# CLINICAL STUDY PROTOCOL

**Study Title**: A Phase 2, Open-label, Single-arm Study Evaluating the Efficacy, Safety and Tolerability of Oncolytic Polio/Rhinovirus Recombinant (PVSRIPO) and the Immune Checkpoint Inhibitor Pembrolizumab in the Treatment of Patients with Recurrent Glioblastoma

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| **Protocol Number:** | **LUMINOS-101 (previously PVSRIPO ICI rGBM 201)** |
| **Clinical Phase** | **Phase 2 Therapeutic Protocol** |
| **US IND Number** | **IND 014735** |
| **Protocol Date:** | **Version 4.0, 26May2021** |
| **Sponsor:** | **Istari Oncology, Inc.**<br>**430 Davis Drive**<br>**Morrisville, NC 27560** |

## Protocol Approval Signature Page

**Sponsor Representative(s)**

**I, the undersigned, have read this protocol and confirm that to the best of my knowledge it accurately describes the planned conduct of the study.**

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| W. Garrett Nichols, MD, MS<br>CMO, Istari Oncology, Inc. | Date |

## Investigator Protocol Agreement

I have read and understand this protocol and agree:

- To assume responsibility for the proper conduct of the study at this site.
- To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by the Sponsor, Istari.
- To conduct the study as outlined herein, in accordance with GCP requirements and the International Conference on Harmonization (ICH) guidelines, the Declaration of Helsinki and complying with the obligations and requirements of Clinical Investigators and all other requirements listed in 21 Code of Federal Regulations (CFR) part 312 and with all applicable local regulations.
- Not to implement any deviations from or changes to the protocol without prior agreement from the Sponsor and prior review and written approval from the Institutional Review Board (IRB)/Institutional Ethics Committee (IEC), except as necessary to eliminate an immediate hazard to study patient(s), or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- To ensure that all persons assisting me with the study are adequately informed about the investigational product(s) and of their study-related duties and functions as described in the protocol.
- To completely inform all participants in this study concerning the pertinent details and purpose of the study prior to their agreement to participate in the study in accordance with GCP and regulatory authority requirements.
- To be responsible for maintaining each patient's consent form in the study file and providing each patient with a signed copy of the consent form.
- To periodic on-site and/or remote monitoring of the source documents and case report forms by Istari or designee.
- To allow access to files for audit or inspection purposes by the Sponsor or by competent regulatory authorities.
- That I am thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol and any additional information provided by the Sponsor including but not limited to the following: the current Investigator's Brochure and approved product label, if applicable.

Hence, I:

- Agree to supply Istari with any information regarding financial arrangements and interests.
- Agree to promptly update any information if any relevant changes occur during the study and for 1 year following completion of the study; and,
- Agree that Istari may disclose this information about such financial arrangements and interests to regulatory authorities.

Investigator Name (Print): _____

Date Signed: _____

Investigator Signature: _____

## NOTICE REGARDING IMMUNOPROGRESSION

**DUE TO KNOWN PVSRIPO-MEDIATED TREATMENT EFFECTS, INCREASED TUMOR SIZE ON RADIOGRAPHIC IMAGING FOLLOWING PVSRIPO ADMINISTRATION MAY OCCUR. EXCEPT IN CASES OF IMMINENT LIFE-THREATENING EMERGENCY, CONSULT WITH SPONSOR OR THEIR DESIGNEE PRIOR TO INITIATING NON-PROTOCOL-SPECIFIED TUMOR TREATMENTS OR PERFORMING SURGICAL RESECTION.**

- PVSRIPO's immune-mediated mechanism of action (MOA) is known to result in atypical tumor imaging responses that can manifest as polycystic or pseudoprogressive appearance, including areas of new enhancement and tumor enlargement (termed immunoprogression) that can persist for up to 6 or more months after infusion. Thus, reliance on radiographic imaging standard criteria (eg, Immunotherapy Response Assessment for Neuro-oncology (iRANO)[1]) for determining disease progression is not optimal for PVSRIPO-based therapy; see **Table 3** for modified response criteria.
- The time to objective radiographic anti-tumor response ranged from approximately 5 to 29 months post-infusion in the Recurrent Glioblastoma (rGBM) PVSRIPO Phase 1 study (Pro00031169; median time to response 15 months).
- For patients to have the best opportunity for an immune-mediated anti-tumor response, experience suggests that patients should be managed as noted below and as outlined in the protocol.

**Treatment emergent adverse events should be managed at the discretion of the principal investigator, with patient safety being the primary consideration. Based on prior experience, the best recommended management of treatment-induced peritumoral edema or of a pseudoprogressive, polycystic, expansive-type of tumor appearance (ie, immunoprogression) 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:**
  - Preferred: Low dose bevacizumab (7.5mg/kg every (q) 3 weeks; few cycles as possible to achieve symptom control)
  - Initiation of low dose steroids (dexamethasone ≤ 4mg/day) if rapid response is clinically indicated
  - Other supportive care treatments, as required (eg, antiepileptics)

## Summary of Major Protocol Amendment Changes

| Protocol Version and Date (from, to) | Summary of Major Changes by Section |
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| Version 3 (23April2021) to Version 4 (21May2021) | • Updated Table 1 to clarify timing of certain laboratory schedule of assessments and their description throughout the text.<br>• Updated Sections 5.2.1 and 5.2.2 to include guidance regarding platelet support both pre-infusion and prior to catheter removal. |

## Protocol Synopsis

<table>
<tr><td>Protocol Title</td><td>A Phase 2, Open-Label, Single-Arm Study Evaluating the Efficacy, Safety and Tolerability of Oncolytic Polio/Rhinovirus Recombinant (PVSRIPO) and the Immune Checkpoint Inhibitor Pembrolizumab for the Treatment of Patients with Recurrent Glioblastoma</td></tr>
<tr><td>Protocol Number</td><td>PVSRIPO ICI rGBM 201</td></tr>
<tr><td>Rationale and Background</td><td>We hypothesize that PVSRIPO, a novel recombinant poliovirus (PV)/rhinovirus, oncolytic viral immunotherapeutic, when infused directly into non-immunogenic rGBM tumors, coupled with immune checkpoint inhibitors (ICI), may serve to generate a potent and specific anti-tumor response given their respective mechanisms of action. The ICI pembrolizumab is a programmed cell death receptor-1 (PD-1)-blocking antibody indicated in a number of malignancies and tumor types that has been reasonably well tolerated, with or without surgical resection, in patients with rGBM. In addition, pre-clinical data and limited clinical use outside of structured trials suggest that PVSRIPO followed by pembrolizumab may be associated with additive to synergistic anti-tumor responses [2-7].</td></tr>
<tr><td>Objectives</td><td>To evaluate the following after intratumoral infusion of PVSRIPO and treatment with at least one dose of pembrolizumab in rGBM patients:
<table>
<tr><th>Objectives</th><th>Endpoints/Evaluation Criteria</th></tr>
<tr><td colspan="2">Primary (efficacy)</td></tr>
<tr><td>Anti-tumor activity:</td><td>• Objective response rate (ORR): Proportion of patients with confirmed CR or PR.<br>• Duration of response (DOR): KM curves showing median DOR for all patients that have confirmed CR or PR.<br>• Durable radiographic response (DRR): Proportion of patients with confirmed response (CR or PR) that lasts for ≥ 6 months. Radiographic outcomes will be assessed via iRANO criteria [1] in this protocol.</td></tr>
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| | **Primary (safety)** | |
| | Safety/tolerability: | • Frequency and severity of treatment-emergent adverse events (TEAE) via Common Terminology Criteria for Adverse Events (CTCAE, v5.0) for patients receiving PVSRIPO and at least one dose of pembrolizumab. |
| | **Secondary (efficacy)** | |
| | Anti-tumor activity: | • Disease Control Rate (DCR): Comprised of patients achieving CR, PR or SD for ≥ 6 months.<br>• Duration of disease control (DDC): KM curves showing median DDC for patients with confirmed CR, confirmed PR or SD for ≥ 6 months. |
| | Survival | • Overall and landmark survival: Proportion of patients alive at ≥ 6, ≥ 12 and at 24 months post-PVSRIPO infusion as calculated by Kaplan-Meier methods. |
| | Progression free survival | • If calculable, progression free survival (PFS) will be estimated based on the time from PVSRIPO infusion to death or confirmed progression, based on the definition of progression noted in **Table 3**. |
| | **Secondary (safety)** | |
| | | • Frequency and severity TEAE via CTCAE for patients receiving PVSRIPO alone without pembrolizumab (any reason). |
| | **Exploratory** | |
| | Identification of biomarkers of anti-tumor response to PVSRIPO followed by pembrolizumab | Assessment of tumor tissue and blood samples for identification of genetic, cytologic, histologic and/or other markers that demonstrate pharmacodynamic activity of PVSRIPO or correlate with anti-tumor response. |
| | Alternative assessment of radiographic response/progression/PFS | Radiographic outcomes will also be analyzed as described in **Table 3** for endpoint determination. All responses must be confirmed via two consecutive MRI assessments at least 4 weeks apart. If on bevacizumab therapy when response first noted, response will be confirmed ≥ 8 weeks. |
| | Patient-reported outcomes | Assessment of patient-reported quality of life and activities of daily living capabilities. |
| **Study Design** | This Phase 2 single-arm trial in patients with rGBM will characterize the efficacy, safety and tolerability of PVSRIPO intratumoral infusion followed by intravenous (IV) pembrolizumab 2 to 4 weeks later, and every 3 weeks, thereafter. | |

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| | The study will begin with a safety lead-in for evaluation of dose-limiting toxicity (DLT) of the combination prior to full open enrollment. As such, the first 3 patients will be sequentially enrolled with a mandatory waiting period of 21 to 28 days after the immediate prior patient's PVSRIPO infusion to allow for DLT assessment. The DLT evaluation period will begin once a patient receives PVSRIPO followed by the first dose of pembrolizumab and continue for 21 days after the first dose of pembrolizumab (± 3 days to account for weekends, patient visit scheduling issues, etc.). The initial DLT assessment will be based on the first 3 patients, which may be expanded up to 6 patients if any DLT are noted in the first 3 (see **Sections 3** and **7.4** for additional detail regarding DLT assessment).<br><br>The first planned dose of pembrolizumab at Week 2 will be delayed by up to 2 additional weeks in patients with any ongoing ≥ Grade 3 peritumoral edema (PTE) or cerebral edema (CE) or ≥ Grade 2 AEs related to PTE or CE following PVSRIPO infusion or signs or symptoms related to these events that require active treatment (eg, treatment-emergent or worsening events such as hemiparesis requiring ≤ 4 mg/day dexamethasone or seizure requiring anti-epileptics; see **Section 5.7.2**), until such events resolve to ≤ Grade 1. If such events have not resolved to ≤ Grade 1 by Week 4 post-PVSRIPO infusion, the patient will not receive pembrolizumab under this trial and will be replaced. Pembrolizumab may be administered any time between Weeks 2 and 4 once events resolve as outlined above, as applicable. The first planned pembrolizumab treatment may also be delayed for administrative or other reasons, including other TEAE that in the Investigator's opinion warrants delayed pembrolizumab treatment, but the first pembrolizumab treatment must occur by Week 4 post-PVSRIPO infusion, as noted above.<br><br>In addition, if a patient requires > 4 mg/day of dexamethasone or equivalent prior to the first dose of pembrolizumab, the patient will not receive pembrolizumab under this trial and will be replaced. These replaced patients will not be considered in the initial DLT assessment of the combination but will be eligible for additional follow-up in this study and will be summarized separately from those receiving the combination. Their information may be utilized in exploratory comparative summaries relative to patients treated with PVSRIPO and ≥ 1 dose of pembrolizumab in this study.<br><br>If ≤ 33% of the initial participants in the safety lead in (≤ 1 of 3 or if > 1 patient of the first 3 experiences a DLT and 3 additional patients are included for DLT assessment, ≤ 2 of 6 patients) experience a DLT, the twenty-four remaining participants will be enrolled. If > 33% of participants (ie, ≥ 3 of 6) experience a DLT, the study will be halted, and the treatment plan modified, or the study may be closed. After the DLT evaluation period and the study is open to full enrollment, ongoing monitoring of TEAEs will occur on at least a monthly basis. |
| **Planned Number of Participants** | Approximately 30 participants receiving PVSRIPO followed by pembrolizumab. Patients receiving PVSRIPO only for any reason will be followed according to the schedule of assessments with MRI and required visits occurring approximately every 9 weeks after Week 11; these patients will be replaced to achieve the approximate total of n=30 receiving PVSRIPO and pembrolizumab. |
| **Study Entry Criteria** | **Inclusion Criteria (IC)**:<br>1. ≥ 18 years of age. |

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| | 2. Actively growing recurrent supratentorial glioblastoma confirmed by biopsy by the site's neuropathologist or designate.<br>o Histologically confirmed recurrent glioblastoma within 6 weeks of PVSRIPO infusion will not require a biopsy to confirm active tumor prior to catheter placement. However, biopsy confirmation of glioblastoma must be provided if not completed within 6 weeks of catheter placement.<br>o Progression of primary glioblastoma or transformation from a lower grade to a higher grade is acceptable for recurrence and as for primary glioblastoma, must be confirmed via prior histology by site pathologist.<br>3. Enhancing lesion ≥1 cm shortest diameter to ≤ 5.5 cm longest diameter in all planes, but occurrence within the prior resection cavity should not contribute to the longest diameter measurement.<br>4. Before catheter placement based on screening MRI and at the time of catheter placement via computed tomography (CT) prior to infusion, neurosurgical investigator must confirm both tumor location (≥ 1 cm from eloquent brain) as well as placement of infusion catheter within or through the progressive enhancing tumor is feasible and at a safe distance relative to eloquent brain function, with the tip of the catheter being placed:<br>a. Within the enhancing portion or in the vicinity of enhancement of target lesion (ie, infiltrative disease)<br>b. ≥ 0.5 cm from ventricles<br>c. ≥ 1 cm deep into the brain<br>d. ≥ 0.5 cm from the corpus callosum<br>Patients with ongoing neurologic symptoms with enhancing disease within 1cm of eloquent brain are excluded.<br>5. First or second relapse supported by MRI or CT scan; relapse is defined as progression following initial/prior therapy(ies).<br>6. Failed previous first line therapy: maximum surgical resection and radiotherapy (RT) (plus concomitant chemotherapy followed by maintenance chemotherapy if unknown or methylated $O^6$-methylguanine-DNA methyltransferase (MGMT) promoter). Patients who begin but do not complete chemotherapy/RT may still be considered for eligibility at the discretion of Sponsor.<br>7. Karnofsky performance status (KPS) ≥ 70 at screening and baseline.<br>8. Undergone prior vaccination against PV and received a boost immunization with trivalent IPOL® (PV Vaccine Inactivated) (Sanofi-Pasteur SA) at least 1 week, but less than 6 weeks, prior to administration of PVSRIPO (within 6 months of PVSRIPO retreatment). Note: Patients who are unsure of their prior vaccination status or have not been vaccinated must provide proof of vaccination and/or evidence of anti-PV immunity prior to enrollment, as applicable.<br>9. Ability to safely discontinue anti-coagulant therapy(ies) prior to biopsy/catheter placement, as required per site/surgical guidelines, in the opinion of the investigator.<br>10. Hemoglobin ≥ 9 g/dl prior to biopsy/catheter placement.<br>11. Platelet count ≥ 100,000/microliter (µL, unsupported); ≥ 125,000/µL (can be supported via platelet transfusion and/or with a Sponsor-approved bone marrow stimulant/colony stimulating factor, eg, romiplostim) at biopsy/catheter placement. |

