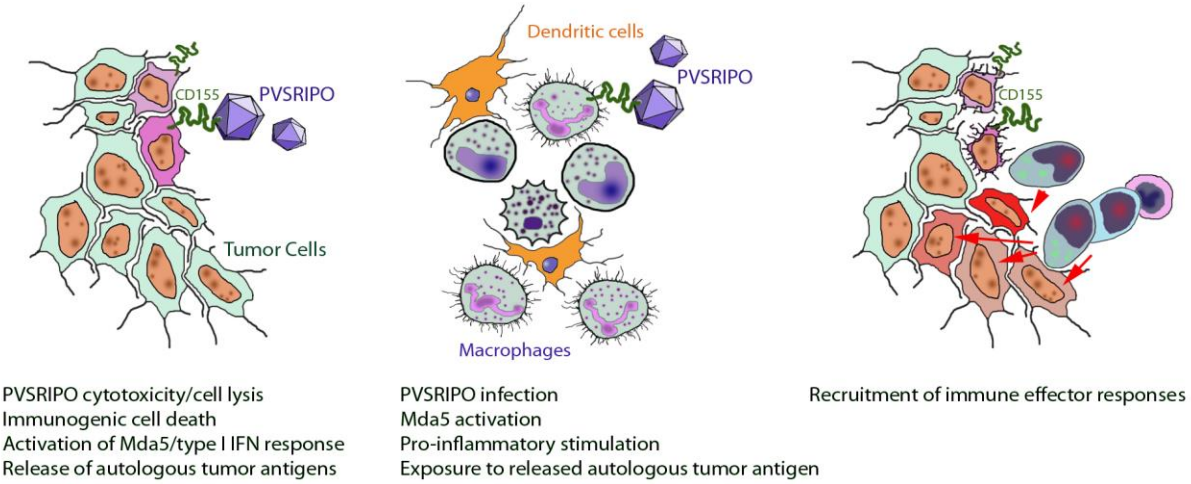


Figure 2. Pathways involved in PVSRIPO oncolytic immunotherapy. PVSRIPO oncolytic immunotherapy is based on combined cytotoxic and immunogenic effects on tumor cells (left), pro-inflammatory effects on antigen-presenting cells (middle) and the generation of adaptive antitumor immunity (right).

7.2.3 PVSRIPO Tumor Tropism

Every aspect of PVSRIPO oncolytic immunotherapy is dominated by its relationship with its host cell receptor, CD155[23]. CD155 is broadly expressed in neuroectodermal malignancies, eg, malignant glioma. Its expression has been empirically confirmed in laboratory malignant glioma cell lines, primary patient explant malignant glioma cells, primary patient explant malignant glioma xenografts maintained in athymic mice, and patient malignant glioma tissues obtained during surgery[24]. CD155 is expressed on tumor cells proper, on proliferating tumor vascular cells, and on macrophages and dendritic cells[25,26].

7.2.4 PVSRIPO Tumor-Specific Cell Killing

Infection of tumor cells with PVSRIPO results in their swift destruction, due to very early translation of highly cytotoxic viral proteins immediately after entry of the viral ribonucleic acid (RNA) into infected host cells[27]. PVSRIPO achieves this through an

unorthodox mechanism of translation initiation[28]. At eukaryotic mRNAs, protein synthesis occurs upon recruitment of a multi-partite protein complex at the canonical 5' 'cap' structure. PV RNAs are un-capped and, hence, rely on cap-independent translation for viral protein synthesis. This involves direct recruitment of ribosomal subunits via binding of the eukaryotic initiation factor (eIF) 4G to viral RNA (**Figure 1**)[29]. eIF4G-binding occurs via the IRES, a *cis*-acting genetic element located in the 5' untranslated region of the viral RNA. In tumor cells, this process is highly efficient, due to un-hinged signal transduction networks that greatly favor ribosome recruitment to viral RNA.

PVSRIPO is deemed acceptable for investigation in humans, including after intracerebral inoculation, because it is incapable of driving viral translation in normal brain cells[17]. This is due to profound neuronal incompetence of the foreign HRV2 IRES in its genome. The foreign HRV2 IRES cannot recruit eIF4G to the viral genome, precluding viral protein synthesis in neuronal cells/the normal brain[30]. By blocking this crucial, early step in the infectious cycle specifically in neurons, PVSRIPO neurovirulence is eliminated.

7.2.5 PVSRIPO Pre-Clinical Experience

In animal tumor models, oncolytic PVs elicit efficient anti-neoplastic effects resulting in tumor regression and, eventually, destruction[27]. There is histologic evidence for direct, virus-mediated tumor cell killing and indirect, host-mediated inflammatory responses directed against tumor. PVSRIPO was subjected to extensive dose-range finding, toxicology, biodistribution, shedding and neutralizing antibody tests with intrathalamic inoculation of up to 5 x 10^9 TCID50 of PVSRIPO in *M. Fascicularis*[31]. These revealed: (i) absence of morbidity and mortality; (ii) absence of neuropathological signs consistent with virus-induced CNS damage; (iii) absence of virus dissemination from the brain or viremia; (iv) absence of extraneural replication; (v) absence of shedding with saliva, urine or stool; (vi) presence of a neutralizing antibody response.

7.2.6 PVSRIPO Clinical Experience

The first-in-human oncolytic PV, PVSRIPO, therapy was initiated at the Preston Robert Tisch Brain Tumor Center (PRTBTC) at Duke University Medical Center (DUMC) in early 2012 as a phase 1 trial in patients with histologically confirmed recurrent WHO grade IV malignant glioma (glioblastoma or gliosarcoma; size of 1-5.5 cm). The study was closed to accrual on 5/31/2017 after 61 patients were treated. Key inclusion criteria in this clinical trial included: ≥ 18 years, adequate performance status/organ function, and prior PV immunity. Key exclusion criteria included: pregnant/breast-feeding females, those who have received radiotherapy less than 12 weeks prior, and those with known immune dysfunction or febrile and/or other systemic illnesses. Patients were given a booster of inactivated PV vaccine ≥1 week, but not > 6 months, prior to PVSRIPO infusion. Consented patients first underwent biopsy of the lesion to confirm

recurrence and then have a catheter placed into the tumor ≥ 1 cm away from the ventricles. The catheter was tunneled 5 cm under the scalp and connected to an infusion pump. The agent was then infused at a rate of 0.5 mL/hr over a period of 6.5 hours. In the investigational new drug (IND) toxicology studies in NHPs, no dose-limiting toxicities were observed, and the maximum tolerated dose was not reached. The starting dose in this first-in-human trial was 1 x 10^8 TCID50, which is 1/10th of the highest non-toxic dose in NHPs from the definitive, IND-directed toxicology study and 1/50th of the highest non-toxic dose in NHPs from the dose-range finding toxicology study. In the dose escalation portion of the phase 1 trial, cohorts of patients were dosed at 1 x 10^8 TCID50 (dose level 1), followed by 3.3 x 10^8 (level 2), 1 x 10^9 (level 3), 3.3 x 10^9 (level 4), and 1 x 10^{10} (level 5). The rationale and outcome associated with subsequent dose reductions to 3.3 x 10^8 (level 2), 5 x 10^7 (level -1), and 1.0 x 10^7 (level -2) are described below.

Dose Escalation Phase: The first 9 patients, who were treated at dose levels 1 through 5, constituted the dose escalation portion of the study. One patient each was treated at dose levels 1 through 3, 2 patients at level 4, and 4 patients at level 5. One of four patients treated at dose level 5 suffered a dose-limiting toxicity of intracranial hemorrhage after catheter removal. This subject was removed from study seven months post-infusion, when he started treatment with lomustine for pathology confirmed tumor recurrence, per protocol guidelines. The subject was still alive (as of 5/31/2017) and remained disease free for an extended period following initial treatment with PVSRIPO followed by the lomustine (approximately 43 months) but has since been re-treated with a second infusion of PVSRIPO at dose level -2 due to evidence of a new lesion.

Three other patients at dose level 5 died of progressive disease 12-20 months post-treatment and had difficulty weaning down steroids following study drug administration due to cerebral edema possibly due to localized inflammation and/or progressive disease. As of 5/31/2017, the 2 patients treated at dose level 1 and 2 did not suffer any significant toxicities and were alive with “No Evidence of Disease” 61+ and 60+ months from treatment, respectively. The patient at dose level 3 and one patient at dose level 4 died of progressive disease 6 months post-infusion, and one patient at dose level 4 died at 16 months post-treatment. Due to observed tumor inflammation at the higher dose levels that could potentially have been related to treatment or tumor growth, which necessitated prolonged steroid use, the dose was de-escalated and six additional patients were treated at dose level 2, as part of the dose expansion phase. In this cohort, one patient died 7 months following therapy after suffering an intracerebral hemorrhage due to head injury from a fall. Five other patients have all died of progressive disease 6-16 months following treatment.

Dose Reductions to Dose Level -1 and -2: Due to the continuing requirement for steroids in all of the additional patients treated at dose level 2 because of localized inflammation, due to treatment and/or tumor growth, a decision was made to de-escalate to dose level -1 (5 x 10^7 TCID50). By 6/16/2016, twenty-four patients had been treated at dose level -1, including 12 patients that were treated more than 12 months prior. Three of these 12 subjects demonstrated tumor reduction without significant

inflammation necessitating prolonged use of bevacizumab and/or additional chemotherapy (as of 5/31/2017, the 3 patients remained alive at 31+, 27+, and 24+ months after PVSRIPO infusion). In contrast, the remaining 9 patients had neurologic or radiographic signs suggestive of either a localized inflammatory reaction to the immune response expected from PVSRIPO or tumor regrowth. These neurologic/radiographic signs of inflammation were considered burdensome and required prolonged use of bevacizumab and/or additional chemotherapy. Seven of the 9 patients with burdensome localized inflammation, most probably due to tumor regrowth, had died, including 5 that died within 12 months of PVSRIPO treatment. Despite the fact that subjects on dose level -1 had been able to remain off significant doses of steroids, we believed that the subjects benefiting the most from PVSRIPO had been those who had experienced minimal inflammation.

As such, on 6/16/2016, we reduced the dose of PVSRIPO to dose level -2 (1.0×10^7 TCID50) to evaluate if the lower dose would limit the occurrence of undesirable burden from the localized inflammation and its treatment on as many subjects (including caregivers) as possible. Based upon additional animal studies, dose level -2 (1.0×10^7 TCID50) is considered to be a therapeutic dose. This dose change was not made due to concerns for the safety of the patients on dose level -1, but due to the hypothesis that dose level -2 would cause less localized inflammatory reaction to the virus inoculum and result in better survival and treatment response. By 2/15/2017, 15 patients had been treated at dose level -2, one of which experienced significant localized intracranial edema that was found to be related to progressive disease upon autopsy (death caused by grade 5 seizure).

After this, an analysis of patients treated on dose level -1 and dose level -2 was completed. Based upon both pre-clinical tests highlighting the role of virus dose in PVSRIPO:host relations and clinical data collected on subjects who were treated with either dose level -1 or dose level -2, it was decided that the phase 2 dose would be dose level -1 (5×10^7 TCID50). It was determined that there was not sufficient pre-clinical or clinical evidence of differences between the 2 dose levels, and most importantly no added benefit attributable to the reduced dose level -2. Additionally, a larger number of patients treated on dose level -1 and with longer follow-up provided additional data to support the decision to pursue the phase 2 study at dose level -1 (5×10^7 TCID50). The need for initial treatment of localized cerebral inflammation either secondary to PVSRIPO or due to tumor growth, and the toxicity profile for the two dose levels, is comparable.

7.3 Study Purpose/Rationale

Outcome for patients with recurrent WHO grade IV malignant glioma is dismal with currently available therapeutic modalities. The blood-brain barrier is a major impediment to therapy and approaches that bypass this barrier may be required. Oncolytic immunotherapy is a promising biologic approach to treatment that not only induces viral mediated tumor destruction, but also harnesses a complex immune response that can serve in disease control. The genetically engineered oncolytic PV (PVSRIPO) has

tropism for tumor cells by virtue of expression of CD155 in tumor cells. To facilitate concentration of the therapeutic agent at the tumor site, while minimizing systemic exposure, PVSRIPO will be delivered directly into the tumor. Significant pre-clinical antitumor activity of PVSRIPO has been observed in several rodent tumor models and in vitro. PVSRIPO was devoid of neuropathogenicity when injected into the thalami of NHPs even at doses as high as 5×10^9 TCID50. Also, the phase 1 trial of PVSRIPO in adults with recurrent WHO grade IV malignant glioma did not yield evidence of viral encephalomyelitis, poliomyelitis or meningitis with a proportion of patients achieving prolonged disease survival up to 92+ months. The main toxicity attributable to PVSRIPO has been post-treatment peri-tumoral inflammation that has required prolonged steroid therapy and/or low-dose bevacizumab to control edema.