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| | 12. Absolute neutrophil count (ANC) ≥ 1000/μL prior to biopsy/catheter placement.<br>13. Creatinine ≤ 1.2 x upper limit of normal (ULN) prior to biopsy/catheter placement.<br>14. Total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) ≤ 2.5 x ULN prior to biopsy/catheter placement.<br>15. PT and aPTT ≤ 1.2 x ULN prior to biopsy/catheter placement.<br>16. If undetectable anti-tetanus toxoid immunoglobulin G (ATT IgG) at screen, Tdap booster vaccine > 1 week prior to biopsy/catheter placement.<br>17. Patients must be willing and able to understand and provide written informed consent.<br><br>**Exclusion Criteria (EC):**<br>1. Received prior therapy with an anti-PD-1, anti-programmed cell death-ligand 1 (anti-PD-L1), anti-programmed cell death-ligand 2 (anti-PD-L2), anti-CD137, or anti-cytotoxic T-lymphocyte-associated antigen (CTLA)-4 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways) ≤ 12 weeks prior to PVSRIPO infusion (note: does not apply for patients treated with pembrolizumab under this protocol who are eligible for PVSRIPO retreatment). Note: patients who had previously permanently discontinued any anti-PD-1 or PD-L1 therapy due to severe or life-threatening immune-related AE are excluded.<br>2. Excluded are:<br>a. Neoplastic lesions in the brainstem, cerebellum, or spinal cord<br>b. Radiological evidence of active/growing multifocal disease: no size increase > 0.5 cm in any direction of any other enhancing non-target lesions present at baseline confirmed via most recent, prior, consecutive MRIs at least 3 months apart. However, two lesions totaling ≤5.5cm of enhancing disease (total diameter) connected by T2 flair are not excluded<br>c. Tumors with ≥ 1 cm of contrast-enhancing tumor component crossing the midline (crossing the corpus callosum)<br>d. Extensive subependymal disease: multiple lesions or lesions covering > 50% of subependymal space. Tumor touching subependymal space allowed<br>e. Extensive leptomeningeal disease: multiple lesions or lesions covering > 50% of leptomeninges. Tumor touching leptomeninges allowed<br>3. Has received systemic immunosuppressive treatments other than systemic corticosteroids (eg, methotrexate, chloroquine, azathioprine) within six months of PVSRIPO infusion.<br>4. Requires treatment with high dose systemic corticosteroids, defined as dexamethasone > 4 mg/day or equivalent, within 2 weeks of PVSRIPO infusion.<br>5. Prior interstitial brachytherapy, implanted chemotherapy, stereotactic radiosurgery or therapeutics delivered by local injection or convection enhanced delivery (CED), including PVSRIPO (except for qualifying patients being retreated with PVSRIPO within this trial). |

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| | 6. Pregnant and/or breast feeding female; patient/female partner of childbearing potential who is unwilling to utilize protocol-defined acceptable form of contraception for duration of study.<br>7. Impending/life-threatening cerebral herniation syndrome, per neurosurgeon/designate.<br>8. Severe, active co-morbidity, defined as follows:<br>a. Infection requiring IV treatment/unexplained febrile illness (maximum temperature ($T_{max}$) > 99.5°F/37.5°C)<br>b. Known immunosuppressive disease/human immunodeficiency virus infection<br>c. Known active hepatitis B (HBV) or C (HCV) infection via positive viral Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA), respectively<br>d. Unstable or severe intercurrent medical conditions such as severe heart disease (New York Heart Association Class 3 or 4)<br>e. Known lung disease with forced expiratory volume in 1st second of expiration < 50%<br>f. Uncontrolled diabetes mellitus (eg, hemoglobin A1C level > 7.0% with treatment)<br>g. History of other malignancy requiring active treatment within 2 years of biopsy/catheter placement with the exception of those with a negligible risk of metastasis or death (eg, resected cutaneous basal cell carcinoma, or other cancers with 5-year overall survival (OS) of > 90%)<br>9. Known albumin allergy.<br>10. Uncontrolled unexplained bleeding and/or hemoptysis within 4 weeks of biopsy/catheter placement.<br>11. Inability to undergo brain MRI with and without contrast. History of severe/anaphylactic reaction to gadolinium contrast agent is excluded. Mild allergy (eg, rash) acceptable with prophylactic acetaminophen and diphenhydramine.<br>12. History of neurological complications due to PV infection.<br>13. Not recovered from toxic side effects (alopecia acceptable) and/or no current or prior tumor treatments within the following timeframe relative to biopsy/catheter placement:<br>a. Chemotherapy or bevacizumab ≤ 4 weeks (except for nitrosourea [6 weeks] or metronomic dosed chemotherapy such as daily temozolomide, etoposide or cyclophosphamide [1 week])<br>b. Tumor treating fields ≤ 7 days<br>c. RT of brain ≤ 12 weeks, except for progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation<br>14. History of agammaglobulinemia.<br>15. Known hypersensitivity to pembrolizumab, or any components of pembrolizumab.<br>16. Active autoimmune disease requiring systemic immunomodulatory treatment within the past 12 months; physiologic replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment. |

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| **Concomitant Treatment** | **Pembrolizumab**: 200 mg given via IV infusion every 3 weeks (q 3 weeks) up to a maximum of 24 months.<br>**As needed at Investigator discretion for the management of events noted below:**<br>**Bevacizumab and/or dexamethasone:**<br>Based on previous studies, the recommended best standard of care (BSC) for control of PTE or CE-related sequalae after PVSRIPO infusion and/or combination of PVSRIPO and pembrolizumab, as needed, are:<br>• Preferred ≥ 2 weeks post-PVSRIPO infusion: low dose bevacizumab (7.5 mg/kg q 3 weeks)<br>  o Treatment with bevacizumab for the shortest course possible is desired over dexamethasone use for control of PTE or CE, when deemed safe to use by the investigator<br>  o Treatment with higher doses of bevacizumab for > 2 cycles is not permitted per protocol<br>• Within 2 weeks of PVSRIPO infusion or thereafter, at the treating clinician's discretion if rapid results are required: low dose dexamethasone (≤ 4 mg/day)<br>  o As low a dose as possible, for the shortest duration possible is desirable for managing PTE or CE. However, participants should primarily be managed with bevacizumab, thereafter<br>• Other supportive care treatments (eg, anti-epileptics for seizure)<br>For the management of any immune-related AE (irAE) after the first dose of pembrolizumab, as low a dose as possible of dexamethasone (ideally ≤ 4 mg/day), for the shortest duration possible, is desirable. Patients requiring > 4 mg/day of dexamethasone or equivalent prior to the first dose of pembrolizumab will be replaced.<br>Any irAE should ultimately be managed at the investigator's discretion with patient safety as the primary consideration. |
| **Investigational Product(s)** | PVSRIPO: Oncolytic Polio/Rhinovirus Recombinant; $5x10^7$ tissue culture infectious dose $(TCID)_{50}$ delivered via CED intratumorally |
| **Reference Product (comparator)** | No active comparator at study outset (may be adapted to include a randomized comparator in the future); comparisons may be made to external controls in similar rGBM populations treated with PVSRIPO alone, pembrolizumab (or other anti-PD-1/ICI) alone or other to treatments/combination of treatments. |
| **Planned Study Sites** | Approximately 10 |
| **Criteria for Evaluation** | Laboratory and clinical evaluation including physical examination, KPS, neurological examination (including via the NANO scale) [8], assessment of AE and survival, as well as radiographic evaluations, will be assessed at regular intervals to evaluate safety and efficacy.<br>Biological samples, including blood/blood cells and tumor biopsy tissue (when available) will be collected to evaluate exploratory assessments of immune/genetic markers and other factors that may impact response. |

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| **Statistical Methods** | This single-arm study is designed to evaluate the efficacy, safety and tolerability of PVSRIPO when followed by pembrolizumab treatment in patients with rGBM. Summary statistics (eg, n, %, confidence intervals [CI]) will be employed to describe the key findings (ORR, DOR, incidence and severity of DLT, Grade 3 or higher AE, etc.).<br>Adverse events considered related to screening or other pre-infusion procedures that are not standard of care (eg, PV booster, biopsy prior to infusion, catheter placement without PVSRIPO infusion) will be summarized separately from TEAE (after PVSRIPO infusion), as will data from patients receiving only PVSRIPO and not at least one dose of pembrolizumab. The number of doses of pembrolizumab received and other baseline characteristics will also be explored as variables for safety and efficacy (eg, age, number of prior recurrences, KPS, NANO score, extent of prior resection, prior bevacizumab and chemotherapy use, tumor genetic profile).<br>All patients enrolled and treated with PVSRIPO will be summarized in all analyses. In addition, a per-protocol analysis will also explore the impact of excluding patients meeting pre-defined criteria from radiographic and survival analyses as outlined in **Section 8**. For comparisons of efficacy or safety relative to PVSRIPO alone or pembrolizumab alone from other data sources, both the overall and per-protocol populations may be considered.<br>The statistical analysis plan (SAP) will provide additional detail regarding planned statistical summary methods and analyses. |
| **Determination of Sample Size** | Based on an interim summary of the Phase 1 study data of PVSRIPO in rGBM (Pro00031169), an ORR of 11.5% was demonstrated (7/61 patients). With 30 subjects treated in this study, there is >75% power to demonstrate a 20% or greater improvement in ORR with the addition of pembrolizumab (ORR > 31%, two-sided P<0.05).<br>The planned evaluable sample size of 30 patients will allow initial assessment of the anti-tumor response via the criteria noted in **Table 3** and survival over time, to inform future study adaptations and/or the sample size and study design of new trials. The design will also allow for assessment of the efficacy, safety and tolerability of PVSRIPO plus pembrolizumab, relative to accumulated experience with PVSRIPO alone (eg, [5], data on file, Istari) and published or other data available with pembrolizumab alone in patients with rGBM (eg, [3]). |
| **Study and Treatment Duration** | Maximum planned duration of follow-up: Up to 24 months (104 weeks). Participants achieving 24 months of follow-up may be offered participation in a roll-over protocol or other mechanism for longer-term follow-up.<br>Planned treatment duration:<br>• Single PVSRIPO infusion at baseline (retreatment allowed for qualifying patients (ie, no ongoing AE ≥ Grade 2 related to peritumoral edema, tolerated initial PVSRIPO infusion well) with enhancing disease ≥ 6 months from initial PVSRIPO infusion (with or without post-infusion resection ≥ 4 weeks after retreatment); see **Section 3**).<br>• Pembrolizumab (200 mg IV infusion), q 3 weeks up to Week 104 (maximum of approximately 102 weeks of treatment), or until irAE meeting criteria for discontinuation or confirmed progression via criteria noted in **Table 3**). |

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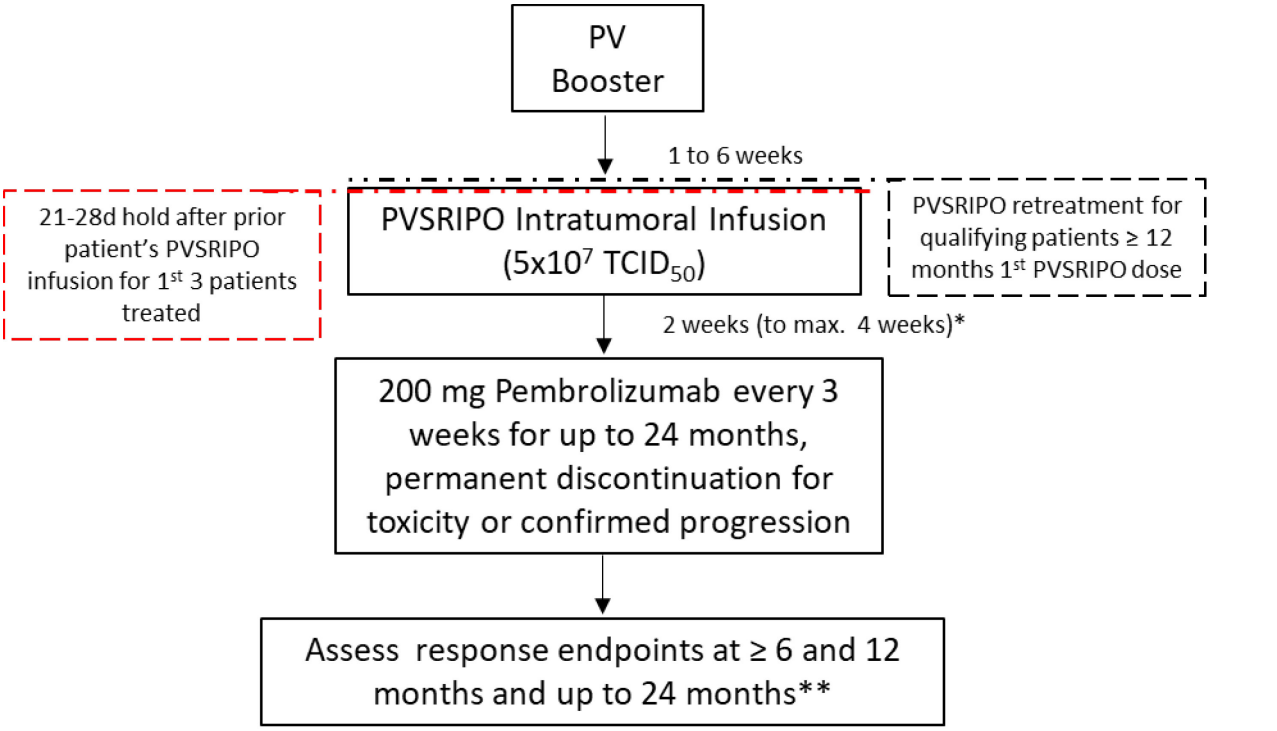
| | **Treatment interruption/discontinuation with pembrolizumab:**<br>Adverse events of an immunologic etiology, including infusion related reactions, have been associated with pembrolizumab exposure. Depending on the severity of the irAE, pembrolizumab should be temporarily withheld or permanently discontinued as outlined in **Section 7.5.3** and the most recent Keytruda® (pembrolizumab) package insert (PI) and "Guide for Keytruda®" provided in the study administration manual (SAM).<br>In general, for an irAE requiring dose interruptions, low-dose dexamethasone (≤ 4 mg/day) should be initiated and tapered when the irAE is ≤ Grade 1.<br>For patients with irAE meeting dose interruption criteria, the next dose of pembrolizumab will be withheld until the irAE has been reduced to ≤ Grade 1 and dexamethasone (or equivalent) dose has been tapered to ≤ 4 mg/day and/or discontinued.<br>Pembrolizumab should be permanently discontinued for severe infusion-related reactions and for irAE that do not resolve within 12 weeks of the last dose of pembrolizumab or dexamethasone (or equivalent) cannot be reduced to ≤ 4 mg/day within 12 weeks.<br>Subjects who discontinue pembrolizumab should continue to be followed until resolution of AEs and for clinical outcomes, as detailed in **Sections 5.4** through **5.6**. |

**Figure 1 Study Schema for Initial Safety/Efficacy Evaluation (n=30)**




* Patients not receiving 1st dose of pembrolizumab by 4 weeks-post PVSRIPO infusion will not receive pembrolizumab under this study; PVSRIPO only patients will continue to be followed as outlined.
**Response/progression utilizing criteria outlined under the SAP section (Table 3) and iRANO (exploratory).

## Table 1 Schedule of Assessments

| Description | Screening[1] | | Treatment and Follow-Up Periods[18] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screen. Period 1 | Screen. Period 2 | Catheter/ PVSRIPO[2] | Post-PVSRIPO infusion, pembrolizumab treatment and Follow-Up (± 7 days)[3] | | | | | | | |
| **Week** | Within 6 wks. of catheter placement | Within 1 wk. relative to catheter placement | | 1 | 2 | U (3-4)[5] | 5 or +3 wk. | 8 or +3 wk. | 11; + every 3 wks. to wk.104 max. | Follow-Up | End of Study visit |
| **Day** | Day -42 to -8 | Day -7 to -1 | 0 (+1 day) | 1 | 14 | (28) | 35 or +21 | 56 or +21 | +21d up to max. 730 days | From discontinuation of pembrolizumab | Anytime for permanent discontinuation |
| **Visit** | 1 | 2 | 3 | 4 | 5 | U | 6 | 7 | ≥ 8 | ≥ 8 | |
| General Evaluations | | | | | | | | | | | |
| Informed Consent | X | | | | | | | | | | |
| Medical History | X | X | X | | | | | | | | |
| Physical Exam[6,7] | | X | | X | X | X | X | X | X | | X |
| Neurologic Exam[7] | | X | | X | X | X | X | X | X | | X |
| NANO assessment[7] | | X | | X | X | X | X | X | X | | X |
| KPS[7] | | X | | X | X | X | X | X | X | | X |
| PRO[4] | | X | | X | | | X | | X | | |
| Adverse Events | | Continuous (including AE due to non-SOC screening procedures) | | | | | | | | | |
| Concomitant Medications | | Continuous (throughout the duration of the study) | | | | | | | | | |
| Lab Evaluations | | | | | | | | | | | |
| PV booster vaccine | X | | | | | | | | | | |
| CBC w/diff | X | X | X | X | X | X | X | X | X | | X |
| CMP | X | X | | X | X | X | X | X | X | | X |
| Pancreatitis labs (amylase, lipase) | | X | | X | X | X | X | X | X | | |
| PT, aPTT | | X | | | | | X | X | | | |
| Pregnancy Test[8] | | X | | | | | | | | | |
| Thyroid Panel[9] | | X | | | X | | | X | X | | |
| ATT IgG[10] | X | | | | | | | | | | |
| LSQ[11] | X | X | | | X | | X | | | | |
| Whole blood, research analyses[12] | X | X | | | X | | X | | X | | X |
| Disease Evaluations | | | | | | | | | | | |
| MRI[13] | X | | | | (X) | X | X | X | X | X | X |
| CT Scan[14] | | | X | | | | | | | | |

| Description | Screening[1] | | Treatment and Follow-Up Periods[18] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screen. Period 1 | Screen. Period 2 | Catheter/ PVSRIPO[2] | Post-PVSRIPO infusion, pembrolizumab treatment and Follow-Up (± 7 days)[3] | | | | | | | |
| **Week** | Within 6 wks. of catheter placement | Within 1 wk. relative to catheter placement | | 1 | 2 | U (3-4)[5] | 5 or +3 wk. | 8 or +3 wk. | 11; + every 3 wks. to wk.104 max. | Follow-Up | End of Study visit |
| **Day** | Day -42 to -8 | Day -7 to -1 | 0 (+1 day) | 1 | 14 | (28) | 35 or +21 | 56 or +21 | +21d up to max. 730 days | From discontinuation of pembrolizumab | Anytime for permanent discontinuation |
| **Visit** | 1 | 2 | 3 | 4 | 5 | U | 6 | 7 | ≥ 8 | ≥ 8 | |
| Biopsy/Tumor research analyses[15] | | | X | | | | | | | | |
| Unscheduled biopsy/tumor tissue collection[16] | | | | | | Continuous | | | | | |
| ORR/DOR/OS/ PFS | | | | | | Continuous | | | | | |
| Treatment(s) | | | | | | | | | | | |
| PVSRIPO[17] | | | X | | | | | | X | | |
| Pembrolizumab[18] | | | | | X | X | X | X | X | | |

Abbreviations: AE: adverse event; aPTT: activated partial thromboplastin time; ATT IgG: anti-tetanus toxoid immunoglobulin G; CBC with diff: Complete Blood Count with differential; CMP: Comprehensive Metabolic Panel; KPSL: Karnofsky performance status score; LSQ: lymphocyte Subset quantitation; NANO: Neurologic Assessment in Neuro-Oncology; PRO: patient-reported outcome; PT: prothrombin time; PV: poliovirus; SOC: standard of care; U: Unscheduled; Wk.: Week

[1] At Screening Period 1, lab evaluations will occur first, followed by patients receiving their single PV booster vaccine.

[2] Catheter placement and PVSRIPO infusion (+1 day window or extension as approved by Sponsor). PVSRIPO infusion occurs only following confirmation of GBM at time of catheter placement or within 6 weeks of visit 3. If diagnosis of GBM is not confirmed/patient not infused, patient is a screen failure. Platelet count should be confirmed prior to catheter placement as well as at time of catheter removal and should remain consistent with inclusion criteria 11. Should the platelet count be less than 125 x $10^9$/L on the day of catheter removal, a platelet transfusion should be administered and platelet count confirmed to be greater than 125 x $10^9$/L prior to catheter removal.

[3] Following Visit 4, all tests and procedures have a ±7-day window except for DLT assessment period for first 3 to 6 patients (21 days ± 3 days) post-first pembrolizumab infusion).

[4] Questionnaires in support of PROs to occur at visits 2, 4, 6, 8 and then every 9-12 weeks per scheduled imaging visit follow-up.

[5] Unscheduled (U): Visit occurs between visits 5 and 6 only if first dose of pembrolizumab is delayed from planned administration at visit 5. If first pembrolizumab infusion is delayed, the schedule of assessments shifts accordingly so the next visit occurs 3 weeks after the date of first pembrolizumab administration.

[6] Includes height, weight, body surface area, vital signs (blood pressure, heart rate, respiratory rate, temperature).

[7] Physical exam, neurologic exam with NANO and KPS daily after PVSRIPO infusion until day of discharge from hospital.

[8] Individuals of childbearing potential, pregnancy test (urine or serum) within 48 h of catheter placement. Repeat every 6 months or any time pregnancy suspected.

[9] Thyroid panel (at a minimum: TSH and free T4; may include free and total T3) should occur every 6 weeks while patient is receiving pembrolizumab.

[10] If undetectable anti-tetanus toxoid immunoglobulin G (ATT IgG) at screen, then Tdap booster vaccine > 1 week prior to biopsy/catheter placement.

[11] LSQ analysis should include the following (both %, and ABS): CD3 T, CD4 T, CD8 T, CD19 B, CD56 NK cells. LSQ should be collected at visit 1 (pre-boost), visit 2 (post-boost), and before pembrolizumab doses at visits 5 and 6.

[12] Collection of whole blood for biomarker analyses where indicated. Collected at visit 1 (pre-boost) and visit 2 (post-boost), before pembrolizumab doses at visits 5, 6, 8, and at End of Study visit for progression.

[13] MRI with and without contrast. Screening MRI conducted within 14 days prior to catheter placement. If neurologic deficit presents at visit 5 and pembrolizumab dose is held to days 15 to 28, MRI should be conducted and precede pembrolizumab administration. After week 11, MRI to occur every 9-12 weeks or more frequently, at the discretion of the treating physician.
[14] CT occurs before infusion to confirm placement criteria and after catheter is removed, post-infusion, to confirm no bleeding.
[15] If possible, up to 3 additional core biopsies will be obtained for biomarker testing (if not available from archival tissue obtained within 6 weeks).
[16] Results from an unscheduled or unplanned biopsy and/or surgical resection should be recorded in the eCRF, and remaining tissue may be requested for analysis.
[17] PVSRIPO retreatment may be allowed for confirmed progression $\geq$ 12 months post-initial PVSRIPO infusion with Sponsor/designee approval.
[18] All visit assessments should be conducted before pembrolizumab administration. Pembrolizumab should be administered every 3 weeks $\pm$ 1 week through week 104, with the first dose at day 14 but up to day 28, if required. If pembrolizumab is permanently discontinued, then safety laboratory evaluations that are not SOC are no longer required 30 days after the date of pembrolizumab discontinuation.

## Table of Contents

## List of Tables



## List of Figures

## List of Abbreviations

| Abbreviation | Stands For |
|---|---|
| AE | Adverse Event |
| ALP | Alkaline Phosphatase |
| ALT | Alanine Aminotransferase |
| ANC | Absolute Neutrophil Count |
| APC | Antigen Presenting Cell |
| aPTT | Activated Partial Thromboplastin Time |
| AST | Aspartate Aminotransferase |
| ATC | Anatomical Therapeutic Chemical |
| ATT | Anti-tetanus toxoid |
| BSC | Best Standard of Care |
| C | Celsius |
| CBC | Complete Blood Count |
| CE | Cerebral Edema |
| CED | Convection-Enhanced Delivery |
| CFR | Code of Federal Regulations |
| CI | Confidence Interval |
| cm | Centimeter |
| CMP | Comprehensive Metabolic Panel |
| CNS | Central Nervous System |
| CR | Complete Response |
| CT | Computed Tomography |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTLA | Cytotoxic T-lymphocyte-associated antigen |
| DCR | Disease Control Rate |
| DDC | Duration of Disease Control |
| dL | Deciliter |
| DLT | Dose Limiting Toxicity |
| DNA | Deoxyribonucleic Acid |
| DOR | Duration of Response |
| DRR | Durable Radiographic Response |
| DSMB | Data and Safety Monitoring Board |
| EC | Exclusion Criteria |
| eCRF | Electronic Case Report Form |
| EDC | Electronic Data Capture |
| EGFR | Epidermal Growth Factor Receptor |
| EGFRvIII | Epidermal Growth Factor Receptor (variant III) |
| EMA | European Medicines Agency |
| EOS | End of Study |
| F | Fahrenheit |
| FDA | Food and Drug Administration |
| FLAIR | Fluid-Attenuated Inversion Recovery |
| g | Gram |
| GBM | Glioblastoma |
| GCP | Good Clinical Practice |
| GTR | Gross Total Resection |
| h | hour |

| Abbreviation | Stands For |
|---|---|
| HBV | Hepatitis B Virus |
| hCG | Human Chorionic Gonadotropin |
| HCV | Hepatitis C Virus |
| HIPAA | Health Insurance Portability and Accountability Act |
| HRV2 | Human Rhinovirus Type 2 |
| HSA | Human Serum Albumin |
| IB | Investigator's Brochure |
| IC | Inclusion Criteria |
| ICF | Informed Consent Form |
| ICH | International Council of Harmonization |
| ICI | Immune Checkpoint Inhibitor |
| ICU | Intensive Care Unit |
| ID | Identification |
| IDH | Isocitrate Dehydrogenase |
| IEC | Institutional Ethics Committee |
| IFN | Interferon |
| IgG | Immunoglobulin G |
| IND | Investigational New Drug |
| IPOL | Poliovirus Vaccine Inactivated |
| IPHP | Investigational Product Handling Plan |
| irAE | Immune-related Adverse Event |
| iRANO | Immunotherapy Response Assessment for Neuro-oncology |
| IRB | Institutional Review Board |
| IRES | Internal Ribosomal Entry Site |
| IV | Intravenous |
| kg | Kilogram |
| KPS | Karnofsky Performance Status |
| LS | Landmark Survival |
| LSQ | Lymphocyte Subset Quantitation |
| MDSC | Myeloid-Derived Suppressor Cell |
| mg | Milligram |
| MGMT | $O^6$-methylguanine-DNA methyltransferase |
| mL | Milliliter |
| mM | Millimolar |
| MOA | Mechanism of action |
| MRI | Magnetic Resonance Imaging |
| NANO | The Neurologic Assessment in Neuro-Oncology scale |
| NK | Natural Killer |
| ORR | Objective Radiographic Response/Objective Response Rate |
| OS | Overall Survival |
| PBS | Phosphate Buffered Saline |
| PD | Progressive Disease |
| PD-1 | Programmed cell death protein 1 |
| PD-L1 | Programmed cell death-ligand 1 |
| PD-L2 | Programmed cell death-ligand 2 |
| PFS | Progression Free Survival |
| PHI | Protected Health Information |