8 OBJECTIVES AND ENDPOINTS

	Objective	Endpoint	Analysis
Primary	Assess the anti-tumor response and duration of response among adults with recurrent WHO grade IV malignant glioma treated with PVSRIPO	ORR: defined as the proportion of patients achieving Complete Response (CR) or Partial Response (PR) based on iRANO criteria DOR: defined as time from CR or PR until confirmed Progressive Disease (PD)	See Section 14.5
Secondary	Assess survival outcomes of adults with recurrent WHO grade IV malignant glioma treated with PVSRIPO relative to a criteria matched external (historical) control group	Landmark Survival: the proportion of patients alive starting ≥ 36 months after 1st PVSRIPO infusion OS: defined as time from PVSRIPO infusion to death from any cause, or last follow-up if patient is alive	See section 14.6
Secondary	Assess the safety of PVSRIPO	The proportion of patients with grade 3, 4, or 5 treatment-related adverse events	See Section 14.6
Secondary	Assess the disease control rate following PVSRIPO infusion	Disease Control Rate (DCR): defined as the proportion of patients deemed non-progressive based on imaging criteria described in the Central Imaging Charter	See Section 14.6
Exploratory	Describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO	Description of imaging changes	See Section 14.7
Exploratory	Assess immunologic responses in peripheral blood and in serum	Change from baseline in immune function	See Section 14.7
Exploratory	Identify genetic predictors of response or failure of response (including in buffy coat) to treatment with PVSRIPO	Identification of genetic markers as predictors of response	See Section 14.7
Exploratory	To obtain the quantitative gut microbiome profile via the OMNIgene®•GUT (OMR-200) kit to determine any potential influence in response to PVSRIPO	Identification of quantitative gut microbiome profile as predictors of response	See Section 14.7
Exploratory	As part of a smaller sub-study only at DUMC, to explore inflammation-associated biomarkers, immune cell phenotype and	Inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence	See Section 14.7

	innate and adaptive immune competence pre- and post-PV boost (prior to infusion) and post-PVSRIPO infusion		
Exploratory	Assess anti-tumor responses and outcomes following PVSRIPO retreatment(s) or treatment with any other non-protocol specified therapy	Description of the proportion of patients who achieve CR, PR, minor response (MR), SD (along with associated durations) following PVSRIPO retreatment	See Section 14.7
Exploratory	Assess anti-tumor responses and outcomes considering site-level experience with PVSRIPO treatment	Description of patient outcomes following PVSRIPO infusion in centers based on the number of prior patients infused	See Section 14.7

9 INVESTIGATIONAL PLAN

9.1 Study Design

In this multicenter, phase 2 study, up to approximately 122 patients will receive PVSRIPO via CED for treatment of recurrent WHO grade IV malignant glioma. Patients ≥ 12 months out from PVSRIPO administration may be eligible for PVSRIPO retreatment upon confirmation of recurrence or progression. Criteria for retreatment eligibility are provided in Section 9.2.1. Prior to protocol version 7, patients enrolled were randomized to receive PVSRIPO alone or PVSRIPO followed by a single dose of lomustine at week 8 post-infusion, which was randomized by center.

9.1.1 Catheter Implantation

Systemic delivery of high molecular weight therapeutic agents to brain tumors is limited by the blood-brain barrier and increased interstitial pressure within the tumor. PV CNS invasion after intravenous (IV) administration via trans-endothelial passage is inefficient[32]. 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 (as required) prior to virus administration for frozen section confirmation of viable tumor and further analysis (Section 9.1.4). The biopsy needle should be placed with stereotactic guidance by a Cosman-Robert-Wells, Magnetic Resonance Imaging (MRI)-compatible, stereotactic head frame or a similar frameless device. Collection of biopsy tissue for clinical pathologic diagnosis will be performed under traditionally accepted conditions according to standard of care at each institution. Up to three additional core biopsies may be obtained (if possible) for molecular genetic testing as described in Section 9.1.4.

In the case of initial PVSRIPO infusion, if the biopsy for clinical pathologic diagnosis does not confirm recurrent tumor, the subject will be withdrawn from the study. Subjects in screening for retreatment whose biopsy does not confirm recurrence will not be retreated with PVSRIPO, but will remain on study in follow-up.

The biopsy needle and catheter will be placed by the operating surgeon using an FDA cleared stereotaxic guidance system guided by a pre-operative MRI. After biopsy, a catheter [Vygon PIC-030 (Sophysa, Inc.; Crown Point, IN)] will be implanted in the operating room using sterile techniques, which may occur under general anesthesia, at the same site or a different site from that used for the biopsy. The catheter will be implanted within, or as close to, an enhancing region of tumor as possible ≥ 1 cm away from the ventricles. Based on prior experiences, a tumor ≤ 1cm from the ventricles can safely and feasibly have a catheter tip placed ≥ 1cm from the ventricles while minimizing the possibility of infusate entering the ventricles. The catheter will be tunneled beneath the scalp for a distance of at least 5 cm to aid in the prevention of infection. A computed tomography (CT) scan will be used to confirm catheter placement post-operatively. Anesthesia administration is discontinued once there is CT confirmation of the catheter placement, as applicable.

Patients who are taking (and are stable on) less than or equal to 4 mg per day of dexamethasone (ie, low-dose corticosteroid) within the 2 weeks prior to admission for any PVSRIPO infusion (ie, patients who meet the relevant eligibility criterion) may continue on this dose. The administration of high-dose corticosteroids will be limited while on study due to concerns that high-dose corticosteroids might negatively impact the efficacy of PVSRIPO. However, high-dose corticosteroids may be administered on a limited basis to patients prior to, during, or after PVSRIPO infusion only when determined to be medically necessary.

9.1.2 PVSRIPO Infusion

The entire volume of the agent to be delivered will be pre-loaded into a syringe and connected to the catheter under sterile conditions in the appropriate clinical care unit (eg neurointensive care unit/intensive care unit 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 into the study for the study drug infusion. This means that the infusion is allowed to start the following day after the biopsy/catheter placement. This will still be considered “day 0” in regard to the protocol and the timing of the subsequent events. At the time of virus injection, emergency drugs, including epinephrine and diphenhydramine will be available and the neurologic status, oxygen saturation, and cardiac rhythm will be monitored. Drug infusion will occur in the appropriate clinical care unit so that all other emergency facilities will be available. Patients will be treated with a prophylactic antibiotic prior to biopsy and catheter insertion per the institution’s neurosurgical standard practice.

Based on Duke's experience, previously published reports[33], and institutional review board (IRB) and FDA-approved trials using similar infusion techniques, patients will be infused at a rate of 0.5 mL/hr. A Medfusion™ 3500 infusion pump (Smiths Medical, Minneapolis, MN) or any other comparable FDA-cleared syringe infusion pump approved by the Sponsor or their designee, will be pre-programmed to a delivery rate of 0.5 mL/hr. The infusate will be loaded in an infusion syringe into the syringe pump at the initial onset to avoid any interruptions in the infusion. The total amount of the infusate delivered to the patient will be 3 mL. The catheter itself (30 cm length, 1 mm interior diameter) cannot be pre-loaded with virus suspension. Therefore, the infusion pump will be programmed for delivery of 3.25 mL (0.25 mL to account for catheter "dead space" with 3.0 mL delivered to the patient). The infusion pump will be programmed for delivery of 0.5 mL/hr over approximately 6.5 hours (stopped when delivered amount is between 3.125 and 3.250 mL). The catheter will be removed in the appropriate clinical care unit. No sedation is required for catheter removal. A non-contrasted CT scan of the brain will be used after catheter removal to confirm there is no bleed post catheter removal.

The infusion catheter (PIC 030) and infusion tubing (PIT 400) are manufactured by Sophysa, Inc. (Crown Point, IN) and supplied to the sites according to the Study Administration Manual (SAM). 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 (see the infusion procedures in the study procedure manual).

Acute Reaction. Any reaction symptoms determined to be an acute reaction to the study drug will be managed by the appropriate clinical care unit.

9.1.3 Gadolinium Distribution Quantitation

Prior to protocol version 8, a non-contrasted MRI imaging, within 4 hours of the completion of infusion, was registered in an attempt to define the shape and position of the contrast agent distribution co-administered with PVSRIPO relative to the patient's brain anatomy. While Gd-DTPA is a widely available MRI contrast agent, there has been speculation that its small molecular weight (938 Dalton) could limit its ability to predict the distribution of the larger molecules typically infused therapeutically with CED [reviewed in Reardon et al., 2011][34]. 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[35]. 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-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[35]. 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[36]. However, as of July 17, 2019, Magnevist®, which was added to the PVSRIPO infusate for tracking purposes, will no longer be manufactured nor be readily available in the US. This change was not related to product quality, safety, or efficacy (see: FDA website for more information). Therefore, for patients enrolled after 10OCT2019, addition of Magnevist® to the infusate was not required and the corresponding post-infusion MRI to occur within 4 hour post-infusion in an effort to track infusate was also removed.

9.1.4 Biopsy Sampling and Analyses

9.1.4.1 Study-Related Biopsy

Prior to catheter placement and PVSRIPO infusion, a biopsy will be performed. The biopsy for the first PVSRIPO infusion will consist of taking multiple samples as described below.

1. A tumor tissue sample will be collected for review via routine histology to confirm tumor recurrence by the site neuropathologist(s) for confirmation of eligibility. For the first PVSRIPO infusion only, the study's central neuropathologist at Duke, Dr. R. McLendon or his designee, will also provide a determination of histology upon receipt of tissue, but this is not necessary for confirmation of eligibility. This will require the sites to ship one H&E stained slide from the first PVSRIPO infusion biopsy to DUMC (see pathology section of the SAM for additional information). Additional unstained slides from this biopsy and others for subsequent PVSRIPO infusion(s) may also be required, depending on the routine pathology/biomarker tests that the site performs.

   Additional pathology/biomarker tests that may be performed include, but are not limited to, the following:

   - Anti-PV receptor
   - EGFRvIII and wild-type EGFR status
   - O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation and MGMT immunohistochemistry
   - Isocitrate dehydrogenase 1 and 2
   - Telomerase reverse transcriptase (TERT)
   - Programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1)

   If any of these tests are routinely performed at the study site per standard of care practice, these tests will not be repeated at DUMC. In these instances, the sites will

enter their local pathology results into the electronic case report form (eCRF). For specific tests outlined in the pathology manual that are not run at the sites, unstained slides will be shipped to DUMC so that these tests can be conducted. Please see the pathology manual for the number and type of slides needed for each test. If a site would prefer to send a tissue block instead of unstained slides, that is permitted. If there is not enough tissue to perform all of these tests, the tests will be run in order of priority per the pathology manual.

2. After acquiring sufficient tissue for standard clinical pathologic testing (#1), up to three additional core biopsies will be obtained, if possible. These additional core biopsies will be frozen in optimal cutting temperature compound and kept at liquid nitrogen temperature. They will be used for genetic analysis, including full genome or full exome sequencing, as well as other molecular genetic testing. The goal of these molecular genetic tests is to identify genetic predictors of response or failure of response to treatment with PVSRIPO. The molecular genetic testing will include, but is not limited to, DNA sequencing, gene amplification, and gene expression. Please see the pathology section of the SAM for information on core biopsy processing and shipping.

9.1.4.2 Archival Tissue

Tissue samples from archival tissue, including initial surgical procedure where WHO grade IV malignant glioma was confirmed and any additional surgical procedures for recurrence prior to enrolling in the PVSRIPO clinical trial, will also be collected. These tissue samples may be tested for, but not limited to, the pathology/biomarkers listed in Section 9.1.4.1. Additionally, 10 unstained slides will be requested from each previous surgical procedure/biopsy for genetic analysis.

If any of the pathology/biomarker tests mentioned in Section 9.1.4.1 are routinely performed at the study site per standard of care practice and the information is available for the applicable archival tissue, these tests will not be repeated at DUMC. In these instances, the sites will enter their local pathology results into the eCRF. For specific tests outlined in the pathology manual that are not run at the sites, unstained slides will be shipped to Duke so that these tests can be conducted. Please see the pathology section of the SAM for the number and type of slides needed for each test. If a site would prefer to send a tissue block instead of unstained slides, that is permitted. If there is not enough tissue to perform all of these tests, the tests will be run in order of priority per the pathology section of the SAM.

9.1.4.3 Future Surgical Procedures

Administration of PVSRIPO is associated with an inflammatory immune response that can result in a pseudoprogressive tumor appearance on radiographic imaging. Following PVSRIPO administration, patients should not undergo tumor resection based on radiographic tumor progression, without prior consultation with the study-designated

neurooncologist(s) and/or ISC except in cases of eminent life-threatening emergency. Should subjects have a resection or biopsy after coming off study, each site will be responsible for requesting samples of this resected/biopsied tissue, which will then be sent to DUMC for pathology/biomarker tissue analysis. If a subject consents, portions of resected/biopsied tissue will be delivered to the DUMC study neuropathologist, Dr. R. McLendon or his designate, for histopathological analyses and to Dr. M. Gromeier or his designate for correlative molecular analyses. The histopathological analyses may include, but are not limited to, the same pathology/biomarker tests listed in Section 9.1.4.1. The correlative molecular analyses will require 10 additional unstained slides. The histopathological and correlative molecular analyses will only occur if a sufficient amount of tissue remains after standard clinical pathologic testing.

If any of the pathology/biomarker tests mentioned in Section 9.1.4.1 are routinely performed at the study site per standard of care practice for the samples collected from future surgical procedures, these tests will not be repeated at DUMC. In these instances, the sites will enter their local pathology results into the eCRF. For specific tests outlined in the pathology manual that are not run at the sites, unstained slides will be shipped to DUMC so that these tests can be conducted. Please see the pathology section of the SAM for the number and type of slides needed for each test. If a site would prefer to send a tissue block instead of unstained slides, that is permitted. If there is not enough tissue to perform all of these tests, the tests will be run in order of priority per the pathology section of the SAM.

In addition, in the event of subject death during or after participation in this study, permission from the subject and next-of-kin to obtain the subject's brain and conduct a post-mortem examination will be requested.

9.1.5 Lomustine (PVSRIPO + Lomustine Arm Only)

Prior to implementation of protocol version 7, for patients randomized to the PVSRIPO with lomustine arm, lomustine was dispensed as an oral therapy at 110 mg/m^2 one time only 8 weeks after PVSRIPO infusion. If a subject was on bevacizumab (had been treated with bevacizumab in the time since PVSRIPO infusion), the lomustine dose was dispensed as an oral therapy at 90 mg/m^2 one time only 8 weeks after PVSRIPO infusion. The dose of lomustine was calculated by the subject's weight at the week 8 study visit. Lomustine was self-administered by the subject. The study team asked the subject for the exact date they took the lomustine and dosage amount taken for documentation.

9.1.6 Safety Considerations

Lomustine safety: Although not applicable after protocol version 7, the major toxicity of lomustine is delayed bone marrow suppression, so blood counts were monitored at a frequency determined by the treating physician. Given that lomustine was administered only once and pulmonary toxicity with lomustine is a cumulative toxicity, a pulmonary function test in the PVSRIPO + lomustine arm was not be required. Long-term use of nitrosoureas has been reported to be possibly associated with the development of secondary malignancies, which should be prevented by the use of a single cycle of

lomustine. Liver and renal function tests were to be monitored periodically, per standard of care practices.

Surgical Complications: The stereotactic tumor biopsies and catheter implantation procedure carries a risk for cerebrospinal fluid leak, infection, hemorrhage, loss of neurologic function, non-neurologic complications and death. These risks depend primarily on the preoperative condition of the patient, the size and location of the tumor and associated diseases. In addition, as is standard with surgical procedures, potential complications for stopping anti-coagulation therapy prior to surgery may include heart attack, stroke or other life-threatening blood clots. The potential risk for the patient will be discussed in detail with the patient and family by the treating neurosurgeon or the neurosurgeon's designee.