| Abbreviation | Stands For |
|---|---|
| PI | Package Insert |
| PR | Partial Response |
| PT | Prothrombin Time |
| PRO | Patient-Reported Outcome |
| PTE | Peritumoral Edema |
| PV | Poliovirus |
| PV1S | Serotype 1 Live-Attenuated (Sabin™) PV Vaccine |
| PVSRIPO | Poliovirus/Rhinovirus Recombinant |
| q | Quaque (each or every) |
| rGBM | Recurrent Glioblastoma |
| RNA | Ribonucleic Acid |
| RT | Radiotherapy |
| SAE | Serious Adverse Event |
| SAM | Study Administration Manual |
| SAP | Statistical Analysis Plan |
| SD | Stable Disease |
| SJS | Stevens-Johnson syndrome |
| SOA | Schedule of Assessments |
| SOC | Standard of Care |
| SOP | Standard Operating Procedures |
| STR | Subtotal Resection |
| SUSAR | Suspected Unexpected Adverse Reaction |
| TAM | Tumor-Associated Macrophage |
| TBK1 | TANK-binding kinase 1 |
| $TCID_{50}$ | Median Tissue Culture Infectious Dose |
| Tdap | Diphtheria, tetanus, and whooping cough (pertussis) booster vaccine |
| TEAE | Treatment Emergent Adverse Event |
| TEN | Toxic Epidermal Necrolysis |
| TERT | Telomerase Reverse Transcriptase |
| $T_{max}$ | Maximum Temperature |
| Treg | Regulatory T Cell |
| TSH | Thyroid stimulating hormone |
| U | Unscheduled |
| µg | Microgram |
| ULN | Upper Limit of Normal |
| US | United States |
| USP | United States Pharmacopeia |
| VEGF | Vascular Endothelial Growth Factor |
| WHO | World Health Organization |
| Wk. | Week |

# 1 Introduction

Glioblastoma (GBM) is the most common primary central nervous system malignancy and has a poor prognosis. Standard first-line treatment includes maximal surgical resection followed by adjuvant radio-chemotherapy, which produces only modest survival benefit and tumors inevitably recur. Therapeutic options at the time of relapse are extremely limited; in recent years, only bevacizumab, a humanized anti-vascular endothelial growth factor (VEGF) monoclonal antibody and tumor treating fields have been approved for recurrent (r) GBM [9]. Despite these therapies and others, the median OS in rGBM patients failing earlier lines of therapy is still only 6 to 10 months, and there are very few long-term survivors [10]. Treatment failure is due in part to the presence of the blood-brain barrier and poor penetration of cytotoxic drugs into the tumor, coupled by the notorious heterogeneity and immunosuppressive nature of rGBM, limiting the therapeutic value of agents that target a single aspect of the disease [9, 11]. Clearly, new approaches are needed to improve outcomes.

Oncolytic virus-based immunotherapy for brain tumors is a unique approach with several advantages over more conventional drugs. For example, certain oncolytic viruses are capable of selective tumor cell killing with a range of inflammatory and immune-stimulatory effects on the tumor itself, the tumor stromal component and the host immune system at large. The objective of oncolytic immunotherapy is to recruit effective immune responses against tumor-associated antigens that can produce lasting immunologic control of cancers. This tumor-specific targeting may be enhanced by immune checkpoint blockade, unleashing the full power of the immune system to recognize and attack the malignancy.

PVSRIPO is a genetically recombinant, non-pathogenic PV/rhinovirus chimera with a tumor-specific and conditional replication phenotype. PVSRIPO is administered by CED directly into the growing tumor; there is no resection performed at baseline. Delivery via CED circumvents the blood-brain barrier, a process by which large molecules (> 400 daltons) are directly infused under pressure into a tumor through a catheter. This delivery method results in adequate distribution of such molecules into the tumor over large areas via inherent interstitial fluid pathways [12, 13].

Immunologically, tumor cells evade immunosurveillance and progress through different mechanisms, including activation of immune checkpoint pathways that suppress antitumor immune responses. The PD-1 signaling pathway is one such pathway that negatively regulates T-cell-mediated, anti-tumor immune responses. Clinical data shows that blockade of PD-1 significantly enhances antitumor immunity, leading to durable clinical response and long-term survival in some patients in a number of cancers (for a review, see [14]).

Therefore, the aim of this study in patients with rGBM is to examine the efficacy and safety/tolerability of PVSRIPO treatment followed by the ICI, anti-PD-1 antibody, pembrolizumab.

## 1.1 Indication

The disease under study is rGBM: a fast-growing and aggressive primary brain tumor that develops from star-shaped glial cells (astrocytes and oligodendrocytes). The OS is around 6 to 10 months depending on the therapeutic agents, number of recurrences, prior treatment failures, tumor mutations, KPS, etc. [10].

### 1.1.1 Current Therapies

Approved agents for the treatment of rGBM may include repeat surgical debulking, RT along with chemotherapeutics and targeted therapies. Examples from these classes include lomustine, temozolomide, bevacizumab, etoposide and procarbazine. Drug eluting wafers like polifeprosan 20 with carmustine and devices such as tumor treating fields are also approved. Despite this, the National Comprehensive Cancer Network Guidelines recognizes that there is no globally accepted standard of care (SOC) nor effective treatment options for rGBM leading to long-term survival; given the poor prognosis, patients are encouraged to enroll in clinical trials for access to novel therapies [10, 15, 16].

### 1.1.2 Investigational Therapies

There are several ongoing studies of experimental therapies alone or in combination with current therapies in recurrent glioblastoma (see: clinicaltrials.gov [17], under “condition or disease” search: recurrent glioblastoma).

## 1.2 Protocol Therapies

### 1.2.1 PVSRIPO Overview

PVSRIPO (investigational product) is a modified version of the serotype 1 live-attenuated (Sabin) PV vaccine (PV1S) with its cognate internal ribosome entry site (IRES) replaced with that of human rhinovirus type 2 (HRV2) (see **Figure 2**). Its immunogenic properties and low potential for long-term sequelae are expected to be similar to the vaccine. PV1S has been safely administered to > 10 billion individuals worldwide without untoward long-term sequelae. The administration of PV1S in humans leads to neutralizing immunity to PV.

The foreign internal ribosome entry site of PVSRIPO causes neuronal incompetence: a failure to recruit host ribosomes, translate viral genomes, and propagate in neurons, which ablates neurovirulence (ie, ability to cause infectious polio) [18].

**Figure 2 Genetic Structure of PVSRIPO**

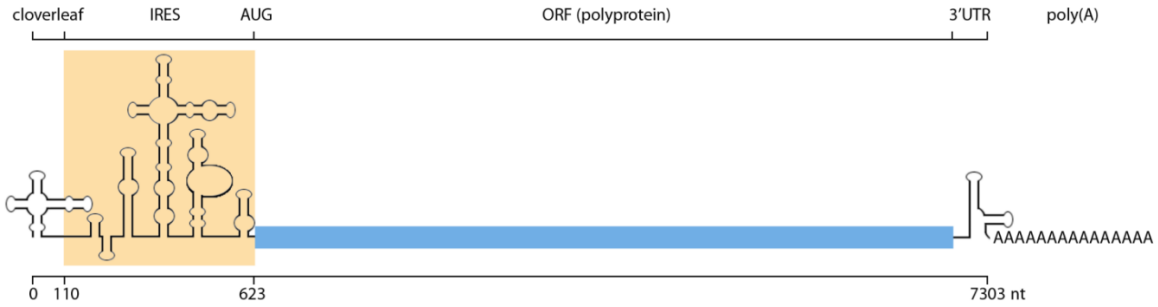



**Figure 2**: PVSRIPO is PV1S containing a heterologous IRES of HRV2. The IRES is a *cis*-acting, non-coding genetic element within the 5' untranslated region of all enteroviruses and is essential for translation of the viral genome. PVSRIPO is a non-enveloped, positive-sense single stranded RNA virus with a genome of ~7300 nucleotides in length. 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.

### 1.2.2 Summary of Nonclinical Studies with PVSRIPO

In animal tumor models, oncolytic PVs elicit efficient anti-neoplastic effects resulting in tumor regression and, eventually, destruction [19]. There is histologic evidence for direct, virus-mediated tumor cell killing and indirect, host-mediated inflammatory responses directed against tumor. PVSRIPO has undergone extensive dose-range finding, toxicology, biodistribution, shedding and neutralizing antibody tests with intrathalamic inoculation of up to 5 x $10^9$ $TCID_{50}$ in Cynomolgus monkeys (M. Fascicularis) [18]. These investigations revealed: (i) absence of morbidity and mortality; (ii) absence of neuropathological signs consistent with virus-induced central nervous system (CNS) damage; (iii) absence of virus dissemination from the brain or viremia; (iv) absence of extraneural replication; (v) absence of shedding in saliva, urine or stool; (vi) presence of a neutralizing antibody response.

### 1.2.3 Summary of rGBM Clinical Studies with PVSRIPO

The first-in-human PVSRIPO study (Pro00031169) investigated PVSRIPO delivered via CED in 61 adult patients with rGBM who failed prior SOC therapies (any recurrence; 1 to 5.5 cm contrast-enhancing supratentorial tumor). Dose levels ranging from $10^7$ up to $10^{10}$ $TCID_{50}$ were examined in a dose escalation phase followed by a dose expansion phase, which established the

recommended Phase 2 dose of $5x10^7$ $TCID_{50}$ [5]. In the dose-expansion phase (n=31), 19% of patients had a PVSRIPO-related AE of grade 3 or higher. Events most often attributed to PVSRIPO included those related to PTE or CE (eg, headache, pyramidal tract syndrome, seizure, dysphasia) and PVSRIPO was well tolerated systemically.

In the ongoing multicenter Phase 2 study (Pro00077024), as of August 2020, 120 adult rGBM patients with similar inclusion criteria as the Phase 1 study (Pro00031169; limited to 2nd recurrence), have been treated with PVSRIPO at $5x10^7$ $TCID_{50}$. Based on a recent safety summary of the ongoing Phase 1 and 2 studies conducted in February of 2020, TEAE with some degree of attribution to PVSRIPO treatment occurring in ≥ 20% of patients included localized inflammation with nervous system disorders related to PTE (eg, headache, pyramidal tract syndrome, seizure, dysphasia) and fatigue.

Based on an earlier published summary of the 61 patients in the Phase 1 study, the estimated percentage of rGBM patients surviving long-term (ie, landmark survival [LS]) in patients treated with PVSRIPO was 21% at 24 through 60 months [5]. This was favorable when compared to criteria matched external controls and a literature summary. The estimated percentage of patients surviving in the external control group was only 4% at 36 months and 2% at 60 months. In the summary of interventional studies of marketed and investigational agents in rGBM patients reported in the recent literature, the weighted mean estimate of patients surviving at 36 months was 2.71% (9 treatment arms) and 0.56% at 60 months (6 treatment arms) (data on file, Istari).

In addition, 4 patients have been successfully treated with PVSRIPO via compassionate use (single patient protocol/Investigator Investigational New Drug [IND]), which was followed by at least one dose of pembrolizumab at the treating investigator's discretion. These limited experiences demonstrate initial feasibility of the approach. To date, no serious adverse events (SAE) or events of increased severity beyond those expected for each agent were noted with the combination (data on file, Istari). Indicators of potential and early onset anti-tumor activity (eg, "soap-bubble" degeneration of PVSRIPO infused enhancing lesion) in these limited experiences also support expansion of this approach in a formal protocol powered to demonstrate an appreciable improvement in response relative to single agent therapy, while rigorously evaluating safety.

### 1.3 Pembrolizumab (Overview in GBM)

Immune checkpoint inhibitors have been studied extensively in GBM (for reviews, see [6, 7]). Clinical studies with pembrolizumab and other PD-1 inhibitors have shown that these agents have been relatively well tolerated in rGBM patients, with low rates of discontinuation due to AEs related to cerebral edema [3, 4, 20, 21]. However, efficacy results with PD-1 inhibitors have

generally showed disappointing results when administered as a single agent in rGBM patients and even in combination with approved agents. For example, in a Phase 2 study in rGBM patients, pembrolizumab alone or in combination with bevacizumab had limited activity [21].

Encouraging improvements in OS were noted in small single agent trials of pembrolizumab in rGBM patients eligible for surgical resection, when administered in the neoadjuvant setting (given both prior to and after resection); n=15 (median OS of 13.57 months [3]) and in another 15 patient trial investigating the same (median OS of 20 months) [4], but not when administered only after resection (median OS of 7.5 months [3]). These findings suggest that other stimuli may be required to recruit immune cells to the tumor even with checkpoint blockade. Preliminary evidence supportive of this concept has been noted via an ongoing investigation with another oncolytic virus (oncolytic adenovirus, DNX-2401) in combination with pembrolizumab. A recent interim analysis reported that 47% of patients receiving the combination (n=48) experienced clinical benefit, noted as stable disease or better [22].

### 1.4 Study Rationale

At this time, there are no immune therapies indicated for GBM, a highly immunosuppressive tumor [11]. Learning from previous studies, the future of immune therapy for GBM appears most hopeful for combinations that seek to increase expression of tumor-specific neo-antigens, coupled with checkpoint inhibition, to overcome the profound immune blockade associated with this disease.

The mechanistic rationale for following PVSRIPO therapy with the PD-1 inhibitor pembrolizumab, in patients with rGBM, is supported by the following:

1. Tumor infection with PVSRIPO causes direct tumor cell killing, which is known to induce the release of tumor-specific antigens that facilitate antitumor immunity [2].
2. PVSRIPO also sub-lethally infects antigen presenting cells (APCs), such as tumor-associated macrophages and dendritic cells. This causes chronic, sub-lethal viral propagation, provoking a persistent type I interferon (IFN)-dominant inflammatory response [2].
3. TANK-binding kinase 1 (TBK1) plays a pivotal role in antiviral innate immunity and the oncolytic virus PVSRIPO works by engaging a TBK1-IFN regulatory factor 3-driven type-I/III IFN response [2]. This powerful signal to T-cell activation is naturally restricted by PD-1. Therefore, combining PVSRIPO with an anti-PD-1 agent is mechanistically logical to provoke a maximal, antitumor, T-cell response in immunologically “cold” tumors, such as rGBM.
4. This direct lytic tumor cell killing, coupled with infection and pro-inflammatory stimulation of APCs, produces tumor antigen-specific, anti-tumor immunity. Thus,

PVSRIPO facilitates recognition and targeting of tumors by the immune system, which should act synergistically with pembrolizumab.

5. Mouse models of GBM support this paradigm; the combination of PVSRIPO with a ICI leads to a greater anti-tumor response than either agent alone (see **Figure 3**).
6. The clinical experience, to date: 4 patients have been treated with PVSRIPO followed by at least one dose of pembrolizumab under expanded access/compassionate use, demonstrating initial feasibility and tolerability of the approach. Indicators of potential and early onset anti-tumor activity in these limited experiences support expansion of this approach in a formal protocol.

Given this supportive information, we hypothesize that mechanistically, use of PVSRIPO and pembrolizumab together may act synergistically to yield superior response and survival rates relative to either agent used alone, in a tolerable manner.

**Figure 3 Mouse Glioma Model Shows Greater Reduction in Tumor Volume with a Murine PVSRIPO in Combination with PD-1 Inhibition than Either Agent Alone**

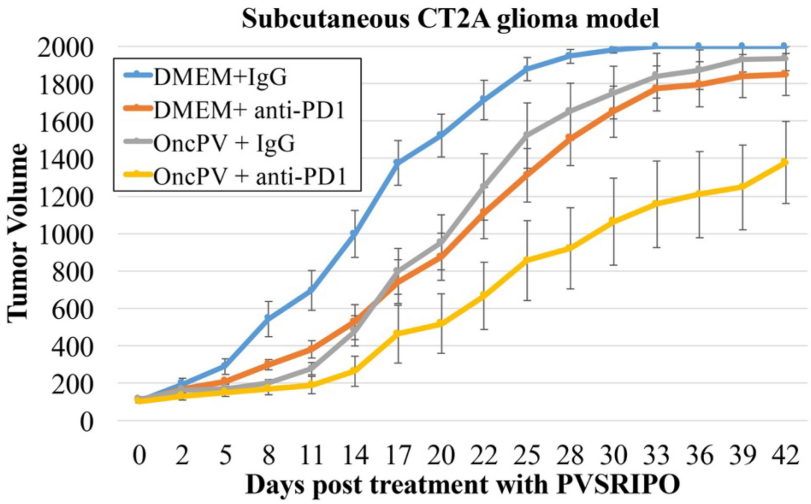



**Figure 3:** CT2A-CD155 tumors were implanted subcutaneously and treated with mock (DMEM)/mRIPO (mouse equivalent of PVSRIPO; denoted as OncPV above; 5 x $10^6$ TCID) on day 0. Anti-PD1/control immunoglobulin G (IgG)2A was administered intraperitoneally on days 0, 3, 6 and 9 (250µg at each interval). Four experimental groups were treated as follows (n=12 each group): (i) Mock/Mock: DMEM (mRIPO control) + IgG2A (250µg; anti-PD1 control); (ii) Mock/anti-PD1: DMEM (mRIPO control) + anti-PD1 (250µg); (iii) mRIPO/Mock: PVSRIPO (5 x $10^6$ TCID) + IgG2A (250µg; anti-PD1 control); (iv) mRIPO/anti-PD1: PVSRIPO (5 x $10^6$ TCID) + anti-PD1 (250µg) (data on file, Istari).

### 1.4.1 Rationale for Dose Selection

**PVSRIPO:** As noted, the dose for PVSRIPO treatment and retreatment (if applicable) was selected based on IND-directed toxicity studies and the Phase 1 study (Pro00031169;

ClinicalTrials.gov Identifier: NCT01491893) in adults with rGBM (n=61) and is supported by the data in over 100 patients in the ongoing multicenter Phase 2 study (Pro00077024; ClinicalTrials.gov Identifier: NCT02986178). The PVSRIPO dose identified in the Phase 1 study, being used in the Phase 2 study and to be utilized in this study is $5x10^7$ $TCID_{50}$.

**Pembrolizumab:** The recommended dose of pembrolizumab is keeping with prior investigations in rGBM [3, 4, 20, 21] that were well tolerated: 200 mg administered as an IV infusion over 30 minutes every 3 weeks. Treatment is planned to continue up to a maximum of Week 104 (approximately 23.5 months), until confirmed disease progression or unacceptable toxicity leading to discontinuation.

# 2 Study Objectives and Endpoints

## 2.1 Efficacy, Safety, and Exploratory Objectives and Endpoints

The study objectives and corresponding endpoints are described in greater detail below and are summarized in **Table 2**. Endpoints evaluated by iRANO criteria will focus on patients treated with PVSRIPO followed by at least one dose of pembrolizumab.

### 2.1.1 Primary Efficacy Endpoints

- ORR: Proportion of patients with confirmed CR or PR.
- DOR: KM curves showing median DOR for all patients that have confirmed CR or PR.
- DRR: Proportion of patients with confirmed response (CR or PR) that lasts for ≥ 6 months.

Objective response (CR or PR), stable disease and confirmed progression will be determined via criteria noted in **Table 3**.

### 2.1.2 Primary and Secondary Safety Endpoints

**Primary:** Safety and tolerability, including assessment of DLT, will be assessed by evaluating the frequency and severity of TEAE via CTCAE (v5.0) when PVSRIPO is followed by at least one dose of pembrolizumab.

**Secondary**: Patients receiving PVSRIPO alone will also be summarized for frequency and severity of TEAE but separately and as a secondary endpoint.

**Table 2 Summary of Study Objectives and Endpoints**

| Objectives | Endpoints/Evaluation Criteria |
|---|---|
| **Primary (efficacy)** | |
| Anti-tumor activity: | • ORR: Proportion of patients with confirmed CR or PR.<br>• DOR: KM curves showing median DOR for all patients that have confirmed CR or PR.<br>• DRR: Proportion of patients with confirmed response (CR or PR) that lasts for ≥ 6 months. Radiographic outcomes will be assessed via iRANO criteria [1] in this protocol. |
| **Primary (safety)** | |
| Safety/tolerability: | • Frequency and severity of TEAE via CTCAE (v5.0) for patients receiving PVSRIPO and at least one dose of pembrolizumab. |
| **Secondary (efficacy)** | |
| Anti-tumor activity: | • DCR: Comprised of patients achieving CR, PR or SD for ≥ 6 months.<br>• DDC: KM curves showing median DDC for patients with confirmed CR, confirmed PR or SD for ≥ 6 months. |
| Survival | • Overall and landmark survival: Proportion of patients alive at ≥ 6, ≥ 12 and at 24 months post-PVSRIPO infusion as calculated by Kaplan-Meier methods. |
| Progression free survival | • If calculable, PFS will be estimated based on the time from PVSRIPO infusion to death or confirmed progression, based on the definition of progression noted in **Table 3.** |
| **Secondary (safety)** | |
| | • Frequency and severity TEAE via CTCAE for patients receiving PVSRIPO alone without pembrolizumab (any reason). |
| **Exploratory** | |
| Identification of biomarkers of anti-tumor response to PVSRIPO followed by pembrolizumab | Assessment of tumor tissue and blood samples for identification of genetic, cytologic, histologic and/or other markers that demonstrate pharmacodynamic activity of PVSRIPO or correlate with anti-tumor response. |
| Alternative assessment of radiographic response/progression/PFS | Radiographic outcomes will also be analyzed as described in **Table 3** for endpoint determination.<br>All response must be confirmed via two consecutive MRI assessments at least 4 weeks apart. If on bevacizumab therapy when response first noted, response will be confirmed ≥ 8 weeks off bevacizumab, via MRI. |
| Patient-reported outcomes | Assessment of patient-reported quality of life and activities of daily living capabilities |

## 2.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints will include the following and will be assessed through a maximum of 24 months post-PVSRIPO infusion:

- DCR: Comprised of patients achieving CR, PR or SD for ≥ 6 months.
- DDC: KM curves showing median DDC for patients with confirmed CR, confirmed PR or SD for ≥ 6 months.
- Overall and landmark survival: Proportion of patients alive at ≥ 6, ≥ 12 and 24 months post-PVSRIPO infusion as calculated by Kaplan-Meier methods.
- Progression free survival: Calculated from the time of PVSRIPO infusion to death or confirmed progression via the definition outlined in **Table 3**.

## 2.3 Exploratory Endpoints

Exploratory endpoints include assessment of changes in markers of immune function from baseline and identification of genetic, cytologic, histologic and/or other markers (ie, biomarkers) that may correlate with response via analysis of biologic samples (includes blood, serum, cells, tumor biopsy).

In addition, radiographic response and PFS as determined via iRANO criteria will also be explored, and patient-reported outcomes will be assessed over the course of treatment.

# 3 Study Design

To establish therapeutic intent and adequately assess the risk/benefit profile, in the first protocol amendment, the study sample size was adjusted to allow for sufficient statistical power to demonstrate a meaningful difference in response, relative to monotherapy, and efficacy was elevated to a primary endpoint.

This is an open-label, single-arm, non-randomized study characterizing the efficacy, safety and tolerability of PVSRIPO delivered via intratumoral infusion followed by pembrolizumab in patients with rGBM.

Approximately 30 eligible participants, 18 years of age or older, will be enrolled and treated with PVSRIPO and at least a single dose of pembrolizumab in this study. The maximum planned duration of follow-up in this study is up to 24 months (104 weeks), and consists of the following treatments:

- Single PVSRIPO infusion at baseline.
    - Note: retreatment with PVSRIPO is allowed for qualifying patients with enhancing disease ≥ 12 months from initial PVSRIPO infusion
- Pembrolizumab (200 mg IV infusion) initiated 14 days after PVSRIPO infusion (up to 28 days, if necessary), given q 3 weeks, through Week 104 (23.5 months of treatment) or until irAE meeting criteria for dose interruption/discontinuation or confirmed progression (must consider clinical and radiographic assessments as outlined in **Section 8.4.6.1** and **Table 3**)

Patients reaching 24 months on this study may be offered participation in a longer-term follow-up/PVSRIPO retreatment protocol or other mechanisms.

The study will occur in the following periods as outlined in **Table 1** and **Figure 1.**

- Screening Period 1: Between 1 and 6 weeks prior to catheter placement
- Screening Period 2: Within 7 days of catheter placement
- PVSRIPO Catheter Placement and Biopsy (if required) Period: Day 0
- PVSRIPO Infusion Period/Baseline: Single PVSRIPO infusion at Day 0 or Day 1 (unless extension granted by Sponsor)
- Post-PVSRIPO infusion, pembrolizumab treatment and follow-up Period: pembrolizumab starting at Week 2 (up to Week 4, max) and every 3 weeks thereafter, to a maximum of Week 104 (approximately 23.5 months maximum pembrolizumab treatment duration)

Participants with enhancing disease ≥ 12 months from a prior PVSRIPO infusion and meet the key IC/EC for retreatment will be eligible for additional PVSRIPO infusion(s).

The study may be adapted or expanded in one or more larger cohorts of participants, in consultation with the DSMB, based on interim review and considering the totality of the study data.

### 3.1 Safety Run-in and DLT Assessment

This study will start with a safety run-in period. The first 3 participants will be sequentially enrolled, with a mandatory waiting period of 21 to 28 days after the immediate prior patient's PVSRIPO treatment before the next patient receives PVSRIPO. The 21- to 28-day waiting period also applies after the third patient receives PVSRIPO, prior to PVSRIPO treatment of the fourth patient.

The DLT evaluation period for the first 3 patients will begin once a patient who received PVSRIPO receives the first dose of pembrolizumab and continue for 21 days (± 3 days to

account for variations in patient visit scheduling, weekends, etc.) after this first dose of pembrolizumab. This wait period allows sufficient time for safety review prior to the next patient receiving their first dose of pembrolizumab. If ≥ 2 of the first 3 patients treated with PVSRIPO followed by 1 dose of pembrolizumab experience a DLT, accrual will be halted, and the treatment plan may be modified, or the study may be closed.

If no DLT occurs in the first 3 participants, the dosing regimen will continue as planned and an additional 27 participants will be enrolled to further characterize safety and anti-tumor activity of treatment with PVSRIPO plus pembrolizumab.

However, if only 1 patient experiences a DLT of the first 3 participants treated, an additional 3 participants will be enrolled (without a waiting period between participants) to undergo DLT evaluation. If ≤ 33% of participants (ie, ≤ 2 of 6 total) experience a DLT, the 24 remaining participants will be enrolled. If > 33% of participants (ie, ≥ 3 of 6) experience a DLT, the study will be halted, and the treatment plan modified, or the study may be closed. After the DLT evaluation period, ongoing monitoring of TEAEs will occur on at least a monthly basis by the Sponsor safety review team and by the DSMB, as specified in the safety plan and applicable charter, respectively.

If one of the first 3 (or 6) participants accrued withdraws from the study during the DLT evaluation period for reasons unrelated to the occurrence of a DLT, the participant will be replaced for the purpose of the initial DLT assessment. For details regarding events and criteria for meeting the definition of a DLT, see **Section 7.4**.

### 3.2 Delay in pembrolizumab administration for ongoing peritumoral or cerebral edema (or other TEAE/reasons)

Peritumoral edema may be associated with PVSRIPO treatment and there is potential for the same after immune checkpoint inhibition. As such, the first planned dose of pembrolizumab (scheduled 2 weeks after PVSRIPO infusion) will be delayed by up to two additional weeks in participants with any ongoing ≥ Grade 3 PTE or CE or ≥ Grade 2 AEs related to PTE or CE following PVSRIPO infusion requiring active treatment, such as new or worsening neurologic signs and symptoms (eg, hemiparesis requiring ≤ 4 mg/day dexamethasone or seizure requiring anti-epileptics), until such events resolve to ≤ Grade 1.