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

Poliomyelitis: PVSRIPO has been tested in 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 is a replication-competent viral agent that, in principle, retains the potential to cause motor neuron damage. Possible complications include transient or permanent mono- or paraparesis, paralysis, and life-threatening respiratory insufficiency.

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

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

Gastrointestinal Infection and Virus Excretion: After oral uptake, PV replicates in the gastrointestinal tract and is excreted by infected individuals with stool. Gastrointestinal

viral replication usually is asymptomatic but may cause mild symptoms of gastrointestinal discomfort. Excretion of PVSRIPO with stool does not occur after intracerebral inoculation in immunized/boosted patients. This is based on thorough testing in 61 patients in the first-in-human phase 1 trial and much historical evidence from investigations in NHPs.

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, which may manifest as neurologic dysfunction. Symptoms of neurologic dysfunction due to cerebral edema may include, but are not limited to, seizures, 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 antiangiogenic agent (according to Special Considerations below), 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, preoperative neurological impairment, and post-operative course prior to virus administration. The risks will be discussed with the subject and the subject's family. Guidelines for reporting serious adverse events (SAEs) related to neurologic dysfunction due to cerebral edema are details in Section 12.8.4.

Special Consideration: In the event a patient demonstrates neurologic or radiographic signs suggestive of a localized inflammatory reaction secondary to the immune response triggered by PVSRIPO that requires a dose of dexamethasone above 4 mg a day after the first 4 weeks post-PVSRIPO infusion, every effort should be made not to increase the dose of dexamethasone further. Instead, patients should be treated with bevacizumab 7.5 mg/kg IV every 3 weeks. If a subject requires planned treatment with bevacizumab > 7.5 mg/kg every 3 weeks, they will be considered off study and enter the follow-up phase. Neuroimaging (MRI) will be performed according to protocol schedule, and at that time, it will be assessed whether further treatment with bevacizumab is needed to control the cerebral inflammation. It is recommended that bevacizumab treatment start no earlier than 4 weeks after PVSRIPO infusion, in consideration of bleeding/wound healing in keeping with the package insert, but is at the investigator's/treating center's discretion/standard procedures following biopsy. Bevacizumab will not be provided by the study. Every attempt should be made to discontinue dexamethasone.

In initial phase 1 and 2 clinical trials, four potential bevacizumab-associated adverse events (AEs) were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed phase 2 and phase 3 studies of bevacizumab, as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated AEs identified in phase 3 trials include congestive heart failure primarily in metastatic breast cancer, gastrointestinal perforations, wound-healing complications, and arterial thromboembolic events. These and other safety signals are described in further detail in the bevacizumab package insert.

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

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

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

Risks of MRIs: Risks and/or discomforts associated with MRI scans include anxiety produced from being in a tight, enclosed space (claustrophobia). In addition, the machine operates using a large and powerful magnet, which attracts certain metals. Therefore, people with these metals in their bodies (specifically pacemakers, infusion pumps, metal aneurysm clips, metal prostheses, joints, rods or plates) will be excluded from the study. Patients will also be checked to make sure that they do not bring any metal objects into the MRI facility. Dental fillings are less affected by the magnetic fields generated and are therefore permitted. Patients will be asked to let the physicians conducting this study know of any metal in their bodies other than dental fillings.

Allergic Reactions to Contrast Agents: For MRI with contrast (considered standard of care for follow-up of rGBM patients), a contrast agent is administered through the vein, which 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 current or history of anaphylactic reactions to gadolinium-based contrast agents will be excluded from the study. Patients with less severe allergies (ie, manageable with prophylaxis) should be pretreated with Tylenol® and Benadryl® prior to injection of the contrast agent, per the treating clinician. Prior to protocol version 8, in addition to the gadolinium (contrast agent) being given for MRI contrast, it was also co-infused with the study drug to assess infusion distribution. As noted previously, production of the contrast agent was discontinued by the manufacturer for non-safety reasons and will no longer be included in the infusate. This approach to gadolinium administration was not FDA approved. The potential risks of intracerebral infusion of gadolinium contrast agents were not completely known but were believed to be small. Risks for infusion of gadolinium contrast agents intrathecally for procedures such as cisternography have been somewhat better studied, and were recently summarized by Selcuk et al (2009)[37]. 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[36,38]. When these contrast agents are used in an appropriately low dose, however, the risk of intrathecal administration appears reasonably low. No neurological sequelae attributable to the procedure were detected in a series of 85 patients[37], in another series of 95 patients at a one year follow-up[39], or in any of 51 patients after over 4 years of mean follow-up in another study[40]. No complication from the intracerebral infusion of gadolinium contrast agents was observed in the 61 patients treated in the phase 1 PVSRIPO clinical trial or this study, to date. Although these results cannot be extrapolated to the procedure proposed in the previous version of this protocol, they indicate that direct exposure of the brain to small amounts of gadolinium contrast agent was 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. In addition, given new information of possible retention of gadolinium-based contrast agents, only MRIs absolutely necessary for the proper management of the subject's condition will be obtained.

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

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

9.1.7 Concomitant Medications

9.1.7.1 Steroids

Corticosteroids should be used at the lowest dose required to control symptoms of edema and mass effect, and discontinued, when/if possible. Use of corticosteroids should be recorded in the eCRF. Every effort should be made to keep the dose of steroids at or lower than an equivalent of 4 mg daily of dexamethasone.

9.1.7.2 Anticonvulsants

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

9.1.7.3 Growth Factors

Routine use of growth factors (ie, granulocyte-colony-stimulating factor [G-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF], and erythropoietin) is not permitted. However, therapeutic use of G-CSF in patients with serious neutropenic conditions, such as sepsis, may be used at the discretion of the treating physician. Use of such growth factors should be recorded in the eCRF.

9.1.7.4 Anti-emetics

The use of anti-emetics will be at the discretion of the treating physician. Use of anti-emetics should be recorded in the eCRF.

9.1.7.5 Proton Pump Inhibitors

The use of proton pump inhibitors (eg, rabeprazole, omeprazole, pantoprazole, lansoprazole or esomeprazole) is allowed on this study.

9.1.7.6 Febrile Neutropenia

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

9.1.7.7 *Pneumocystis jiroveci* Pneumonia (PJP) Prophylaxis

The use of medication (ie, Bactrim®) for PJP prophylaxis in patients on chronic steroids is recommended but is at the discretion of the treating physician.

9.1.7.8 Neurosurgical Procedures

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

9.1.8 Randomization

With the amendment under protocol version 7 removing the lomustine arm, there is no randomization; approximately 122 patients will receive PVSRIPO. Prior to protocol version 7, for the initial patients enrolled, randomization was 1:1 to PVSRIPO or PVSRIPO plus a single dose of lomustine. Randomization, which was stratified by center, was performed through a permuted block algorithm.

9.2 PVSRIPO Retreatment

Additional infusions of PVSRIPO are allowed (ie, retreatment) upon progression or recurrence of tumor in patients who are ≥ 12 months out from the initial and subsequent PVSRIPO infusions. In order to qualify, the retreatment eligibility criteria listed in Section 9.2.1 must be met. Details about that retreatment plan are provided in section 9.2.2 and in the Retreatment Schedule of Study Tests and Procedures (Table 2). All cases for consideration of PVSRIPO retreatment are to be discussed with the study designated neurooncologist/ISC for approval prior to proceeding.

9.2.1 Retreatment Eligibility Criteria

To be eligible for PVSRIPO retreatment(s), patients must continue to satisfy all general IC and EC, including the following IC/EC adaptations/additions:

1. Adaptation to IC 1: Histologically confirmed (≤ 3 months of planned treatment) measurable (≥ 1 cm and ≤ 5.5 cm of contrast-enhancing) supratentorial primary brain tumor that has recurred/progressed ≥ 12 months after initial PVSRIPO infusion
2. Adaptation to IC 12: New boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) ≥ 1 week, but ≤ 6 months prior to PVSRIPO retreatment
3. Addition: No treatment-limiting signs and symptoms (eg, cerebral edema) from prior PVSRIPO administration, such that PVSRIPO retreatment should be well tolerated in the opinion of the investigator/neurosurgeon

9.2.2 PVSRIPO Retreatment Plan

Catheter implantation (Section 9.1.1), PVSRIPO infusion (Section 9.1.2), and biopsy sampling (Section 9.1.4) will occur according to the procedures previously described in this protocol, unless otherwise noted. Rationale for PVSRIPO dose selection is given in Section 9.3. All retreated patients will follow the Schedule of Study Tests and Procedure for PVSRIPO Retreatment (Table 2). No blood for immunologic analysis or stool samples will be collected after retreatment and the PV boost must occur ≥ 1 week from plan re-treatment but within 6 months. Other changes are noted in the retreatment schedule of tests and procedures (see Table 2).

9.3 Rationale for Selection of Dose, Regimen, and Treatment Duration

The dose for PVSRIPO treatment and retreatment (if applicable) was selected based on IND-directed toxicity studies[31] and on experience from the phase 1 study in adults with recurrent WHO grade IV malignant glioma. Dose-range finding and toxicology studies in NHP documented the absence of viral encephalomyelitis, poliomyelitis and meningitis with intracerebral injection of PVSRIPO up to a dose of 5×10^9 TCID50[31]. In the adult WHO grade IV malignant glioma study, 4 patients were dosed at the maximum intended dose of 10^{10} TCID50 without signs of viral encephalomyelitis, poliomyelitis or meningitis. The intended dose of 5×10^7 TCID50 corresponds to the dose selected for future studies during the phase 1 adult WHO grade IV malignant glioma study. The most significant adverse response observed in that study was localized intracranial inflammation resulting from the immune response and/or tumor growth.

There is empirical evidence for beneficial effects of lymphodepletion with DNA-damaging chemotherapy, eg, lomustine or temozolomide, in conjunction with immunotherapy regimens. Lymphodepletion is believed to generate an 'immunologic reset' by broadly eliminating suppressive T-cell subsets and favoring preferential expansion of T-cell populations with pro-inflammatory activation phenotypes during immune recovery. While the immunologic benefits of lymphodepletive chemotherapy is well documented, it is equally well known that standard cycle-therapy (eg, with one year of lomustine) produces long-term immune suppression by abrogating the expansive phase of immune recovery following the initial cycle of lomustine. Furthermore, information stemming from patients treated with lomustine post-PVSRIPO on the first-in-human phase 1 study suggests significant clinical and radiographic responses occurring after the first cycle, with no evidence for further benefit with additional cycles of lomustine thereafter. Immune monitoring of patients at the nadir (4 weeks) after lomustine revealed the following: 1) a contraction of a specific, high-impact subset of (immunosuppressive) T-regulatory cells; and 2) beginning expansion of effector T-cells. Any such beneficial effect is likely to be lost upon standard multicycle chemotherapy, as subsequent lymphodepletions will eventually dampen the immune system's capacity to target the tumor. As such, it is was hypothesized that the combination of one cycle of chemotherapy (lomustine) with PVSRIPO could improve patient's survival. However, as noted during an initial review of the first 50 patients treated on study on November 14, 2018 (~50% treated with lomustine at week 8) did not note any indicators of this

potential advantage. Furthermore, a marked increase in hematological toxicities was noted and randomization to a single dose of lomustine is discontinued under protocol version 7.

9.4 Rationale for Correlative Immune Function Studies

While tumor-selective PVSRIPO propagation is an important mediator of cytotoxicity, significant intra- and peritumoral inflammation likely ensues. This raises the possibility that an immunogenic response is being generated, certainly against the virus itself and with great likelihood against the tumor as well (**Figure 2**)[22]. While the exact host immune response to PVSRIPO oncolysis is currently unknown (but is being investigated), host innate antiviral defenses are likely to trigger a broad immune effector cascade that needs to be examined in patients receiving PVSRIPO therapy. Blood will therefore be collected for immune function sub-studies before and at periodic intervals following treatment with PVSRIPO.

9.5 Definition of Evaluable Subjects and On-Study Subjects

Evaluable patients for efficacy analyses will be all who initiates a PVSRIPO infusion. Any patient who has undergone biopsy and catheter placement will be evaluable for toxicity.

Patients will be considered on study for as long as they do not initiate a cancer therapeutic intervention other than the ones included on this trial, that is, PVSRIPO, bevacizumab at 7.5 mg/kg IV every 3 weeks as described in 'special considerations' in Section 9.1.6, and a single dose of lomustine for patients randomized to the PVSRIPO + lomustine arm, prior to protocol version 7. For example, patients will come off study if, after consult with the study-designated neurooncologist(s) or ISC, the treating physician feels it is necessary to modify the dosing or interval of bevacizumab or if any chemotherapy, small molecule inhibitor, radiation therapy, immunotherapy, hospice care, etc. is initiated at the recommendation of the treating physician. Errors in bevacizumab dosing administration will not be grounds for removing a subject from the study; these occurrences will be reviewed by the Sponsor and discussed with the site Principal Investigator. Patients will continue to be followed per protocol (Section 12.3).

9.6 Early Study Termination

This study can be terminated at any time for any reason by the Sponsor. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow-up should occur in accordance with Section 12.6, which describes procedures and process for prematurely withdrawn patients.

10 STUDY DRUG

10.1 PVSRIPO

10.1.1 Names, Classification, and Mechanism of Action

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

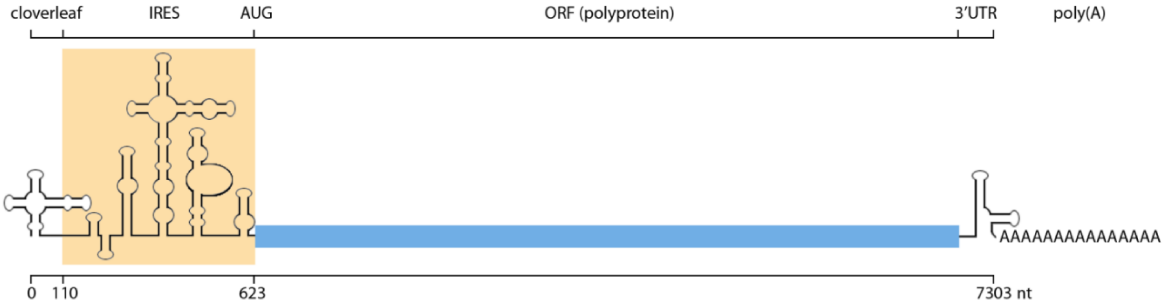


Figure 3. Genetic structure of PVSRIPO

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

10.1.2 Packaging and Labeling

PVSRIPO is formulated in 50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% HSA. It is provided in sterile, single use containers. PVSRIPO utilized in this study was manufactured at the Biopharmaceutical Development Program/SAIC-Frederick at National Cancer Institute (NCI)-Frederick.

10.1.3 Supply, Receipt, and Storage

The study agent and vehicle will be supplied directly to the site by the Sponsor or their designee. The study agent will be shipped via approved methods in the appropriate packaging on dry ice.

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. Details on the supply, receipt, and storage of PVSRIPO will be outlined in the SAM.

10.1.4 Dispensing and Preparation

For aliquot preparation, the agent will be thawed slowly on ice (4°C) and kept at that temperature. Thawed vials of PVSRIPO are stable at 4°C for 48 hours. PVSRIPO contained in the clinically intended delivery apparatus (ie, ready for infusion) is stable at room temperature for 18 hours but should be used as soon as possible. The manufacturer will provide the study agent's potency (as TCIDs) and will also supply the appropriate vehicle for aliquot preparation. Aliquot preparation will occur in each institution's designated investigational pharmacy preparation center. 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, eg syringes, vials, etc., will be disposed of as biological waste. The final desired aliquot of the study agent infusate will be prepared at the intended volume, which is sufficient for priming the infusion tubing and for infusion. The capped infusion syringe containing infusate (and primed infusion tubing if primed in pharmacy) will be transported to the study site in a 'ziplock' bag placed in a portable cooler or similarly contained transport device with a frozen ice block/ice pack, in order to maintain a temperature of approximately 4°C. There is no need to monitor the temperature during transport to the bedside.

Details on dispensing and preparing study agent will be outlined in the Investigational Product–handling portion of the SAM.

10.1.5 Compliance and Accountability

Drug accountability records will be maintained for all clinical trial supplies. All empty and partially used clinical trial supplies will be destroyed in accordance with institutional guidelines. Each site's designated investigational pharmacy will maintain detailed documentation of the receipt and/or destruction of the study agent, which will then be provided to the Sponsor or designee. 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.

10.1.6 Disposal and Destruction

All surgical materials used in the procedure and (potentially) coming in contact with the study agent will be disposable. These materials will be disposed of as biological waste

using established procedures. Used sharps will be disposed in biohazard sharps container and incinerated for final disposal.

10.2 Study Agent: Lomustine (applicable to patients enrolled under Protocol Version 6 or earlier)

10.2.1 Lomustine Overview

Lomustine (Gleostine®, CCNU [chloroethyl-cyclohexyl-nitrosourea]), is an oral nitrosourea alkylating agent. This class of drugs works by inhibiting DNA replication, RNA transcription, and nucleic acid function[41]. The drug is believed to inhibit DNA replication, RNA transcription, and nucleic acid function and it also may modify cellular proteins[41]. Specifically, lomustine possesses a chloroethyl group that alkylate nucleic acids and cell proteins and form DNA-DNA or DNA-protein crosslinks, which lead to the global effects of impaired nucleic acid functioning[41]. Lomustine is approved for treatment of primary brain tumors in patients who have already received appropriate surgical and/or radiotherapeutic procedures (see Gleostine® label).

10.2.2 Lomustine Packaging and Labeling

Lomustine capsules are available in individual bottles of 5 capsules each and are supplied in either 100 mg, 40 mg or 10 mg capsules, with each strength being distinguishable by the color of the capsules[41]. The total dose prescribed by the physician can be obtained (to within 10 mg) by determining the appropriate combination of the enclosed capsule strengths[41]. For patients randomized to receive lomustine prior to protocol version 7, the appropriate number of capsules of each size should were placed in a single vial to which the patient information label explaining the differences in the appearance of the capsules is affixed[41].

10.2.3 Lomustine Supply, Receipt, and Storage

Prior to protocol version 7, lomustine was supplied to the site by the Sponsor or their designee. Lomustine as supplied in capsules of 10 mg, 40 mg, and 100 mg strengths. Capsules were stable for the lot life indicated on package labeling when stored at room temperature in well closed containers. Avoid excessive heat (over 40°C, 104°F). Lomustine is a cytotoxic drug. Follow applicable special handling and disposal procedures.

10.2.4 Lomustine Dispensing and Preparation

For subjects randomized to the PVSRIPO + lomustine arm prior to protocol version 7, lomustine was dispensed as an oral therapy within 10mg of but not to exceed 110 mg/m^2 once, 8 weeks after PVSRIPO infusion. If a subject was on bevacizumab (had

been treated with bevacizumab in the time since PVSRIPO infusion), the lomustine dose was dispensed as an oral therapy within 10 mg of but not to exceed 90 mg/m^2 once, 8 weeks after PVSRIPO infusion. The dose of lomustine was calculated by the weight at the week 8 study visit. Lomustine was self-administered by the patient. The study team asked the subject for the date they took the lomustine and dosage amount taken.

Lomustine should have been taken with fluids on an empty stomach with no food or drink for 2 hours afterwards in order to decrease incidence of nausea. Premedication with anti-emetics were given at the discretion of the treating physician. Patients were told to avoid exposure to broken capsules and to wear disposable gloves when handling the capsules.

10.2.5 Lomustine Disposal and Destruction

If subjects did not take the dose that they are dispensed, they were instructed to return the unused amount to the clinical site for accountability and destruction.

11 SUBJECT ELIGIBILITY

11.1 Inclusion Criteria

Prior to submission of screening MRI for central review and patient for eligibly approval, sites should confirm the following:

1. Patients must have a recurrent (first or second recurrence only, including this recurrence; transformation from a lower grade tumor to a WHO grade IV malignant glioma will be considered a first recurrence) supratentorial WHO grade IV malignant glioma based on imaging studies with measurable disease (a minimum measurement of 1 cm and maximum of 5.5 cm of contrast-enhancing tumor) with prior histopathology consistent with a WHO grade IV malignant glioma confirmed by the site's neuropathologist or the neuropathologist's designate.
    a. Assuming patient meets all other criteria, site neurosurgeon must confirm placement of infusion catheter tip can occur within or as close as possible to an enhancing region of tumor that is ≥ 1cm from ventricles and at a safe distance relative to eloquent brain function.
    b. Tumor size and location requirements per protocol must be confirmed as qualifying and safe to proceed by the reviewer(s) designated by the Sponsor.
2. If the subject is male and sexually active, he is eligible to enter and participate in this study if his partner(s) meets the criteria outlined in 2a or if he or his partner(s) is using one of the methods of birth control outlined in 2b. If the subject is female, she is eligible to enter and participate in this study if she meets the following criteria:
    a. Non-childbearing potential (ie, physiologically incapable of becoming pregnant, including any female who is postmenopausal or surgically sterile).

Surgically sterile females are defined as those with a documented hysterectomy and/or bilateral oophorectomy or tubal ligation. Postmenopausal for purposes of this study, is defined as 1 year without menses); or

   b. Childbearing potential, has a negative serum pregnancy test at screening, and agrees to use one of the following methods of birth control: approved hormonal contraceptives (eg, birth control pills, patches, implants, or infusions), an intrauterine device, or a barrier method of contraception (eg, a condom or diaphragm) used with spermicide.
   c. If the male has had a vasectomy or is using a condom with spermicide, the female partner does not need to use additional birth control noted in 2a and 2b.

3. Age ≥ 18 years of age at the time of entry into the study.
4. Karnofsky Performance Status (KPS) Score ≥ 70%.
5. Prothrombin and Partial Thromboplastin Times ≤ 1.2 x normal prior to biopsy.
6. Total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase ≤ 2.5 x normal prior to biopsy.
7. Neutrophil count ≥ 1000 prior to biopsy.
8. Hemoglobin ≥ 9 prior to biopsy.
9. Platelet count ≥ 100,000/μL unsupported is necessary for eligibility on study; however, because of risks of intracranial hemorrhage with catheter placement, platelet count ≥ 125,000/μL is required for the patient to undergo biopsy and catheter insertion, which can be attained with the help of platelet transfusion.
10. Creatinine ≤ 1.2 x normal range prior to biopsy.
11. Positive serum anti-PV titer prior to biopsy.
12. The patient must have received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week, but less than 6 weeks, prior to administration of the study agent.
13. At the time of biopsy, prior to administration of virus, the presence of recurrent tumor must be confirmed by histopathological analysis.
14. A signed informed consent form (ICF) approved by the IRB will be required for patient enrollment into the study. Patients or their legally authorized representative (LAR) 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.
15. Able to undergo brain MRI with and without contrast.

11.2 Exclusion Criteria

1. Females who are pregnant or breast-feeding.
2. Patients with an impending, life-threatening cerebral herniation syndrome, based on the assessment of the study neurosurgeons, their designate, and the reviewer designated by the sponsor.
3. Patients with severe, active co-morbidity, defined as follow:
   a. Patients with an active infection requiring intravenous treatment or having an unexplained febrile illness (Tmax > 99.5°F/37.5°C)

   b. Patients with known immunosuppressive disease or known human immunodeficiency virus infection
   c. Patients with unstable or severe intercurrent medical conditions such as severe heart disease (New York Heart Association Class 3 or 4)
   d. Patients with known lung (forced expiratory volume in the first second of expiration [FEV1] < 50%) disease or uncontrolled diabetes mellitus
   e. Patients with albumin allergy
   f. Patients with existing or history of anaphylactic reaction to gadolinium
4. Patients with a previous history of neurological complications due to PV infection.
5. Patients who have not recovered from the toxic effects of prior chemo- and/or radiation therapy. Guidelines for this recovery period are dependent upon the specific therapeutic agent being used.
6. Patients may not have received tumor treating fields (≤ 1 week), chemotherapy or bevacizumab ≤ 4 weeks [except for nitrosourea and lomustine (≤ 6 weeks); metronomic dosed chemotherapy, such as daily temozolomide, etoposide or cyclophosphamide (≤ 1 week)] prior to starting the study drug.
7. Patients may not have received immunotherapy ≤ 4 weeks prior to starting the study drug unless patients have recovered from side effects of such therapy.
8. Patients may not be less than 12 weeks from radiation therapy of the brain, unless progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation.
9. Prior to enrollment, has not completed all standard of care treatments, including surgical procedure and radiation therapy (at least 59Gy)
   a. 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
   b. If the MGMT promoter in their tumor is known to be methylated or the MGMT promoter methylation status is unknown at time of screening, patients must have received at least one chemotherapy regimen prior to participating in this trial
   c. If enrolling at 2nd recurrence, must have failed treatments initiated at 1st recurrence
10. Patients with neoplastic lesions in the brainstem, cerebellum, or spinal cord; radiological evidence of multiple areas of active (growing) disease (active multifocal disease); tumors with contrast-enhancing tumor component crossing the midline (crossing the corpus callosum); extensive subependymal disease (tumor touching subependymal space is allowed); or extensive leptomeningeal disease (tumor touching leptomeninges is allowed).
11. Patients with undetectable anti-tetanus toxoid immunoglobulin G (IgG).
12. Patients with known history of agammaglobulinemia.
13. Patients on greater than 4 mg per day of dexamethasone within the 2 weeks prior to admission for PVSRIPO infusion.
14. Patients with worsening steroid myopathy (history of gradual progression of bilateral proximal muscle weakness, and atrophy of proximal muscle groups).

15. 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.
16. For patients randomized prior to V7, a known history of hypersensitivity to lomustine, dacarbazine, or any components of lomustine.
17. Patients with active autoimmune disease requiring systemic immunomodulatory treatment within the past 3 months.

Notes:
For exclusion criterion 9, with regard to prior treatments, “completed standard of care treatments” at diagnosis means maximal safe resection, radiotherapy as specified and concomitant temozolomide. Discuss with study designated neuro-oncologist if there are any questions regarding duration of treatment. If the patient is being considered at 2nd recurrence, he/she must have failed any treatments initiated at 1st recurrence within the timeframe specified for a given agent.

For exclusion criterion 10, with regard to “active multifocal disease”, a lesion would be considered inactive if there was no size increase >0.5cm in any direction on 2 consecutive MRI at least 3 months apart. With regard to defining “extensive” subependymal or leptomeningeal disease, as noted, tumor touching these spaces is allowed, but multiple lesions within these spaces or lesions covering more than 50% of these spaces are not.

For clarification of what constitutes an unrelated malignancy requiring current active treatment with regard to exclusion criterion #15, if a patient is treated for an unrelated malignancy other than the exceptions noted within the past 3 years, a letter from their treating oncologist for the unrelated malignancy must be on file confirming that said unrelated malignancy does not require current active treatment (prophylactic therapy like tamoxifen is permitted) and that the patient is stable with low risk of recurrence/death within 3 years from this other malignancy (ie, disease is stable). If this letter is not on file, a consult with the Sponsor’s medical designee is required prior to submitting the patient for consideration of enrollment in the trial.

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

12.1 Screening Examination

The screening examination will take place within 2 weeks before catheter placement. An informed consent must be signed by the patient or their LAR before any screening procedure takes place.

Up to 6 weeks before receiving the initial PVSRIPO infusion, the patient must have the following:

- A serum test for lymphocyte subset quantitation (LSQ) and anti-tetanus toxoid IgG
- Whole blood drawn (10-20 mL) for serum collection for determination of PV titer (the results of this screening PV titer must be known prior to the PVSRIPO infusion), whole blood (~4-6 ml) for isolation of buffy coat and other immunologic analysis (~76.5 mL)
    - **NOTE:** Blood draw for LSQ quantitation, PV titer and immune cell analysis will not be collected prior to any PVSRIPO retreatment(s)
- As part of a smaller sub-study only at DUMC, subjects at this center may have an additional 6 ml of blood drawn prior to PVSRIPO infusion to explore inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence.
    - **NOTE:** Blood draw for participants in the DUMC sub-study will not be collected prior to PVSRIPO retreatment(s)
- For newly enrolled subjects who have not received PVSRIPO under a previous version of the protocol, a stool sample will be obtained via the OMNIgene (OMNIgene®•GUT (OMR-200)) stool sample kit
    - The complete collection kit will be dispensed to the subject for the sample to be obtained prior to the subject receiving the PVSRIPO infusion.
    - The subject may return the pre-paid postage kit directly to the laboratory conducting the analyses. All instructions will be provided.
    - **NOTE:** Stool samples are not collected prior to PVSRIPO retreatment(s)

Following this blood draw, the patient will receive a booster of inactivated PV vaccine (≥ 1 week but ≤ 6 weeks of the initial PVSRIPO infusion; ≥ 1 week but ≤ 6 months of any PVSRIPO retreatments).