If such events have not resolved to ≤ Grade 1 by 4 weeks post-PVSRIPO infusion, or the patient has not received pembrolizumab for any reason by 4 weeks post-PVSRIPO infusion, the participant will not receive pembrolizumab under this trial and will be replaced. If a participant requires > 4 mg/day of dexamethasone or equivalent prior to the first dose of pembrolizumab, the

participant will not receive pembrolizumab under this trial and will also be replaced. These replaced participants will not be considered in the initial DLT assessment of the treatment with PVSRIPO and pembrolizumab.

# 4 Study Population

## 4.1 Inclusion Criteria

All tumor size and location-related eligibility criteria listed below will be independently verified by the Sponsor or their designee prior to patient enrollment.

A patient will be eligible for inclusion in the study if he/she meets all the following criteria prior to Day 0 (ie, catheter placement for PVSRIPO infusion) and after catheter placement, where specified:

1. ≥ 18 years of age.
2. Actively growing recurrent supratentorial glioblastoma confirmed by biopsy by the site's neuropathologist or designee.
    - Recurrent glioblastoma histologically confirmed within 6 weeks prior to PVSRIPO infusion will not require a biopsy to confirm active tumor prior to catheter placement. However, biopsy confirmation of glioblastoma must be provided prior to PVSRIPO infusion if histological confirmation is not completed within 6 weeks prior to catheter placement.
    - For the purpose of this protocol, "recurrence" is defined by either progression of primary glioblastoma or transformation from a lower grade to a higher grade. Note that in the case of transformation, this change must be confirmed via histology by site pathologist.
3. Enhancing lesion ≥1 cm shortest diameter to ≤ 5.5 cm longest diameter in all planes; occurrence within the prior resection cavity should not contribute to the longest diameter measurement.

4. Before catheter placement based on screening MRI and at the time of catheter placement via CT prior to infusion, neurosurgical investigator must confirm both tumor location (≥ 1 cm from eloquent brain) as well as placement of infusion catheter within or through the progressive enhancing tumor is feasible and at a safe distance relative to eloquent brain function, with the tip of the catheter being placed:
    a. Within the enhancing portion or in the vicinity of enhancement of target lesion (ie, infiltrative disease)
    b. ≥ 0.5 cm from ventricles
    c. ≥ 1 cm deep into the brain
    d. ≥ 0.5 cm from the corpus callosum
5. First or second relapse supported by MRI or CT scan; relapse is defined as progression following initial/prior therapy(ies).
6. Failed previous first line therapy: maximum surgical resection and RT (plus concomitant chemotherapy followed by maintenance chemotherapy if unknown or MGMT promoter). Patients who begin but do not complete chemotherapy/RT may still be considered for eligibility at the discretion of Sponsor.
7. KPS ≥ 70 at screening and baseline.
8. Undergone prior vaccination against PV and received a boost immunization with trivalent IPOL® (Sanofi-Pasteur SA) at least 1 week, but less than 6 weeks, prior to administration of PVSRIPO (within 6 months of PVSRIPO retreatment). Note: Patients who are unsure of their prior vaccination status/who have not been vaccinated must provide proof of vaccination and/or evidence of anti-PV immunity prior to enrollment, as applicable.
9. Ability to safely discontinue anti-coagulant therapy(ies) prior to biopsy/catheter placement, as required per site/surgical guidelines, in the opinion of the investigator.
10. Hemoglobin ≥ 9 g/dL prior to biopsy/catheter placement.
11. Platelet count ≥ 100,000/μL (unsupported); ≥ 125,000/ μL (can be supported via platelet transfusion) at biopsy/catheter placement and/or with a Sponsor-approved bone marrow stimulant/colony stimulating factor, eg, romiplostim).
12. ANC ≥ 1000/μL prior to biopsy/catheter placement.
13. Creatinine ≤ 1.2 x ULN prior to biopsy/catheter placement.
14. Total bilirubin, ALT, AST, ALP ≤ 2.5 x ULN prior to biopsy/catheter placement.
15. PT and aPTT ≤ 1.2 x ULN prior to biopsy/catheter placement.
16. If undetectable ATT IgG at screen, Tdap booster vaccine > 1 week prior to biopsy/catheter placement.
17. Patients must be willing and able to understand and provide written informed consent.

## 4.2 Exclusion Criteria

A patient will not be eligible for inclusion in this study if he/she meets any of the following criteria prior to Day 0 (PVSRIPO catheter placement):

1. Received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways) ≤ 12 weeks prior to PVSRIPO infusion (Note: does not apply for patients treated with pembrolizumab under this protocol who are eligible for PVSRIPO retreatment). Patients who had previously permanently discontinued any anti-PD-1 or PD-L1 therapy due to severe or life-threatening immune-related AE are excluded.
2. Excluded are:
    a. Neoplastic lesions in the brainstem, cerebellum, or spinal cord
    b. Radiological evidence of active/growing multifocal disease: no size increase > 0.5 cm in any direction of any other enhancing non-target lesions present at baseline confirmed via most recent, prior, consecutive MRIs at least 3 months apart. However, two lesions connected by T2 flair are not excluded.
    c. Tumors with ≥ 1cm of contrast-enhancing tumor component crossing the midline (crossing the corpus callosum)
    d. Extensive subependymal disease: multiple lesions or lesions covering > 50% of subependymal space. Tumor touching subependymal space allowed.
    e. Extensive leptomeningeal disease: multiple lesions or lesions covering > 50% of leptomeninges. Tumor touching leptomeninges allowed.
3. Has received systemic immunosuppressive treatments other than systemic corticosteroids (eg, methotrexate, chloroquine, azathioprine) within six months of PVSRIPO infusion.
4. Requires treatment with high dose systemic corticosteroids, defined as dexamethasone > 4 mg/day or equivalent, within 2 weeks of PVSRIPO infusion.
5. Prior interstitial brachytherapy, implanted chemotherapy, stereotactic radiosurgery or therapeutics delivered by local injection or CED, including PVSRIPO (except for qualifying patients being retreated with PVSRIPO within this trial).
6. Pregnant and/or breast feeding female; patient/female partner of childbearing potential who is unwilling to utilize protocol-defined acceptable form of contraception for duration of study.
7. Impending/life-threatening cerebral herniation syndrome, per neurosurgeon/designate.
8. Severe, active co-morbidity, defined as follows:
    a. Infection requiring IV treatment/unexplained febrile illness ($T_{max}$ > 99.5°F/37.5°C)
    b. Known immunosuppressive disease/human immunodeficiency virus infection
    c. Known active hepatitis B or C infection via positive viral DNA or RNA, respectively
    d. Unstable or severe intercurrent medical conditions such as severe heart disease (New York Heart Association Class 3 or 4)
    e. Known lung disease with forced expiratory volume in 1$^{st}$ second of expiration < 50%
    f. Uncontrolled diabetes mellitus (eg, hemoglobin A1C level > 7.0% with treatment)
    g. History of other malignancy requiring active treatment within 2 years of biopsy/catheter placement with the exception of those with a negligible risk of

metastasis or death (eg, resected cutaneous basal cell carcinoma, or other cancers with 5-year OS of > 90%)

9. Known albumin allergy.
10. Uncontrolled unexplained bleeding and/or hemoptysis within 4 weeks of planned PVSRIPO infusion.
11. Inability to undergo brain MRI with and without contrast. History of severe/anaphylactic reaction to gadolinium contrast agent is excluded. Mild allergy (eg, rash) acceptable with prophylactic acetaminophen and diphenhydramine.
12. History of neurological complications due to PV infection.
13. Not recovered from toxic side effects (alopecia acceptable) and/or no current or prior tumor treatments within the following timeframe relative to biopsy/catheter placement:
    a. Chemotherapy or bevacizumab ≤ 4 weeks (except for nitrosourea [6 weeks] or metronomic dosed chemotherapy such as daily temozolomide, etoposide or cyclophosphamide [1 week])
    b. Tumor treating fields ≤ 7 days
    c. RT of brain ≤ 12 weeks, except for progressive disease outside of the radiation field or 2 progressive scans at least 4 weeks apart or histopathologic confirmation
14. History of agammaglobulinemia.
15. Known hypersensitivity to pembrolizumab, or any components of pembrolizumab.
16. Active autoimmune disease requiring systemic immunomodulatory treatment within the past 12 months; physiologic replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment.

### 4.2.1 Additional Exclusion Criteria per Sponsor or Designee: Notification of Enrollment Goal Met

1. The study is targeting balanced enrollment of patients with and without any prior anti-angiogenic or anti-VEGF-targeted agent uses (eg, bevacizumab, cediranib, aflibercept, vandetanib, cabozantinib, sunitinib). As such, once 50% of planned patients (ie, 15 patients) are enrolled into either group, enrollment into the other group may end and future patients with that criterion may not be eligible. For example, after 15 patients who received prior anti-VEGF therapy are enrolled and treated with PVSRIPO and ≥ 1 dose of pembrolizumab, enrollment may be limited to only patients without prior anti-VEGF therapy use.
2. The study is targeting enrollment of ≥ 50% of patients who failed a prior chemotherapy regimen. If the study enrolls 40% of subjects who did not fail a prior chemotherapy regimen (ie, 12 of the first 30 subjects had no prior chemotherapy, which is not required if MGMT promotor unmethylated), enrollment into this group may be halted and future patients who did not have any prior chemotherapy may not be enrolled.

## 4.3 Patient Identification (ID)

Patients will be identified by a unique numeric patient ID assigned at time of screening.

## 4.4 Removal, Replacement, or Early Withdrawal of Patients from Therapy or Assessment

Participants may voluntarily withdraw from the study at any time.

The Investigator may also withdraw a participant from the study at any time based on his/her discretion. Reasons for Investigator-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 (in keeping with the criteria outlined in **Table 3**)
- Pregnancy
- Upon request of the participant
- If, in the Investigator's medical judgment, further participation would be injurious to the participant's health or wellbeing
- Development of intolerable symptoms
- Protocol deviation
- Non-compliance of the participant
- Administrative issues

## 4.5 Handling of Withdrawals

Participants withdrawing from the study prior to receiving PVSRIPO infusion, either voluntarily or due to ineligibility, will be considered non-evaluable in all but the screening analysis set (see **Section 8**); these patients will be replaced. Minimal information will be retained in the study database for screen failures.

Patients who are treated with > 4 mg/day dexamethasone (or equivalent) prior to receiving the first dose of pembrolizumab and patients who do not receive ≥ 1 dose of pembrolizumab after PVSRIPO will also be replaced and will not contribute to the PVSRIPO plus pembrolizumab (ie, combination) datasets. These subjects will continue to be followed for safety and efficacy and will be summarized separately.

If patients do not enter the voluntary follow up period, all information as described at the next scheduled study visit if <11 weeks after PVSRIPO treatment or at the End of Study (EOS) visit, should be obtained, if possible and as applicable.

Handling of early withdrawals for analyses are outlined in **Section 8**.

### 4.6 Sponsor's Termination of Study

This study can be terminated at any time for any reason by the Sponsor. If this occurs, all participants should be notified as soon as possible to schedule their last study visit.

## 5 Investigational Plan and Study Procedures

### 5.1 Screening Period

The patient's most recent MRI, diagnosis and all other recent and relevant information should be considered against the study eligibility criteria prior to formally considering a patient for signing of informed consent and commencement of subsequent screening assessments and procedures. For example, subjects with tumor diameters > 5.5 cm (and which are not part of a prior resection cavity) noted on their most recent MRI should not be screened for inclusion on this study.

#### 5.1.1 Screening Period 1 (within 6 weeks to 8 days of catheter placement) (Visit 1)

For potentially qualifying patients, the first screening examination period will take place within 6 weeks, to at least 1 week, prior to catheter placement for PVSRIPO infusion and the patient will have the assessments below completed (see **Table 1**).

**Administrative:**

- An informed consent form (ICF) must be signed by the patient before any screening procedures are completed.
- Medical History (includes details of tumor diagnosis, characteristics including molecular genetic testing, tumor treatment and surgical history, and evaluation of pre-existing conditions)

**Clinical:**

- AE assessment of any non-SOC screening procedures

**Laboratory Assessments:**

- A blood test for lymphocyte subset quantitation (LSQ) and anti-tetanus toxoid (ATT) IgG (local lab)
    - Note: if ATT IgG is negative, must have a tetanus (Tdap) booster vaccine administered > 1 week from planned catheter placement for PVSRIPO infusion.
- Complete blood count (CBC) with differential (SOC/local lab)
- Comprehensive metabolic panel (CMP; SOC/local lab)
    - A blood draw for immunologic, genetic or other analyses will occur (whole blood).
    - These samples will be transferred to the designated laboratory in keeping with the SAM
    - After completion of blood draws for immune analysis, a single booster immunization of trivalent inactivated IPOL® (Sanofi-Pasteur SA; obtained via site)
        - ≥ 1 week but ≤ 6 weeks of the initial PVSRIPO infusion
        - ≥ 1 week but ≤ 6 months for any PVSRIPO retreatment

### 5.1.2 Screening Period 2 (as close to infusion as possible but within 7 days prior to catheter placement, unless otherwise noted) (Visit 2)

Whenever possible, when confirming eligibility based on the study IC/EC, the least invasive screening procedures (eg, KPS) noted below and in **Table 1** should be performed first and the most invasive, performed last.

As close to the planned PVSRIPO infusion as possible but at least within 7 days before catheter placement, the following will be conducted:

**Clinical assessments:**

- Medical history (since Screening 1)
- Physical examination
- Neurologic and NANO assessment
- KPS
- Patient-Reported Outcome (PRO) Assessment
- AE Assessment
- Concomitant Medications

**Radiographic assessments:**

- Baseline MRI (or imaging with volumetric quantitation ability) of the brain with and without gadolinium contrast (within 14 days of biopsy/catheter placement)

**Laboratory assessments:**

- CBC with differential
- CMP
- Pancreatitis labs (amylase, lipase)
- Prothrombin time (PT; SOC/local lab) and activated partial thromboplastin time (aPTT; SOC/local lab)
- Thyroid panel (to include at a minimum: TSH, free T4 (thyroxine); may include free and total T3 (triiodothyronine); SOC/local lab)
- LSQ
- Whole blood collected for research analyses

**Within 48 hours of but prior to planned catheter placement:**

- Urine or serum-based pregnancy test for women of childbearing potential (urine or serum acceptable; a serum-based pregnancy test should be conducted in the event of equivocal results noted with urine test)

## 5.2 PVSRIPO Treatment Period

See **Table 1** for the complete study schedule of assessments during the PVSRIPO treatment period and pembrolizumab treatment and follow-up period.