The screening examination will take place within 14 days before catheter placement, but as close to biopsy as possible. Whenever possible, when confirming eligibility based on the study inclusion/exclusion criteria, the least invasive screening procedures (eg, KPS) should be performed first and the most invasive last.

Pre-treatment evaluations within 14 days before catheter placement, but as close to biopsy as possible, to determine eligibility will include the following, unless otherwise indicated:

- Clinical assessment (including medical history) and physical examination, including a neurologic assessment and KPS within 14 days of catheter placement
- Laboratory Evaluations:
    - Complete blood count (CBC) with differential
    - Comprehensive metabolic panel (CMP)
    - Prothrombin time (PT) and activated partial thromboplastin time (aPTT)
    - Beta-human chorionic gonadotropin (HCG), if appropriate (within 14 days of catheter placement and 48 hours of PVSRIPO infusion)
    - Whole blood for immunologic analysis (~ 96.5 mL)

        - **NOTE:** Whole blood for immunologic analysis will not be collected prior to PVSRIPO retreatment(s)
- As part of a smaller sub-study only at DUMC, subjects at this center may have an additional 6 ml of blood drawn prior to PVSRIPO infusion to explore inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence.
    - **NOTE:** Participants in the DUMC sub-study will not have additional blood collected prior to PVSRIPO retreatment(s)
- Baseline MRI of the brain with and without gadolinium contrast within 14 days of starting treatment, but as close to biopsy as possible. The Sponsor designee will review the MRI to confirm eligibility for the first PVSRIPO infusion and retreatment(s) with PVSRIPO.

If a subject does not receive the initial PVSRIPO infusion, minimal records regarding the subject and the reason for screen failure will be documented in the eCRF. If a subject is found to be eligible based on the evaluations above, they will be enrolled. At the time of biopsy (if required), prior to each administration of virus, the presence of recurrent tumor must be confirmed by histopathological analysis. Subjects in screening for initial PVSRIPO infusion that do not have recurrent tumor will be ineligible and will not receive PVSRIPO; these subjects will be excluded from all efficacy and post-infusion safety analyses and are considered screen failures. Subjects must meet the criteria outlined in Section 9.2.1 in order to be eligible for retreatment with PVSRIPO.

12.2 Treatment/Retreatment Period

Day 0 (Infusion Period)

- Biopsy (if required) and catheter placement
    - *For initial PVSRIPO Infusion*: After obtaining tissue for standard clinical pathologic testing and confirmation of recurrence, up to three additional core biopsies may be obtained (if possible) for molecular genetic tests (Section 9.1.4)
    - *For PVSRIPO Retreatment*: Tissue for tumor typing and clinical pathologic testing per SOC should be obtained and results reported on the eCRF when available (Note: results from any other biopsy/surgical resection for the duration of a patient's follow-up should be recorded in eCRF)
- CT of the brain to confirm catheter placement ≥ 1 cm from the ventricles prior to beginning infusion
- PVSRIPO infusion (infusion may be same day or a plus 1-day window)

- Day 0 (after the infusion)CT of the brain (without contrast) after catheter removal to confirm there is no bleed post catheter removal

12.3 Follow-up Post-PVSRIPO Treatment/Retreatment

Day 1 post-infusion

- Clinical assessment and physical examination, including a neurologic assessment and KPS, to be performed **daily** until discharged from the hospital (must be documented within 24h (± 4h) of actual discharge
- CBC with differential
- CMP
- 3 ml of blood to explore inflammation-associated biomarkers/immunologic analysis (**NOTE**: collection on Day 1 or Day 2 post-infusion acceptable; Participants retreated with PVSRIPO will not have blood drawn for biomarker analyses)

Week 1 (+/- 3 days)

- Clinical assessment and physical examination, including a neurologic assessment and KPS
- CBC with differential
- CMP
- Participants in the DUMC sub-study should have a 6ml blood draw to explore inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence
    - **NOTE:** Participants in DUMC sub-study retreated with PVSRIPO will not have additional blood drawn for biomarker analyses.

Week 2 (+/- 1 week)

- Clinical assessment and physical examination, including a neurologic assessment and KPS
- CBC with differential
- CMP
- Whole blood (96.5 mL) for immunologic analysis
    - **NOTE:** Participants retreated with PVSRIPO will not have blood drawn for biomarker analyses

Week 4 (+/- 1 week)

- Clinical assessment and physical examination, including a neurologic assessment and KPS
- MRI of the brain with gadolinium contrast
- CBC with differential
- CMP

Week 8 (+/- 1 week)

- Clinical assessment and physical examination, including a neurologic assessment and KPS
- MRI of the brain with gadolinium contrast
- CBC with differential
- CMP
- Whole blood (96.5 mL) for immunologic analysis

    - **NOTE:** Participants retreated with PVSRIPO will not have blood drawn for biomarker analyses provided they are > 8 weeks out from initial PVSRIPO infusion
- Beta-HCG, if appropriate and if randomized to the lomustine arm prior to protocol version 7
    - **NOTE:** Participants randomized to the lomustine arm that are retreated with PVSRIPO are not required to repeat the pregnancy test at 8 weeks.
- Prior to protocol version 7, patients randomized to the lomustine cohort received a single oral dose (while on lomustine, additional CBCs and CMPs were obtained at a frequency determined by the treating physician). The lomustine dose may have been taken the day of the week 8 visit or 1 day after the visit.
- A stool sample will be obtained via the OMNIgene®•GUT (OMR-200) kit. NOTE: for patients who already received PVSRIPO under a previous version of the protocol and for whom the Week 8 visit has passed, they will be provided with the OMNIgene®•GUT stool collection kit for their post-infusion stool sample at their next clinic visit.
    - **NOTE:** Participants retreated with PVSRIPO will not have stool collected for biomarker analyses provided they are > 8 weeks out from initial PVSRIPO infusion

After protocol version 7, the Week 12 (+/- 1 week) visit and all protocol associated assessments associated with this visit outlined below have been removed and are not required, as they were primarily associated for comparisons between PVSRIPO with or without lomustine use. This includes:

- Clinical assessment and physical examination, including a neurologic assessment and KPS
- CBC with differential
- CMP

For patients randomized to the lomustine arm prior to protocol version 7, this visit was 4 weeks post lomustine dose (+/-1 week).

Weeks 16, 25, 34, 43, 52 (+/- 1 week)

- Clinical assessment and physical examination, including a neurologic assessment and KPS
- MRI of the brain with gadolinium contrast
- CBC with differential
- CMP

After week 52, patients will follow-up at the discretion of the treating physician, receiving periodic MRIs, but will still be considered on study.

Data obtained at each visit/phone call should be entered in the eCRF approximately every 1 to 2 months, in keeping with the patient's follow-up until the patient is off study,

deceased, or no longer willing to participate. Data to be entered in the eCRF by the PI or their designee will include, but are not limited to, the following:

- Patient status of deceased or alive
  - Alive status to include need for treatment with other modalities (eg dosing or interval of bevacizumab is modified, chemotherapy, small molecule inhibitor, radiation therapy, immunotherapy, hospice care, surgery) and details of these treatments
- MRIs to be conducted per SOC
  - All SOC MRIs obtained must be sent to the sponsor-designated central imaging center for assessment of tumor size, volume, and other characteristics at least every 6 months
- Corticosteroid use (dose/frequency/route) since last 6-month time point
- Neurosurgical procedures for reasons other than required for tumor progression (ie, for managing hydrocephalus) since last visit

While patients are on study, their malignant glioma may not be treated with any modality for cancer other than PVSRIPO or a single dose of lomustine (for those randomized to the lomustine arm prior to protocol version 7), or reduced dose of bevacizumab described in 'special considerations' in Section 9.1.6. Patients will be considered progressive and off study upon initiation of treatment of the tumor with another modality or initiation of hospice care (see Section 9.5).

12.4 Follow-Up for Off-Study Patients

When subjects are considered off study, they will no longer be obligated to undergo study-related tests and procedures. However, these subjects may enter the voluntary off-study follow-up period, and the data described above, as for those after week 52 and in the sections that follow, will still be collected from these subjects, as feasible, until death or loss to follow-up. Subjects will be followed for serious adverse event (SAE) for 30 days after coming off study. The information above and following additional data from off-study subjects will be collected and reported, if possible, but is not mandatory and will not be considered a deviation if the data cannot be obtained. Subjects' medical records will be reviewed for the remainder of their life, in order to collect data on the following:

- Patient status, including survival
  - Status for those alive will include information pertaining to progression and treatment with other modalities (eg, dosing or interval of bevacizumab is modified, chemotherapy, small molecule inhibitor, radiation therapy, immunotherapy, hospice care, surgery) and details of these treatments
- MRIs to be conducted per SOC
  - All SOC MRIs obtained should be sent to the sponsor-designated central imaging center for assessment of tumor size, volume, and other characteristics at least every 6 months

- Corticosteroid use (dose/frequency/route) since last 6-month time point
- Neurosurgical procedures for reasons other than required for tumor progression (ie, for managing hydrocephalus) since last visit

In addition, in the event of subject death during or after participation in this study, permission from the subject and next-of-kin to obtain the subject's brain and conduct a post-mortem examination will be requested.

Patients who have completed the required follow-up, may be considered for a separate, but related, long-term rollover study. If they qualify for the rollover study, all subsequent follow-up will occur under that protocol. PVSRIPO retreatment will be allowed in patients who meet the retreatment eligibility criteria. Patients will continue to contribute to the safety and efficacy calculations under this study, which will be analyzed together (cumulative safety and efficacy since initial PVSRIPO infusion) and separately as a retreatment cohort (safety and efficacy as of the date of retreatment).

12.5 End of Study

The study will be considered complete once enrollment has been met, follow-up procedures outlined in Section 12.3 have been conducted on all subjects, and data analysis is concluded. The study may also be terminated early for any reason by the Sponsor.

Subjects that are lost to follow-up will be documented in the patient record and in the 21 CFR Part 11 database. In the compliant database, the subject will be marked as "Patient Lost to Follow-up."

12.6 Early Withdrawal of Subject(s)

12.6.1 Criteria for Early Withdrawal

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

- Progressive disease as documented by MRI, physical examination, or tissue sample at any time after the completion of therapy requiring treatment with non-protocol therapy
- Pregnancy
- Upon request of the subject
- If, in the investigator's medical judgment, further participation would be injurious to the subject's health or wellbeing
- Development of intolerable symptoms
- Protocol deviation
- Non-compliance of the subject

- Administrative issues

12.6.2 Follow-up Requirements for Early Withdrawal

Subjects should be seen in clinic or contacted at a minimum of every 3 months until death, if possible, in an effort to obtain the follow-up data as noted in Section 12.4.

12.6.3 Replacement of Early Withdrawal(s)

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

12.7 Study Assessments

12.7.1 Clinical Assessment

Standard clinical assessment, including medical history and baseline symptoms, will be obtained and documented per institutional guidelines.

12.7.2 Physical Examination

Standard physical examination, including KPS and neurological examination will be conducted and documented per institutional guidelines.

12.7.3 Radiographic Review

A screening MRI, which includes a standard MRI with all Brainlab® or similar images, will be done within 14 days of infusion but as close to biopsy as possible. A CT will be obtained and reviewed by the neurosurgeon to confirm catheter placement prior to beginning the PVSRIPO infusion. Starting at week 4, an MRI of the brain with gadolinium enhancement will be obtained at all subsequent visits, except for the week 12 visit (Note: week 12 visit occurred prior to protocol v7).

Given that imaging following PVSRIPO differs greatly from what is typically seen following chemoradiation treatment, treatment with antiangiogenic compounds or even immunotherapy-like vaccines or checkpoint inhibitors, determination of progression using Macdonald criteria, Response Assessment in Neuro-Oncology (RANO) criteria, or immunotherapy RANO (iRANO) criteria are not appropriate in this trial. PVSRIPO can trigger an inflammatory immune response that is observed on imaging, which can make distinguishing between the inflammatory immune response and progressive disease

difficult. Therefore, an exploratory objective of this study is to describe radiographic imaging post-PVSRIPO treatment. In the phase 1 PVSRIPO clinical trial, an initial increase in the extent of FLAIR abnormalities could be observed after PVSRIPO, followed by a decrease in the extent of FLAIR abnormalities. In addition, an initial increase in size of the enhancing lesion with multiple cystic degeneration was observed ("swiss cheese" or "honeycomb" appearance), as well as an extension of enhancing disease toward previously non-enhancing infiltrative disease, followed by a tumor retraction. Depending on the size of the tumor, the changes could be observed up to 12 months post-PVSRIPO infusion. The qualitative description of the changes observed in this trial will help to better characterize imaging following PVSRIPO infusion and refine imaging assessment criteria specific to PVSRIPO.

Imaging based responses will be noted by the assessing clinician as well as independent central imaging reviewers, blinded to the patient outcome, in keeping with an imaging charter to be finalized prior to image assessment.

In addition, routine teleconferences with participating investigators at each site will be conducted by the Sponsor or designee in order to discuss radiographic issues, as well as enrollment, subject updates, and study conduct. Except in case of eminent life-threatening emergency, consult with the study-designated neurooncologist(s)/ISC prior to administration of non-protocol-specified treatments and/or surgical resection, if radiographic progression is suspected.

For lesion measurement, the axial post contrast T1 slice where the enhancing lesion is at the largest on the baseline screening MRI (pre-infusion MRI), should be identified as the "target lesion." Please note that as per RANO criteria for determination of target lesion, the enhancing lesion should measure at least 1 x 1 cm and resection cavity or cystic area should not be included in the baseline tumor volume measurement. Over 7 years of experience with PVSRIPO have demonstrated that on follow-up imaging, an initial change in configuration of the target lesion is expected, often with increase in size of the lesion; this is due to the process of intratumoral administration itself and the immunotherapy effect of PVSRIPO, which often triggers microcystic degeneration of the previously densely enhancing area, as well as an extension of an enhancing immune reaction into previously non-enhancing infiltrative disease. Such changes will not be described as progressive disease, but as "immunoprogression" (a term coined for PVSRIPO, to distinguish from pseudoprogression, which is often reserved to imaging changes occurring after radiation therapy). This is often followed months later by a tumor retraction.

Complete response and partial response of the treated lesion will be defined using the same criteria as in iRANO. However, in the case a patient is initiated on protocol specified dosage of bevacizumab for management of symptoms of immunoprogression and imaging demonstrates a complete or partial response of the target lesion while on bevacizumab, this response must be maintained for at least 8 weeks after coming off low dose bevacizumab to be considered a treatment related radiographic response.

It is to note that with time, a number of patients develop a cystic/necrotic area with a thin rim of residual enhancement the same size (replacing the target lesion) or sometimes slightly larger than the previously densely enhancing target lesion, this should be labeled as stable disease.

Given the radiographic changes post PVSRIPO making it impossible to properly identify true disease progression based solely on imaging criteria, per protocol, patients should *not* be taken off study for progressive disease based solely on imaging criteria. As per protocol, patients can come off study due to progression only once it has been shown that they are not improving clinically with protocol allowed dose of bevacizumab only and therapy in addition to low dose bevacizumab must be added.

12.7.4 Laboratory Evaluations

The timing of laboratory assessments that will be obtained during the course of the study is given above in Table 1. A list of each evaluation is below.

- CBC with differential
- CMP
- PT, aPTT
- Beta-HCG, if applicable, within 48 hours prior to receiving the PVSRIPO infusion and for patients who received lomustine prior to protocol version 7, prior to the cycle of lomustine (lomustine arm)
- Immunologic/Genetic/Gut Microbiome testing: may include tests noted in Section 14.7.

12.7.5 Correlative Assessments

- Anti-tetanus toxoid IgG titer within 6 weeks prior to treatment will be obtained by each institution and recorded in the eCRF.
- The screening eligibility anti-PV titer results will be sent back to the sites for entry into their eCRF by the sponsor-designated laboratory.
- LSQ (**NOTE:** Not required prior to PVSRIPO retreatment)
- Anti-tetanus toxoid IgG
- Prior to initial PVSRIPO infusion, a 96.5 mL sample of whole blood for immunologic assays and PV titer (at screening only) will be collected according to Section 12.3. From these samples, immune signatures may be identified using patient peripheral blood mononuclear cells to 1) track longitudinally with clinical outcomes in PVSRIPO-treated patients to determine the precise mechanism responsible for the therapeutic effects, and 2) predict which patients will derive the greatest benefit from PVSRIPO therapy based on their baseline immune profiles.
- At screening for initial PVSRIPO infusion only, an additional 4-6 ml of whole blood will be obtained for isolation and storage of the buffy coat for future molecular

genetic to determine the genetic characteristics of these samples according to Section 12.

- As part of a smaller sub-study only for patients enrolled at DUMC, 6ml of blood will be obtained to explore inflammation-associated biomarkers, immune cell phenotype and innate and adaptive immune competence pre-PV booster, post-PV booster and 1 week after their initial PVSRIPO infusion only.
- For newly enrolled patients prior to receiving initial PVSRIPO infusion only, at screening and again post-PVSRIPO infusion (Week 8), a kit will be provided for collection of a stool sample via the OMNIgene®•GUT (OMR-200) kit to obtain the quantitative gut microbiome profile to determine any potential influence in response to PVSRIPO or PVSRIPO/lomustine (for patients treated prior to protocol version 7).

Note: For patients who received PVSRIPO under a previous version of the protocol, no baseline/pre-PVSRIPO infusion stool sample will be obtained, and patients should be dispensed the stool collection at their Week 8 visit. If these patients have already passed the Week 8 visit, they will be given the OMNIgene kit at their next clinic visit for provision of their post-PVSRIPO infusion sample.

Results from these research samples may not be immediately analyzed. However, if results become available during the course of the study or thereafter, they may be shared with the investigator in consideration of future patient management.

Tumor tissue will be stored and analyses may be performed by qualified site personnel, or tumor tissue will be sent to the Department of Pathology at DUMC. Please refer to Section 9.1.4 and pathology section of the SAM for details about the pathology/biomarker correlative assessment analysis.

12.8 Adverse Events

An AE is any untoward medical occurrence in a subject receiving study drug(s) and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any preexisting medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with catheter placement or the use of PVSRIPO, lomustine (for patients randomized to this arm prior to protocol version 7), bevacizumab, or corticosteroids whether or not related to use of any of these drugs. The institutional PI is responsible for the identification and documentation of AEs and serious AEs (SAEs), as defined below. At each study visit, the institutional PI or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

Events that do not meet the definition of an AE include:

- Medical or surgical procedures (eg, endoscopy, appendectomy). The condition that leads to the procedure is, however, an AE.

- Situations where an untoward medical occurrence did not occur (eg, social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of preexisting diseases or conditions present or detected at the start of the study that do not worsen.

Adverse events related to the non-standard of care screening procedures (eg, polio vaccination booster) and biopsy/catheter placement procedure will be reported (non-treatment emergent adverse events). Treatment emergent adverse events will be noted from the time of infusion through the time the subject comes off study (as defined in Section 9.5), all AEs must be recorded in the subject's medical record and AEs case report form. Subjects will be followed for SAEs for 30 days after coming off study.

AEs will be assessed according to the CTCAE (Common Terminology Criteria for Adverse Events) version 4.03. If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:

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

Attribution of AEs will be determined for each study intervention individually (catheter placement, PVSRIPO, lomustine (only for patients randomized to this arm prior to protocol version 7), bevacizumab, and corticosteroids).

12.8.1 AEs of Special Interest

See section 9.1.6 for management of anticipated AEs.

12.8.2 Reporting of AEs

Any AE occurrence (spontaneously volunteered and enquired for, as well as observed AEs) during the study must be documented in the patient's medical records in accordance with the investigator's normal clinical practice and on the AE page of the eCRF. SAEs that occur during the study must be documented in the patient's medical record, on the AE eCRF, and on the SAE form.

The investigator should attempt to establish a diagnosis of the event based on the signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE (and SAE if serious) and not the individual signs/symptoms.

If an abnormal laboratory finding or other abnormal assessment meets the definition of an AE, then the AE eCRF page must be completed as appropriate. In addition, if the abnormal assessment meets the criteria for being serious, the SAE form must also be completed. A diagnosis, if known, or clinical signs or symptoms if the diagnosis is unknown, rather than the clinically significant laboratory finding or abnormal assessment, should be used to complete the AE/SAE page. If no diagnosis is known and clinical signs or symptoms are not present, then the abnormal finding should be recorded. Only laboratory results considered clinically significant by the clinical investigator that are not part of or supportive of a diagnosis should be reported as an AE. If an SAE report is completed, pertinent laboratory data should be recorded on the SAE form, preferably with baseline values and copies of laboratory reports.

Reporting of any SAEs to applicable regulatory authorities will be the responsibility of the Sponsor or designee in compliance with local regulations. The SAE page should be completed as thoroughly as possible and signed by the investigator before transmittal to the assigned safety representative. It is very important that the investigator provide an assessment of the causal relationship between the event and the study drug(s) at the time of the initial report, as this will be useful for submissions to regulatory authorities.

A database of all AEs (not just those considered related to the study drug(s)) will be maintained in 21 CFR Part 11 compliant database. The event will be categorized by organ system, relationship to treatment, its grade of severity, and resolution. The Sponsor/designee, along with an independent DSMB, will periodically review the collective AEs 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.

12.8.3 Serious Adverse Events (SAEs)

An AE is considered “serious” if it is one of the following outcomes:

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

12.8.4 Reporting of SAEs

SAEs must be reported by the PI to the Sponsor or designee within 24 hours of discovery while patients are on study and for 30 days after patients are taken off study.

The PI is required to provide an assessment of the causal relationship between the event and the study drug(s) at the time of the initial report. The related SAE page should be completed as thoroughly as possible and signed by the PI before transmittal to the Sponsor or designee. The PI should not wait to for additional information to fully document the related SAE before notification, although additional information may be requested.

Instances of death not due to disease progression, congenital abnormality, or an event that is of such clinical concern as to influence the overall safety assessment, if brought to the attention of the PI at any time after cessation of study drug(s) and linked by the PI to this study, should be reported to the Sponsor or their designee. Where applicable, information from relevant laboratory results, hospital case records, and autopsy reports should be obtained.

Contact information for processing SAEs can be found in the study administration manual (SAM).

The Sponsor is responsible for appropriate reporting of SAEs to the necessary regulatory bodies where appropriate. The Sponsor will report to the Investigators all suspected unexpected serious adverse reactions (SUSARs) associated with PVSRIPO. The Investigator (or Sponsor where required) must report any SUSARs to the IRB according to the Institution's regulations.

REPORTING OF SERIOUS ADVERSE EVENTS RELATED TO CEREBRAL EDEMA

- Events related to cerebral edema that meet criteria for a serious adverse event (SAE) should be reported as described in the protocol and study procedures (ie, within 24h of learning of the event). Investigators who have concerns about the protocol-specified medical management of cerebral edema, or the determination of possible progressive disease, should contact the study designated neuro-oncologist to discuss the case as noted.
- When reporting the SAE, describe the following in the narrative description of the event:
    - Specific signs and symptoms of edema, such as seizures, headache, confusion, lethargy, unresponsiveness, coma, or focal neurologic deficits (eg, pyramidal tract syndrome, hemiparesis)
- In addition, any specific signs or symptoms considered to be out of proportion for the extent of edema may be reported as additional SAEs, if indicated.
- The narrative description should include the following:
    - Time to onset following PVSRIPO infusion
    - Maximum severity of signs and symptoms
    - Treatment(s) undertaken
    - Time to resolution as the information becomes available

12.8.5 Toxicity Monitoring

Patients will be monitored for toxicity while on study. AEs will be categorized and graded in accordance with the NCI CTCAE (Version 4.03). Unacceptable AEs, as defined in Section 12.8.6, will be identified during this period of toxicity monitoring.

12.8.6 Definition of Unacceptable Adverse Event (ie, Adverse Events of Special Interest)

An unacceptable AE (ie, adverse event of special interest; AESI) is any grade 3 or grade 4 toxicity considered related (possibly, probably or definitely) to a protocol treatment (includes surgical biopsy/infusion procedure, PVSRIPO, lomustine (for patients randomized to this arm prior to protocol version 7), bevacizumab, corticosteroids) that is not reversible (ie, does not improve to ≤ grade 2 with exception of neurologic events, which must only improve from highest grade level to next lower grade level) within 2 weeks. This includes any treatment-related life-threatening event or any treatment-related death. Any grade 2 or higher serious autoimmune toxicities—particularly those affecting vital organs (eg, cardiac, renal, CNS)—will also be considered unacceptable AEs if they occur within 2 weeks of any protocol treatment, whether the AE requires intervention.

Exceptions to unacceptable AEs noted above are as follows, with or without supportive treatment:

- Events associated with the biopsy procedure/catheter placement: seizures or hemorrhages occurring during anesthesia or the biopsy/catheter insertion prior to administration of the agent if grade 2 or lower (grade 3 or higher surgical complication from insertion of catheter is considered an unacceptable AE).
- Seizures: Due to the nature of the disease under investigation in this protocol, patients may have preexisting 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 Common Terminology Criteria (CTC), and will be reported as such in this protocol, seizures will not be considered an unacceptable AE if, in the opinion of the institutional PI, they have not increased in frequency or can be attributed to another recognized cause of increasing seizure frequency such as subtherapeutic anticonvulsant 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 or inflammation. A new neurologic deficit or worsening of a known neurologic deficit, which resolves (ie, returns to baseline) within 2 weeks after initiation of medical therapy (eg, corticosteroids or bevacizumab at 7.5 mg/kg IV every 3 weeks) will not be considered an unacceptable AE.
- Thromboembolism: Due to the high incidence of deep vein thrombosis (DVT) in this patient population, patients may have undiagnosed preexisting DVTs or be susceptible to the development of DVTs due to the underlying disease process. Although DVT may be defined as a grade 3 or 4 toxicity under NCI CTC, and will

be reported as such in this protocol, DVT will not be considered an unacceptable AE in this protocol.

- Syndrome of inappropriate antidiuretic hormone (SIADH): Due to the high incidence of SIADH in this patient population, patients may be susceptible to the development of SIADH due to the underlying disease process. Although SIADH may be defined as grade 3 toxicity under NCI CTC, and will be reported as such in this protocol, SIADH will not be considered an unacceptable AE in this protocol unless it is refractory to medical management.
- Muscle weakness and weight gain: Due to the high incidence of generalized muscle weakness (ie, steroid myopathy) and weight gain in patients taking steroids in this patient population, patients may be susceptible to the development of generalized muscle weakness or weight gain, which is due to steroids alone. Although generalized 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, generalized muscle weakness or weight gain will not be considered an unacceptable AE in this protocol if the patient has required steroids greater than the 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 preexisting neurologic deficits or have an onset of new neurologic deficits due to tumor progression. Although such neurologic deficits may be defined as unacceptable AEs 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 an unacceptable AE if unequivocal tumor progression can be documented radiographically or histologically.

12.8.6.1 Guidelines for Monitoring Unacceptable Adverse Events

Given that both long- and short-term toxicities are of interest in this study, it is not feasible to suspend accrual while toxicity is assessed, as is often done in phase 1 trials. If the following criteria are satisfied or there are other reasons for concern about the safety of patient treatment (eg, treatment-related toxic death; concerns are raised by the Data and Safety Monitoring Board [DSMB] or the Sponsor or their designee), 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.

Prior to protocol v7, each treatment group will be monitored independently of the other by the DSMB. However, if there are reasons for accrual suspension in either arm, accrual to both arms will be suspended pending a comprehensive review of patient experiences.

Tabulated below are the conditions under which accrual will be temporarily suspended and data carefully reviewed to determine the appropriate action, including permanent

study termination, continuation with patient accrual after appropriate amendment, or continuation with patient accrual with no modification of the protocol. Accrual will also be suspended whenever a death occurs that is possibly, probably, or definitely related to protocol treatment.