### 5.2.1 Day 0 (Visit 3; catheter placement)

The following will occur on the day of PVSRIPO infusion:

**Clinical assessments (prior to PVSRIPO infusion):**

- Medical history, including any changes noted since Screening Period 2

**Laboratory assessments:**

- CBC with differential (Platelet count should be confirmed prior to catheter placement as well as at time of catheter removal and should remain consistent with inclusion criteria 11. Should the platelet count be less than 125 x $10^9$/L on the day of catheter removal, a platelet transfusion should be administered and platelet count confirmed to be greater than 125 x $10^9$/L prior to catheter removal)

- Pregnancy test for individuals of childbearing potential prior to infusion if no results on file within 48 h of but before planned infusion

**Biopsy(ies):**

If there is no confirmation of diagnosis via histology based on a tissue sample obtained within 6 weeks prior to planned infusion, a biopsy will be conducted prior to catheter placement and PVSRIPO infusion to confirm active tumor/high-grade glioma. After the biopsy is performed, the catheter is placed but PVSRIPO may not be infused until the qualifying diagnosis is made.

If the biopsy at catheter placement does not confirm active tumor/high-grade glioma by the site pathologist, the patient will not receive PVSRIPO and will be withdrawn from the study as a screen failure. The catheter should be removed and followed by a CT scan to confirm no bleeding.

Note: for all patients, including patients with tumor transformed from a lower grade to a higher grade, glioblastoma must be confirmed via prior histology by the site pathologist prior to biopsy/catheter placement.

Stereotactic biopsy(ies) and catheter placement will be performed via the site's standard of care prior to PVSRIPO administration, unless specified below.

- After biopsy for confirmation of diagnosis (if required), if there is sufficient tissue available, up to three additional core biopsies will be obtained to undergo planned future molecular genetic testing as described in **Section 5.7.4**. If no additional biopsies are obtained but archival tissue is available within 6 weeks prior to infusion, that tissue will be requested/utilized for future testing.
    - For patients retreated with PVSRIPO, tissue for tumor typing and clinical pathologic testing per SOC should be obtained prior to the infusion and results reported on the electronic case report form (eCRF) when available
    - Note: results from any analyses conducted on tissue from other biopsies or unplanned surgical resections for the duration of a patient's follow-up should be recorded in eCRF and remaining tissue may be requested for analysis

- The biopsy needle and catheter will be placed by the trained operating surgeon with stereotactic guidance using a MRI-compatible stereotactic head frame or a similar frameless device, and an Food and Drug Administration (FDA) cleared stereotaxic guidance system guided by a pre-operative MRI.
- Site neurosurgeon(s) must be approved by the Sponsor or their designee prior to performing these procedures in study. This may require reviewing documentation of PVSRIPO-related administration experience or training, documentation of CED experience with study specific training, or related qualifications.
- After biopsy(ies), a Sponsor-approved catheter (per the PVSRIPO Investigational Product Handling Plan [IPHP] or similar FDA cleared device with Sponsor approval) will be implanted in the operating room using sterile techniques, which may occur under general anesthesia at the discretion of the treating neurosurgeon, at the same site or a different site from that used for the biopsy.
- The catheter will be tunneled beneath the scalp for a distance of at least 5 cm to aid in the prevention of infection.
- The catheter will be placed within or through the progressive enhancing tumor, at a safe distance relative to eloquent brain function, with the tip of the catheter being placed:
    - Within the enhancing portion or in the vicinity of enhancement of target lesion (ie, infiltrative disease)
    - $\geq$ 0.5 cm from ventricles
    - $\geq$ 1 cm deep into the brain
    - $\geq$ 0.5 cm from the corpus callosum
- A CT scan will be used to confirm catheter placement criteria post-operatively.
- If anesthesia is utilized in the catheter placement procedure, use should be discontinued once there is CT scan confirmation of appropriate catheter placement.

Patients will be treated with a prophylactic antibiotic prior to biopsy and catheter insertion per the site's neurosurgical standard practice.

### 5.2.2 PVSRIPO Infusion (Day 0 or Day 1 [Visit 4])

Note: At the time of PVSRIPO infusion, emergency drugs, including epinephrine and diphenhydramine will be available and the neurologic status, oxygen saturation, and cardiac rhythm of the patient will be monitored. Drug infusion will occur in the neuro-intensive care unit (ICU) or neuro step down unit so that all other emergency facilities will be available.

- CBC with differential (Platelet count should be confirmed prior to PVSRIPO infusion as well as at time of catheter removal and should remain consistent with inclusion criteria 11. Should the platelet count be less than 125 x $10^9$/L on the day of catheter removal, a platelet transfusion should be administered and platelet count confirmed to be greater than 125 x $10^9$/L prior to catheter removal)

- PVSRIPO infusion should occur on the same day (Day 0) or the day after catheter placement (Day 1) to allow flexibility due to the longer infusion (6.5 hr) and complexity of scheduling the necessary components for the infusion (eg, operating room time, surgeon, support team, pharmacy time, radiology appointments). However, under extenuating circumstances (eg, unavoidable scheduling issues, delayed diagnosis from biopsy, issues with investigational product or other critical supplies), so long as the patient remains in the neuro-ICU or neuro step-down unit, the time from catheter placement to PVSRIPO infusion may be extended up to 72 h post-placement with Sponsor approval. This timeframe is in keeping with other agents delivered safely via CED via similar procedures for even longer periods of time, which was not associated with increased risk of infection [23, 24]. If infusion is delayed, it will be recorded in the eCRF and patient's source documents along with the rationale and the Sponsor's approval.
  - See **Section 6.2.4** for additional details pertaining to infusion of PVSRIPO

**Post-infusion assessments (Immediately following infusion):**

- After catheter removal, a CT scan of the brain (without contrast) will occur to confirm there is no bleed
- AE assessment

## 5.3 Post-PVSRIPO Treatment, Pembrolizumab Treatment and Follow-up Periods

During the post-PVSRIPO infusion treatment period and pembrolizumab treatment and follow-up periods, the following will occur.

**Day 1 post-PVSRIPO infusion (continuation of Visit 4):**

**Clinical Assessment:**

- Conducted daily until discharge and documented within 18 h of discharge:
  - Physical examination
  - Neurologic and NANO assessment
  - KPS
  - PRO assessment
  - AE assessment
  - Concomitant Medications

**Laboratory Evaluation:**

- CBC with differential
- CMP
- Pancreatitis labs (amylase, lipase)

**Discharge:**

- Patients may be discharged after the above assessments are conducted and when patient is clinically stable post-PVSRIPO infusion

**Week 2 (± 1 week; assessments conducted before pembrolizumab treatment; Visit 5):**

**Clinical Assessment:**

- Physical examination
- Neurologic and NANO assessment
- KPS
- AE assessment
- Concomitant Medications

**Radiographic Assessment:**

- MRI with and without contrast agent prior to planned pembrolizumab treatment only if patient presents with neurologic deficit or other symptoms that warrant withholding of pembrolizumab treatment and MRI would be considered informative at discretion of Investigator

**Laboratory Assessment:**

- CBC with differential
- CMP
- Pancreatitis labs (amylase, lipase)
- Thyroid panel
- LSQ
- Whole blood for research analyses

**Planned Treatment:**

- Pembrolizumab (200 mg IV infusion)

### 5.3.1 Delayed 1st Pembrolizumab Treatment: Week 3 to 4 visit (if needed) and Shift in Schedule of Assessments (Unscheduled Visit)

Note: If the 1st pembrolizumab infusion is delayed from Week 2 (Visit 5) due to ongoing AE, etc., the schedule of assessments (days, weeks) shifts accordingly so the next planned visit would occur 3 weeks after the date of 1st pembrolizumab infusion. For example, if a patient's 1st treatment with pembrolizumab occurs at Week 3 (Study Day 21), their next scheduled clinic visit would be Week 6 (Study Day 42).

The Week 3 to Week 4 visit is only required if the first planned dose of pembrolizumab is delayed from Week 2.

The first pembrolizumab treatment is planned for Day 14 (2 weeks after PVSRIPO infusion); however, pembrolizumab treatment will be delayed by up to two additional weeks post-PVSRIPO infusion in patients with any ongoing ≥ Grade 3 PTE or CE or ≥ Grade 2 AEs related to PTE or CE following PVSRIPO infusion or for any related events requiring active treatment (eg, TEAE such as new or worsening neurologic signs and symptoms like hemiparesis requiring ≤ 4 mg/day dexamethasone or seizure requiring anti-epileptics), until such events resolve to ≤ Grade 1. If such events have not resolved to ≤ Grade 1 within 28 days (4 weeks) post-PVSRIPO infusion, the patient will not receive pembrolizumab under this trial and will be replaced.

The first pembrolizumab treatment may also be delayed by up to two additional weeks post-PVSRIPO infusion for administrative or other reasons, including other TEAE that in the Investigator's opinion warrants delayed pembrolizumab treatment, but the 1st planned treatment should occur as close to Day 14 as possible.

If the first pembrolizumab treatment is delayed for any reason, it may be given anytime between Weeks 2 through 4 so long as the event(s) leading to the delay have resolved as noted above.

There is a ± 7-day visit window for study visits after the first week of the study to account for these adjustments and to align future doses of pembrolizumab with the study visits and assessments.

> Patients requiring > 4 mg/day of dexamethasone or equivalent prior to the first dose of pembrolizumab will not receive pembrolizumab under this trial.

Patients receiving PVSRIPO only will be followed according to the schedule of assessments with MRI and required visits occurring approximately every 9 weeks after Week 11.

After the first pembrolizumab treatment, subsequent pembrolizumab treatments will occur every 3 weeks (± 1 week) thereafter, up to Week 104 (24 months). Treatment with pembrolizumab may be interrupted and/or discontinued for irAE, as specified in **Section 7.5.3** and discontinued for confirmed PD. NOTE: If pembrolizumab is permanently discontinued, then safety laboratory evaluations that are not SOC are no longer required after 30 days from the date of pembrolizumab discontinuation.

If this visit is required due to delayed pembrolizumab treatment at Week 2, the following assessments at Week 3 to Week 4 should occur (prior to the first dose of pembrolizumab, as applicable):

**Clinical Assessment:**

- Physical examination
- Neurologic and NANO assessment
- KPS
- AE assessment

**Radiographic Assessment:**

- MRI of the brain with and without gadolinium contrast per SOC

**Laboratory Assessment:**

- CBC with differential
- CMP
- Pancreatitis labs (amylase, lipase)

### 5.3.2 Week 5 or + 3 weeks from 1st pembrolizumab treatment visit (± 1 week; assessments occur before pembrolizumab treatment; Visit 6)

**All assessments should occur before the planned pembrolizumab administration.**

**Clinical assessment:**

- Physical examination
- Neurologic and NANO assessment
- KPS
- PRO Assessment
- AE assessment
- Final DLT assessment (Day 21 (± 3 days) post- 1st pembrolizumab treatment) for the first 3 to 6 patients, as applicable, if received 1st dose at Week 2, as scheduled

**Radiographic Assessment:**

- MRI of the brain with and without gadolinium contrast per SOC

**Laboratory Assessment (before pembrolizumab treatment):**

- CBC with differential
- CMP
- Pancreatitis labs (amylase, lipase)
- PT and aPTT
- LSQ
- Whole blood for research analyses

**Planned Treatment:**

- Pembrolizumab (200 mg IV infusion)

### 5.3.3 Week 8 or + 3 weeks from last visit (± 1 week; Visit 7)

The following will be conducted:

**Clinical Assessment:**

- Physical examination
- Neurologic and NANO assessment
- KPS
- AE assessment

- MRI of the brain with and without gadolinium contrast per SOC

**Laboratory Assessments:**

- CBC with differential
- CMP
- Pancreatitis labs (amylase, lipase)
- PT and aPTT
- Thyroid panel

**Planned Treatment:**

- Pembrolizumab (200 mg IV infusion)

### 5.3.4 Week 11 (or + 3 weeks from last visit; Visit 8) to Week 104 post-infusion

After PVSRIPO infusion and while on pembrolizumab treatment, study visits occur 3 weeks (± 1 week) through a maximum of 104 weeks that include the following:

**Clinical Assessment**:

- Physical examination
- Neurologic and NANO assessment
- KPS
- PRO Assessment
- AE assessment

**Radiographic Assessment:**

- MRI of the brain with and without gadolinium contrast. MRI should occur approximately every 9-12 weeks per SOC until through a maximum of 104 weeks

**Laboratory Assessments:**

- CBC with differential
- CMP
- Pancreatitis labs (amylase, lipase)
- Pregnancy test (every 6 months or any time pregnancy suspected)
- Thyroid Panel
- Whole blood for research analyses

**Planned Treatment:**

- Pembrolizumab (200 mg IV) treatment is planned to occur every 3 weeks up to a maximum of Week 104 (24 months).
    - Patients who remain on pembrolizumab will report for infusions every 3 weeks. If pembrolizumab is permanently discontinued, then safety laboratory evaluations that are not SOC are no longer required after 30 days from the date of pembrolizumab discontinuation. See **Sections 5.5** and **5.6** for patients who permanently discontinue or interrupt pembrolizumab treatment before and after study Week 11.
- In general, patients who discontinue pembrolizumab treatment and/or are no longer in the per protocol population (ie, have initiated non-study tumor treatments; see **Section 8**) should be followed per SOC, as possible/feasible.
- If approved by Sponsor or designee, patients with confirmed progression meeting the key safety and infusion related eligibility criteria may be eligible for retreatment with PVSRIPO, ≥ 3 weeks after their last pembrolizumab treatment under this protocol.
    - Treatment with pembrolizumab or other treatments after PVSRIPO retreatment will be at the discretion of the investigator and will be recorded in the eCRF.

## 5.4 Early Discontinuation/End of Study Visit

It is expected that all participants treated in the study, regardless of completion or discontinuation of study intervention or addition of non-protocol interventions, will remain in the study for long-term follow-up, per SOC.