Table 3. Accrual of Unacceptable Adverse Events

Number of Patients Accrued Within a Group	Number of Patients with Unacceptable Adverse Events Within Group Requiring Accrual Suspension
2-5	> 2
6-8	> 3
9-12	> 4
13-17	> 5
18-25	> 6
> 25	> 7

These guidelines have not been adjusted for differential length of follow-up of accrued patients. The probability of accrual suspension as a function of the true unacceptable toxicity rate is tabulated below based on simulation studies. These statistics were generated assuming toxicity outcome was known at the time of accrual and ignored issues such as time to toxicity, accrual rate, and length of follow-up.

Table 4. Probability of Unacceptable Adverse Events

Underlying Unacceptable Adverse Event Rate	Probability of Accrual Suspension	Expected Number of Accrued Patients
0.05	0.0002	30.9
0.10	0.0027	30.5
0.15	0.12	29.3
0.20	0.33	26.6
0.25	0.58	23.0
0.30	0.80	18.8
0.35	0.92	15.0
0.40	0.97	12.3

In addition to the Sponsor, their designee, and the medical monitor, the FDA and/or the DSMB for this study may be part of this decision-making in the event that there are unacceptable toxicities or inflammatory issues.

12.8.7 Notification of Pregnancy

Because all patients are required to have a boost immunization of trivalent inactivated IPOL™, there should be no risk of transmission of a mother to her fetus after receiving

intracranial PVSRIPO. As such, patients who become pregnant after receiving PVSRIPO will continue to be monitored in the same manner, that is, per protocol, unless the assessment is contraindicated during pregnancy. Partners who become pregnant should be asked to sign a Pregnant Partner Information Form, and information regarding the pregnancy and its outcome may be collected.

All pregnancies in female patients and female partners of male patients receiving study drug will be recorded from dosing until 6 months after the patient has completed therapy with PVSRIPO, lomustine (for patients randomized to this arm prior to protocol version 7), bevacizumab, or corticosteroids.

Should a patient or male patient's partner become pregnant or suspect she is pregnant while participating in this study, or in the 6 months following when the patient receives the last dose of therapy (PVSRIPO, lomustine (for patients randomized to this arm prior to protocol version 7), bevacizumab or corticosteroids), the treating investigator should be informed immediately. All pregnancies will be reported on a pregnancy report form and submitted to the CRO along the same timelines as an SAE.

The IRB and the Sponsor will also be informed of the pregnancy. The patient will be asked to provide information on the outcome of the pregnancy, including premature termination should the case arise. For pregnancies in a female partner of a male patient, consent to follow-up on the pregnancy will be obtained in agreement with the United States (US) Health Insurance Portability and Accountability Act (HIPAA) and other local laws and regulations. Pregnancy outcomes will be reported on a pregnancy follow-up report form.

Spontaneous miscarriage and congenital abnormalities will be reported as SAEs. The follow-up period for all pregnancies will be deemed to have ended when the health status of the child has been determined on its birth and followed up for 8 weeks after the birth for any potential abnormalities. Full details will be recorded on the pregnancy follow-up report form.

12.9 External Data and Safety Monitoring Board (DSMB)

In an effort to ensure external oversight, Duke established an external DSMB for the phase 1 clinical trial performed at DUMC. Istari licensed PVSRIPO from Duke and the current study is monitored by this DSMB as well. The external DSMB will be responsible for safeguarding the interests of trial subjects and assessing the safety of the interventions at Duke during the trial. The DSMB will provide recommendations about stopping or continuing enrollment in the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection, recruitment, and retention of subjects and their management. Additional details regarding the responsibility of the DSMB and its chair may be found in the charter document.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Monitoring

The study will be monitored by the Sponsor or its designee to ensure subject safety and to ensure that the study is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice (GCP), and applicable regulatory requirements. In addition, in accordance with GCP and International Council of Harmonization (ICH) guidelines, the study monitors will carry out source document verification at regular intervals to ensure that the data collected in the eCRFs are accurate and reliable. The frequency of such visits will depend on the enrollment rate and other factors, such as the incidence of certain AEs and findings during previous monitoring visits, and will be outlined in the study monitoring plan.

The investigator must permit the Sponsor, their designee (including the appointed monitor), the IRB, the Sponsor's internal auditors, and representatives from regulatory authorities and government funding agencies direct access to all study-related documents and pertinent hospital or medical records for confirmation of data contained within the eCRFs. In addition, the PI and the site staff will be available to meet with the monitor during monitoring visits; such visits will be arranged in advance with the investigational site. The investigator and site staff will cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved. In addition, during these visits, the monitor will review the investigational site file and regulatory documentation and verify that the investigational product has been stored appropriately and dispensed and administered correctly to eligible participants.

Deviations should be reported to the relevant IRB per IRB guidelines. Deviations required to be reported by ICH guidelines will be included in the study report.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of regulatory agencies or the Sponsor or designee. All study documents must be made available upon request to the monitoring team and other authorized regulatory authorities, including but not limited to the National Institute of Health, NCI, the IRB responsible for overseeing the study at that institution, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

13.2 Audits

The study may be audited by an audit team originating from the Sponsor or its designee. The Investigator will agree to cooperate with the auditor to ensure than any problems detected in the course of the audit visits are resolved.

13.3 Data Management and Processing

13.3.1 Case Report Forms (CRFs)

The Sponsor or designee will be responsible for activities associated with data management of the study. This will include setting up a relevant database, along with appropriate validation of data and resolution of queries. An eCRF will be the primary data collection document for the study and is developed in conjunction with statistical oversight. Data collected during the study will be recorded in the participant's eCRF in a timely manner following acquisition of new source data. Only the PI, the study coordinator, the data management team, and the clinical trials manager/qualified site designee are permitted to make entries, changes, or corrections in the eCRF.

An audit trail will be maintained automatically by the electronic case report form (eCRF) management system. All users of this system will complete user training, as required or appropriate per sponsor requirements and other regulations.

13.3.2 Data Management Procedures and Data Verification

Study centers will enter data directly into an electronic data capture (EDC) system by completing the eCRF via a secure Internet connection. Data entered into the eCRF must be verifiable against source documents at the study center. Any changes to the data entered in the EDC system will be recorded in the audit trail and will be FDA CFR 21 Part 11 compliant.

Completeness of entered data will be checked automatically by the EDC system and users will be alerted to the presence of data inconsistencies via programmed edit checks. Missing or implausible data will be brought to the attention of the institutional PI or designee requiring an appropriate response (ie, confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

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

13.3.3 Study Closure

Following completion of the studies, the site PIs and their qualified designees will be responsible for ensuring the following activities:

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

14 STATISTICAL METHODS AND DATA ANALYSIS

All efficacy and safety measures over the course of the study will be presented, as appropriate. Continuous data will be summarized by means, standard deviations (SDs), medians, maximum, minimum, 25th and 75th percentiles, and number of subjects. Categorical data will be summarized by counts and percentages.

A Statistical Analysis Plan (SAP) will be prepared to provide details regarding the definition of analysis variables and analysis methodology to address the study objectives. Should a statistical approach described in the SAP differ from those described in this protocol, the SAP will take precedence and supersede methods describe herein.

14.1 Analysis Sets

The Safety Population will consist of all enrolled patients who receive their initial PVSRIPO treatment. This will be the primary population used for efficacy and safety analyses.

All safety and efficacy analyses will be based on all treatment groups combined. Additional safety and efficacy data will also be summarized by the randomized treatment group prior to protocol version 7, as well as for patients who received additional infusions of PVSRIPO.

14.2 Historical Control Group

The external criteria matched historical control group that will be used for survival comparisons will include recurrent WHO grade IV malignant glioma patients who have been treated at the institutions participating in this multicenter trial (where data are available) or from other available datasets. Patient demographics, disease characteristics, and outcomes for the historical controls will be summarized by center and overall, as applicable.

Initially, the historical group only includes patients from DUMC; however, this group may be augmented with historical patient data from other sources and, in particular, from participating centers once they join the study. From DUMC, the historical group includes recurrent WHO grade IV malignant glioma patients from the DUMC PRoGREss registry (IRB# Pro00027120; Primary and Recurrent Glioma Registry) who would have been eligible to receive PVSRIPO if the treatment had been available. The DUMC historical group includes 96 patients of whom 95 are deceased. Median survival is 11.7 months (95% confidence interval [CI]: 9.8-13.2). The 24-month survival estimate is 13.5% (95% CI: 7.6%-21.2%). Details about the construction of this comparison group, as well as a summary of patient characteristics, are provided in Appendix A: Selection and Description of the Historical Control Cohort at DUMC.

These data may be updated to include historical patient data from the other sites or sources. Selection of the final control group will be based on eligibility criteria similar to those of this protocol. Where possible, historical control data may also be supplemented through a prospectively defined review of the literature or other sources. The SAP will describe any additional data used for the external control database.

14.3 Patient Demographics and Other Baseline Characteristics

Socio-demographic, medical history, and clinical characteristics of patients enrolled and treated on this study will be summarized overall and by treatment group.

14.4 Treatments

For the PVSRIPO in combination with lomustine arm, the number of patients who did not receive one cycle of lomustine at the required dose level will be calculated and the reason for not receiving lomustine as required summarized for those randomized to the lomustine arm prior to protocol version 7.

The number of patients who require bevacizumab administration will be tabulated along with the number of doses administered.

The number and percentage of patients who require additional treatment (concomitant medications) for control of inflammation or to deal with disease progression will be tabulated along with the type of treatment administered.

14.5 Primary Objective

The primary objective of this study is to assess the anti-tumor response based on iRANO criteria, as well as response duration among adults with recurrent WHO grade IV malignant glioma treated with PVSRIPO at first or second recurrence.

The primary endpoints of this study are ORR and DOR. For evaluation of ORR, the number (%) of patients with best response of CR or PR will be calculated, along with the 95% confidence interval for the percentage. The distribution of DOR will be described using the Kaplan-Meier curve.

14.6 Secondary Objectives

Overall Survival: OS, including landmark survival rates (focusing at ≥ month 36) will be summarized using the Kaplan-Meier method. The survival response will be compared to the external control as outlined in the SAP.

The distribution of demographics, baseline characteristics, confounding factors, and the differences between the PVSRIPO group and the historical control group will be assessed. Cox, or other time-to-event models will be used to adjust for any potential confounding factors. Examples of potential variables for adjustment are treatment, center, gender, age, maximal extent of resection at diagnosis, prior bevacizumab treatment, KPS at the time of eligibility for PVSRIPO treatment, the number of prior recurrences, prior number of patients treated at a center (ie, center experience) and molecular diagnostic markers. The SAP will present details about the models to be used.

In addition to the models mentioned above, a propensity score analysis may be used to compare the PVSRIO and historical control groups. The SAP will describe the propensity score analysis.
Disease Control Rate: For evaluation of DCR, the percentage of patients classified as non-progressive based on the imaging criteria described in the Central Imaging Charter will be estimated, along with its 95% confidence interval.

Safety: A secondary objective is to assess the safety of each PVSRIPO treatment cohort in recurrent WHO grade IV malignant glioma patients. Within each cohort for those randomized prior to protocol version 7, as well as for those patients retreated with PVSRIPO, the proportion of patients who experience grade 3, 4, or 5 AEs that are possibly, probably, and definitely related to protocol treatment will be estimated.

In addition, for each type of toxicity, the maximum grade experienced by each patient will be summarized with frequency distributions. Adverse experience tabulations will be generated: one tabulation will include all toxicities regardless of attribution, and one tabulation is for each study-related intervention will include only toxicities that are possible, probably, and definitely related to catheter placement, PVSRIPO, lomustine (for patients randomized to this arm prior to protocol version 7), bevacizumab, or corticosteroids.

14.7 Exploratory Objectives

An exploratory objective of this study is to describe changes visualized on imaging due to intratumoral inoculation of PVSRIPO to help develop future imagining/response classification criteria specific to PVSRIPO. Two components of the inflammatory reaction will be summarized: the enhancing lesion and the edema (FLAIR) area. Summary statistics will be presented by treatment group (PVSRIPO alone and PVSRIPO/lomustine to account for patients randomized to this arm prior to protocol version 7) at screening, baseline, and for each visit at which MRIs are conducted, up to week 52.

Another exploratory objective is the assessment of immunologic changes stemming from the administration of PVSRIPO alone or in combination with lomustine for those randomized to this treatment arm prior to protocol version 7 (run as a sub-study at Duke). Peripheral blood will be collected at defined intervals (see Table 1) for correlative

immune-monitoring studies in serum and in peripheral blood monocytes. These include (i) analyses of innate and inflammatory immune events such as high mobility group 1 box (HMGB1), inflammatory cytokines, natural killer (NK) and NK T-cells, and regulatory immune subsets (Tregs & myeloid-derived suppressor cells (MDSCs); (ii) analyses of adaptive immune responses (lineage, maturation, induction and activation/functional status of tumor antigen-specific T-cells). Other markers of inflammation (eg, cytokines) will also be explored at all centers (Day 1 after PVSRIPO administration).

Another exploratory objective is the identification of tumor genetic markers that are predictors of response (ran as a sub-study at Duke). Cox and logistic models will explore the relationship of genetic markers to survival time.

Another exploratory objective is to obtain the quantitative gut microbiome profile via the OMNIgene®•GUT (OMR-200) kit to determine any potential influence in response to PVSRIPO or PVSRIPO/lomustine (for patients randomized to this arm prior to protocol version 7).

Still another exploratory objective is to perform future molecular genetic analyses of the buffy coat to determine the genetic characteristics of these samples to assess the potential influence in response to PVSRIPO or PVSRIPO/lomustine (for patients randomized to this arm prior to protocol version 7).

Anti-tumor response/outcomes to PVSRIPO retreatment will be described. The type, frequency and duration of anti-tumor responses, along with use of non-protocol-specified therapies will be summarized. Further details will be outlined in the SAP.

Last, response/ other outcomes based on the amount of prior experience an investigational site has with PVSRIPO and in what order a patient is treated at a site, will be described. For example, summaries may consider patient outcomes based on centers treating ≥ 4 vs ≤3, etc. and/or number of prior patients infused at a center (eg, first 3 patients at a site vs. patients treated after a center has treated ≥ 4 patients). Further details will be outlined in the SAP.

14.8 Additional Data Summaries

Additional collected data will be summarized for each scheduled visit up to week 52, including disease status, vital signs, KPS, physical and neurological examinations, and laboratory values.

14.9 Interim Analysis

An interim analysis will be conducted part way through the study in support of a pre-Biologics License Application (BLA) submission, a BLA submission or equivalent. Specifically, this interim analysis will be conducted after approximately 50 or more patients have been treated with PVSRIPO and followed for approximately 21 months or

more. Specific parameters and analysis methodology will be outlined in the SAP and finalized prior to conducting the interim analysis.

Feasibility

We anticipate that participating centers will be able to treat patients with PVSRIPO per procedures developed at DUMC. However, one of the purposes of the multicenter study is to informally assess that feasibility. Feasibility, based on safety, will be assessed when ≥ 10 patients have been treated by other institutions (details specified in the SAP).

14.10 Sample Size Calculation

The sample size of the study is approximately 122 participants. A formal sample size calculation was not performed. The following table shows the ORR in percent and the corresponding 95% confidence intervals as a function of the number of objective responses, if for example 120 patients were enrolled.

Number of Subjects	Number of objective responses	ORR (%)	95% CI
120	10	8.3%	3.4%, 13.3%
120	12	10.0%	4.6%, 15.4%
120	14	11.7%	5.9%, 17.4%
120	16	13.3%	7.3%, 19.4%
120	18	15.0%	8.6%, 21.4%
120	20	16.7%	10.0%, 23.3%

15 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

15.1 Regulatory and Ethical Compliance

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

15.2 Institutional Review Board

The protocol, ICF, advertising material, and additional protocol-related documents must be submitted to the appropriate IRB at each institution for review. The study may be initiated only after the institutional PI has received written and dated approval from the IRB. The written approval of the IRB together with the approved ICF must be filed in the study files.

The institutional PI must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the ICF. The institutional PI must obtain protocol re-approval from the IRB within 1 year of the most recent IRB approval. The Investigator will report promptly to the IRB any new information that may adversely affect the safety of the patients or the conduct of the study. The Investigator will submit written summaries of the study status to the IRB as required. On completion of the study, the Institutional Ethics Committee (IEC)/IRB will be notified that the study has ended.

15.3 Informed Consent

The ICF must be written in a manner that is understandable to the subject population. Prior to its use, the ICF must be approved by the IRB.

The institutional PI or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects who cannot read or understand English or are visually impaired. Potential subjects will have the opportunity to contact the institutional PI or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study. Documentation of the informed consent should be documented in the participant's medical records and should meet the requirements of GCP.

Before conducting any study-specific procedures, the institutional PI must obtain written informed consent from the subject or a LAR. The original ICF will be stored with the subject's study records, and a copy of the ICF will be provided to the subject. The institutional PI is responsible for asking the subject whether the subject wishes to notify

his/her primary care physician about participation in the study. If the subject agrees to such notification, the investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

If new information becomes available that may be relevant to the patient's willingness to continue participation in the study, the ICF will be updated and approved by the applicable IRB and other regulatory authorities, if required. Study participants will be informed about this new information and reconsent obtained.

Participants who are rescreened are required to sign a new ICF. For pregnancies in a female partner of a male patient, consent to follow-up on the pregnancy will be obtained in agreement with HIPAA.

15.4 Study Documentation

Study documentation includes, but is not limited to, source documents, CRFs, monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the mandated "Regulatory Binder," which includes, but is not limited to, approved protocol versions, approved ICFs, Form FDA 1572s, and College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA) laboratory certifications.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

The PI is responsible for ensuring that all source documents comply with the ICH E6 (R2) guidelines [ie, in keeping with attributable, legible, contemporaneous, original, accurate and complete (ALCOA-C)].

15.5 Privacy, Confidentiality, and Data Storage

The institutional PI will ensure that subject privacy and confidentiality of the subject's data will be maintained. If required, Research Data Security Plans will be approved by the appropriate institutional Site Based Research group.

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

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key, accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a dedicated 21 CFR Part 11 compliant database (Medidata Rave), which is housed in an encrypted and password-protected file on a secure network drive. Access to electronic databases and IT infrastructure will be managed by the Sponsor or their qualified designee.

Monitors, auditors, and other authorized agents of the Sponsor and/or its designee, the IRB(s) approving this research, and the US FDA, as well as that of any other applicable agency(ies), will be granted direct access to the study patients' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the patients to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the patients' identities will remain confidential unless prior consent is obtained.

All personal data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precautions to ensure confidentiality of those data, and in accordance with HIPAA, applicable to national and/or local laws and regulations on personal data protection.

Upon completion of the study, research records will be archived and handled by each institution's policy. Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

15.6 Data and Safety Monitoring

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

15.7 Protocol Amendments

All protocol amendments must be released by the Sponsor or their designate. All protocol amendments will be submitted for approval to the IRB of record by the Sponsor and approved by the site's IRB(s) prior to implementation, as applicable. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an

immediate hazard. However, the PI and/or Sponsor must inform the IRB and all other applicable regulatory agencies of such action immediately.

15.8 Records Retention

Essential documents are those documents that individually and collectively permit evaluation of the study and the quality of the data produced. After completion of the study (end of study defined as the date of the last follow-up contact with the last patient), all documents and data relating to the study will be kept in an orderly manner by the investigator in a secure study file. This file will be available for inspection by the Sponsor or its representatives. Essential documents should be retained for 2 years after the final marketing approval for the indication being investigated or at least 2 years since the discontinuation of clinical development of the study drug for the indication being studied, and the FDA has been notified. It is the responsibility of the Sponsor or their designee to inform the study center when these documents no longer need to be retained. The investigator must contact the Sponsor before destroying any study-related documentation. In addition, all patient medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution, or medical practice.

No study document should be destroyed without prior written agreement between the Sponsor and the investigator. If the investigator wishes to assign the study records to another party or move them to another location, the investigator must notify the Sponsor or their designee in writing of the new responsible person and/or the new location.

16 REFERENCES

1. Ohgaki H: Epidemiology of brain tumors. Methods Mol Biol 472:323-42, 2009
2. Hall WA, Fodstad O: Immunotoxins and central nervous system neoplasia. J Neurosurg 76:1-12, 1992
3. Imperato JP, Paleologos NA, Vick NA: Effects of treatment on long-term survivors with malignant astrocytomas. Ann Neurol 28:818-22, 1990
4. Heimberger AB, Sun W, Hussain SF, et al: Immunological responses in a patient with glioblastoma multiforme treated with sequential courses of temozolomide and immunotherapy: Case study. Neuro-Oncology 10:98-103, 2008
5. McLendon RE, Rosenblum MK, Bigner DD: Russell and Rubinstein's Pathology of Tumors of the Nervous System. Seventh edition. London, Hodder Arnold, 2006, pp 1132
6. Stupp R, Wong ET, Kanner AA, et al: NovoTTF-100A versus physician's choice chemotherapy in recurrent glioblastoma: a randomised phase III trial of a novel treatment modality. Eur J Cancer 48:2192-202, 2012
7. Stupp R, Mason WP, van den Bent MJ, et al: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987-96, 2005

8. Chinot OL, Wick W, Mason W, et al: Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med 370:709-22, 2014
9. Gilbert MR, Dignam JJ, Armstrong TS, et al: A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med 370:699-708, 2014
10. Friedman HS, Prados MD, Wen PY, et al: Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 27:4733-40, 2009
11. Wick W, Puduvalli VK, Chamberlain MC, et al: Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. J Clin Oncol 28:1168-74, 2010
12. Batchelor TT, Mulholland P, Neyns B, et al: Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. J Clin Oncol 31:3212-8, 2013
13. Taal W, Oosterkamp HM, Walenkamp AM, et al: Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. Lancet Oncol 15:943-53, 2014
14. Stupp R, Taillibert S, Kanner AA, et al: Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. Jama 314:2535-43, 2015
15. Groothuis DR: The blood-brain and blood-tumor barriers: a review of strategies for increasing drug delivery. Neuro Oncol 2:45-59, 2000
16. Stupp R, Hegi ME, Mason WP, et al: Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10:459-66, 2009
17. Gromeier M, Alexander L, Wimmer E: Internal ribosomal entry site substitution eliminates neurovirulence in intergeneric poliovirus recombinants. Proc Natl Acad Sci U S A 93:2370-5, 1996
18. Gromeier M, Bossert B, Arita M, et al: Dual stem loops within the poliovirus internal ribosomal entry site control neurovirulence. J Virol 73:958-64, 1999
19. Campbell SA, Lin J, Dobrikova EY, et al: Genetic determinants of cell type-specific poliovirus propagation in HEK 293 cells. J Virol 79:6281-90, 2005
20. Dobrikova EY, Broadt T, Poiley-Nelson J, et al: Recombinant oncolytic poliovirus eliminates glioma in vivo without genetic adaptation to a pathogenic phenotype. Mol Ther 16:1865-72, 2008
21. Brown MC, Dobrikov MI, Gromeier M: Mitogen-activated protein kinase-interacting kinase regulates mTOR/AKT signaling and controls the serine/arginine-rich protein kinase-responsive type 1 internal ribosome entry site-mediated translation and viral oncolysis. J Virol 88:13149-60, 2014
22. Brown MC, Gromeier M: Cytotoxic and immunogenic mechanisms of recombinant oncolytic poliovirus. Curr Opin Virol 13:81-5, 2015

23. Mendelsohn CL, Wimmer E, Racaniello VR: Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell 56:855-65, 1989
24. Merrill MK, Bernhardt G, Sampson JH, et al: Poliovirus receptor CD155-targeted oncolysis of glioma. Neuro Oncol 6:208-17, 2004
25. Castriconi R, Daga A, Dondero A, et al: NK cells recognize and kill human glioblastoma cells with stem cell-like properties. J Immunol 182:3530-9, 2009
26. Freistadt MS, Fleit HB, Wimmer E: Poliovirus receptor on human blood cells: a possible extraneural site of poliovirus replication. Virology 195:798-803, 1993
27. Gromeier M, Lachmann S, Rosenfeld MR, et al: Intergeneric poliovirus recombinants for the treatment of malignant glioma. Proc Natl Acad Sci U S A 97:6803-8, 2000
28. Pelletier J, Sonenberg N: Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature 334:320-5, 1988
29. Sweeney TR, Abaeva IS, Pestova TV, et al: The mechanism of translation initiation on Type 1 picornavirus IRESs. EMBO J 33:76-92, 2014
30. Merrill MK, Dobrikova EY, Gromeier M: Cell-type-specific repression of internal ribosome entry site activity by double-stranded RNA-binding protein 76. J Virol 80:3147-56, 2006
31. Dobrikova EY, Goetz C, Walters RW, et al: Attenuation of neurovirulence, biodistribution, and shedding of a poliovirus:rhinovirus chimera after intrathalamic inoculation in Macaca fascicularis. J Virol 86:2750-9, 2012
32. Arita M, Ohka S, Sasaki Y, et al: Multiple pathways for establishment of poliovirus infection. Virus Res 62:97-105, 1999
33. Laske DW, Youle RJ, Oldfield EH: Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. Nat Med 3:1362-8, 1997
34. Reardon DA, Desjardins A, Peters KB, et al: Phase 2 study of carboplatin, irinotecan, and bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy. Cancer 117:5351-8, 2011
35. Salford LG, Brun A, Nirfalk S: Ten-year survival among patients with supratentorial astrocytomas grade III and IV. J Neurosurg 69:506-9, 1988
36. Li L, Gao FQ, Zhang B, et al: Overdosage of intrathecal gadolinium and neurological response. Clin Radiol 63:1063-8, 2008
37. Selcuk H, Albayram S, Ozer H, et al: Intrathecal gadolinium-enhanced MR cisternography in the evaluation of CSF leakage. AJNR Am J Neuroradiol 31:71-5, 2010
38. Arlt S, Cepek L, Rustenbeck HH, et al: Gadolinium encephalopathy due to accidental intrathecal administration of gadopentetate dimeglumine. J Neurol 254:810-2, 2007
39. Tali ET, Ercan N, Krumina G, et al: Intrathecal gadolinium (gadopentetate dimeglumine) enhanced magnetic resonance myelography and cisternography: results of a multicenter study. Invest Radiol 37:152-9, 2002

40. Aydin K, Terzibasioglu E, Sencer S, et al: Localization of cerebrospinal fluid leaks by gadolinium-enhanced magnetic resonance cisternography: a 5-year single-center experience. Neurosurgery 62:584-9; discussion 584-9, 2008
41. Gleostine, pp Reference ID: 3509888

17 APPENDICES

17.1 Appendix A: Selection and Description of the Historical Control Cohort at DUMC

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 1 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 1 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 (first or second recurrence, including this recurrence) supratentorial WHO grade IV malignant glioma 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 any PVSRIPO study or received PVSRIPO
4. KPS ≥70 at the time patient could have been enrolled in this PVSRIPO study
5. Absence of rapid clinical decline
6. Pre-treatment steroid usage ≤ 4 mg per day of dexamethasone within the 2 weeks prior to PVSRIPO infusion.

Within this historical control cohort 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.

Table 5. Characteristics of the Patients within the Historical Control Group

Patient Characteristics	N	%
All	96	100.0
Gender		
Female	36	37.5
Male	60	62.5
Diagnosis		
GBM	96	100.0
Age when first eligible for PVSRIPO, mean (SD)	55.5	(11.5)
Age when first eligible for PVSRIPO, median (range)	55	(23-77)
Maximal resection/surgical procedure at GBM diagnosis[a]		
Gross Total Resection (GRT)	63	65.6
Subtotal Resection (STR)	23	24.0
Biopsy	10	10.4
Number of prior progressions until first eligible for PVSRIPO		
1	85	88.5
2	11	11.5
KPS when first eligible for PVSRIPO		
100	8	8.3
90	61	63.5
85	1	1.0
80	24	25.0
70	2	2.1
Bevacizumab prior to when first eligible for PVSRIPO		
No	49	51.0
Yes	47	49.0
Bevacizumab failure prior to when first eligible for PVSRIPO		
No	60	62.5
Yes	36	37.5
Vital status		
Alive	1	1.0
Dead	95	99.0

GBM = glioblastoma; KPS = Karnofsky performance status; PVSRIPO = polio/rhinovirus recombinant; SD = standard deviation;
[a] 2 patients with a maximal resection of "STR/GTR?" were coded as a STR, and 1 patient with "GTR to main tumor, STR to satellite lesion" was coded as a GTR.

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