Before withdrawing a participant from the study, investigators should clarify and document the specific reason for the withdrawal. For example, the following scenarios may apply:

- Discontinuation of study intervention(s), but completes remaining study assessments and agrees to collection of relevant follow-up information (ie, remains in study for long-term follow-up – this is the default scenario)
- Discontinuation of study intervention and refuses study specific assessments, but agrees to collection of relevant follow-up information (remains in study for vital status and SOC MRI reporting)
- Patient withdrew consent for any further study communication or follow-up from study site (ie, completely withdraws from study)

If patient will not remain in follow-up and is permanently discontinuing the study, all assessments at the next scheduled study visit should be conducted (or as noted in the termination/end of study visit if after Week 11) or the information obtained, if possible.

## 5.5 Follow-Up Period of Off Study Participants/Patients Permanently Discontinuing Pembrolizumab

If a patient permanently discontinues pembrolizumab prior to or at Week 11, they should report to the clinic as scheduled for follow-up as noted in **Table 1.**

Patients who no longer meet the criteria for the per protocol population (see **Section 8**) or patients who permanently discontinue pembrolizumab for confirmed progression or irAE requiring permanent discontinuation after Week 11 will remain in follow-up, where assessments will occur as outlined in the SOA noted in **Table 1.** However, the frequency should occur per SOC (ie, approximately every 9 weeks), as feasible.

Entry of all applicable data and uploading of scans should occur no later than every 3 months. If SOC follow-up occurs less frequently (ie, greater than 3 months between visits/phone call), data should be transmitted within 2 weeks (± 1 week) of acquisition.

Data to be entered in the eCRF during this period may include, but is not limited to the following:

- Patient status: deceased or alive
- Use of additional anti-cancer therapies and/or concomitant medications used to manage PVSRIPO-related inflammation (ie, corticosteroids, bevacizumab) and/or adjustments to such therapies since the last entry.
    - Entries should include start/stop date, dose, route, and frequency.
- MRIs conducted per SOC
    - Any SOC MRIs (deidentified) may be requested by the Sponsor or Sponsor designee. For all SOC MRI, the assessment of tumor size, volume, and other characteristics should be recorded on the eCRF no less than every 6 months as well as in the event of disease progression.
- Neurosurgical procedures required for reasons other than tumor progression (ie, for managing hydrocephalus) since last visit and details of these procedure(s).

Results of any molecular, genetic, or histologic testing from all biopsies/tissue obtained since last recorded entry.

## 5.6 Follow-up for Patients Temporarily Interrupting Pembrolizumab

Patients temporarily interrupting pembrolizumab for qualifying irAE as noted in **Section 7.5.3** should report to the clinic as scheduled for follow-up as noted in **Table 1.**

## 5.7 Study Assessments

The results of all study assessments outlined in **Table 1** will be recorded on the eCRF and study source documents, as applicable.

### 5.7.1 Physical Examination

Standard physical examination per SOC, to include at least the following:

- Height (carried over from baseline)
- Weight
- Body Surface Area
- Vital Signs, including:
    - Blood pressure
    - Heart rate
    - Respiratory rate
    - Temperature

In addition, a neurological examination, and assessment of NANO and KPS scores will be conducted and documented per institutional guidelines to contribute to assessment of clinical and neurologic status.

### 5.7.2 Radiographic Imaging

A baseline MRI of the brain with and without gadolinium contrast must be conducted within 14 days of catheter placement for PVSRIPO infusion, but as close to biopsy/catheter placement as possible, and thereafter as scheduled in **Table 1**. Allowance will be made for the use of volumetric, contrast-enhancing imaging modalities as well, with Sponsor approval. Eligibility based on radiographic IC/EC will be based on the screening MRI obtained within 14 days of catheter placement; this MRI (and if required, recent prior MRIs) will be provided to the Sponsor or their designee for eligibility confirmation.

In addition, a CT scan will occur immediately after catheter placement in an effort to ensure proper positioning, and immediately after catheter removal post-PVSRIPO infusion, to confirm there is no bleed.

All MRI will be assessed by the investigator; tumor measurements and response will be recorded in the eCRF according to the response criteria outlined **Section 8.4.6.1** summarized in **Table 3**.

All de-identified images may be requested by the Sponsor for independent radiographic assessment and/or long-term storage.

### 5.7.3 Laboratory Assessments

Laboratory assessments that will be conducted during the study are listed below:

- CBC with differential
- CMP (to include: Na, K, bicarbonate, Cl, BUN, Cr, AST, ALT, Alk phos, total bilirubin, amylase, lipase)
- PT and aPTT
- Thyroid Panel
    - TSH
    - Thyroxine (free T4)
    - May include free and total T3 (triiodothyronine)
- Pregnancy test (beta- human chorionic gonadotropin (hCG); urine or serum) for women of childbearing potential within 14 days of catheter placement and 48 h of PVSRIPO infusion (and approximately every 6 months while on study treatments)
- Whole blood for biomarker research analyses (may include but is not limited to immune and genetic analyses)
  Note: Whole blood for research analyses will not be collected prior to any retreatment with PVSRIPO, as applicable

All required laboratory assessments must be conducted in accordance with this protocol. The timing and frequency of laboratory assessments are specified in **Table 1**.

- The laboratory tests (hematology, chemistry, coagulation, pregnancy testing) will be performed by the study center's usual local laboratory (or similarly qualified laboratory under special circumstances with Sponsor approval)
- Laboratory reports will be filed with the source documents, with the Investigator noting if any abnormalities are clinically significant and/or suggestive of an AE
- Site personnel must enter results into the eCRF for the specified lab parameters (with units and reference ranges, which should be provided to the Sponsor or their designee at start-up) at the specified timepoint.

Laboratory tests may be performed at additional times during the study as part of standard care and are not required to be reported. However, any laboratory test performed in association with an AE must be recorded in the participant's eCRF and if not at a protocol-specified timepoint, as an unscheduled visit.

### 5.7.4 Patient-Reported Outcomes

To better understand the patient experience in this trial, two individual, standardized paper instruments, EORTC-QLQ-30 and EORTC QLQ-BN20 [25], will be administered during routine

clinic visits for the purposes of evaluating PROs at selected timepoints as defined in the Schedule of Assessments. Patients will be instructed on the use of the questionnaires upon enrollment into the trial and will be provided these questionnaires for completion upon designated clinic visits. A separate manual on the use of these questionnaires will be provided to each investigational site.

### 5.7.5 Other Assessments

Additional pathology/biomarker tests that may be performed on archival tissue and tissue obtained from any resections or biopsies post-baseline include but are not limited to the following:

- PV receptor (CD155) expression
- Epidermal Growth Factor Receptor (EGFR) vIII and wild-type EGFR status
- MGMT promoter methylation/MGMT immunohistochemistry
- Isocitrate Dehydrogenase (IDH) 1 and 2
- Telomerase reverse transcriptase (TERT)
- PD-1 and PD-L1
- TP53 mutational status and p53 expression
- Tumor mutational burden
- Microsatellite instability-high status
- IFN-γ
- Granzyme B
- Immune cells [including, natural killer (NK) cells, NK T-cells, Regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and tumor antigen-specific T-cells]
- T-cell receptor sequencing

Research to identify signatures of potential PVSRIPO response will be conducted using patient blood samples and will occur as outlined in **Table 1**. These samples may be stored for future testing or analyzed by the Sponsor or their designee.

## 6 Therapies Under Investigation

Information pertaining to the study-associated treatments are outlined below. Any required updates to **Section 6** not impacting the safety of patients may be made via administrative memorandum until a protocol amendment can be issued.

## 6.1 Method of Assigning Patients to Treatment

All patients will be sequentially assigned to therapy via a unique numeric patient ID as they are enrolled. There is no randomization, as this is a single-arm trial.

## 6.2 Investigational Product: PVSRIPO

### 6.2.1 Name, Classification and Mechanism of Action

PVSRIPO is a modified version of the serotype 1 live-attenuated (Sabin™) PV vaccine and is classified as an oncolytic viral immunotherapeutic.

### 6.2.2 Packaging, Labeling and how Supplied

PVSRIPO is formulated in 50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% human serum albumin (HSA) in phosphate buffered saline (PBS). It is provided in sterile, single use glass vials with a green flip off top and each vial contains approximately 0.5 mL of stock PVSRIPO ($2.24 \times 10^9$ $TCID_{50}$).

#### 6.2.2.1 Potential Risks with Use

Detailed risks associated with PVSRIPO administration and use are outlined in greater detail in the Investigator's Brochure (IB). Based on an expanded Phase 1 trial and an ongoing multicenter Phase 2 trial, PVSRIPO may result in neurologic changes/exacerbation of existing and new-onset neurologic signs and symptoms due to peritumoral inflammation/edema within 12 months of PVSRIPO infusion. These changes are best managed with low dose dexamethasone and/or bevacizumab as described in **Section 6.5.4**.

Intracranial hemorrhage at infusion catheter removal has also been noted. This has been mitigated by inclusion criterion **Number 11**, requiring platelet counts ≥ 125,000/µl (supported via transfusion acceptable) at the time of catheter placement/biopsy.

It is unknown if PVSRIPO can cause fetal harm; as such, female study participants and female partners of male study participants who are of childbearing potential should be advised to utilize effective forms of contraception for at least 30 days prior to and after any PVSRIPO infusion and for up to 4 months after the last dose of study drug received. Effective forms of birth control include using at least 2 methods, one of which should be a barrier method. Examples include:

- Condom
- Oral, injected, or implanted hormonal contraception
- Placement of an intrauterine device or intrauterine system

- Occlusive cap (eg, diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository
- Vasectomy or surgical castration ≥ 6 months prior to biopsy

**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.

It is recommended that adverse events related to potential PTE be managed as outlined in **Section 6.5.4** (ie, low dose bevacizumab and/or dexamethasone as indicated). However, for unexpectedly severe cases of PTE or CE with associated clinical symptoms requiring treatment that are not managed successfully per the recommended practices or other measures, surgical resection of the lesion infused with PVSRIPO, approximately 4 weeks post PVSRIPO and approximately 2 weeks following the first dose of pembrolizumab, could occur at the discretion of the treating clinician and neurosurgeon. This resection is not planned as a part of the protocol and will be considered a DLT, as noted in **Section 7.4**. For more information on PVSRIPO, see the IB.

### 6.2.3 Shipment, Receipt and Storage

PVSRIPO and diluent will be supplied directly to the site by the Sponsor or their designee. PVSRIPO is shipped on dry ice at approximately -80°C with temperature monitoring and is stored long-term at ≤ -80 °C. Refer to the IPHP for additional handling, excursion and long-term storage conditions.

The diluent for PVSRIPO is sterile 0.2% HSA in PBS. Each diluent vial contains approximately 6.0 mL. The diluent is shipped and should be stored long-term under refrigerated conditions (between 2 to 8°C), with temperature monitoring. Refer to the IPHP for additional details.

### 6.2.4 Preparation, Dispensing and Administration

Details on the preparation, dispensing and transport of the prepared PVSRIPO infusate solution to deliver a final dose of 5 x $10^7$ $TCID_{50}$ to the patient are provided in greater detail in the GBM IPHP and Infusion Guidelines. In brief, the stock PVSRIPO is diluted with 0.2% HSA in PBS

(diluent), mixed via gentle inversion and presented in a 10 mL Infusion Syringe such that when 3.0 mL is infused on study Day 0, a total dose of 5 x $10^7$ $TCID_{50}$ is administered to the patient.

PVSRIPO infusate preparation will occur in each institution's designated investigational pharmacy or equivalent. All handling of the investigational product will occur in a biosafety cabinet or a similarly contained environment.

The infusion syringe specified in the IPHP is loaded into an FDA cleared infusion pump approved by the Sponsor (eg, Medfusion®3500 syringe infusion pump, Alaris™ Syringe Module Syringe Infusion Pump). Participants will receive PVSRIPO infusate by means of CED over a period of 6.5 h, at a rate of 0.5 mL/h through the tubing and catheter embedded into the patient's tumor and affixed to the scalp (see **Section 5.2.1** for details on catheter insertion). The infusion tubing and catheter are supplied by or must be approved by the Sponsor or their designee prior to use. Please refer to the PVSRIPO IPHP for additional details.

## 6.3 Accountability and Compliance of Investigational Products

Drug accountability records will be maintained for all clinical study agents and supplies. All empty and partially used clinical study agents and supplies will be destroyed with appropriate documentation in accordance with institutional guidelines. All investigational product (PVSRIPO) will be administered in accordance with the conditions of this protocol. Only authorized site personnel may supply or administer investigational product. Each site's designated investigational pharmacy will maintain detailed documentation of the receipt, dispensing and/or destruction of the investigational product, which will then be provided to the Sponsor or designee. Only participants enrolled in the study may receive investigational product, in accordance with all applicable regulatory requirements. Upon completion of the study, this material will undergo final inspection and reconciliation.

### 6.3.1 Disposal and Destruction

All materials coming into contact with PVSRIPO, including the infusion syringe/any overage after infusion, tubing, dressings, or coverings used to protect the infusion/priming site, will be disposed of as biological waste in the preparation and treatment rooms in accordance with institutional guidelines.

## 6.4 Additional Study Therapy: Pembrolizumab

### 6.4.1 Pembrolizumab Classification and Mechanism of Action

Pembrolizumab is lawfully marked, PD-1 blocking antibody, immune check point inhibitor.

### 6.4.2 Packaging, Labeling and how Supplied

Pembrolizumab will be labeled according to the manufacturer's specifications and is commercially supplied for injection as follows: 100 mg/4 mL (25 mg/mL) solution in a single-dose vial; see the Keytruda® (pembrolizumab) PI for details.

#### 6.4.2.1 Potential Risks with Use

Based on its mechanism of action, pembrolizumab can cause severe, life-threatening irAE. Refer to **Section 7.5.3** for dose interruption/stopping criteria for irAE as well as the Keytruda® (pembrolizumab) PI or "Guide for Keytruda®".

In addition, pembrolizumab may cause fetal harm when administered to a pregnant woman. Animal models link the PD-1/PD-L1 signaling pathway with maintenance of pregnancy through induction of maternal immune tolerance to fetal tissue. Women of childbearing potential should be advised of the potential risk to a fetus and to use effective contraception during treatment with pembrolizumab and for 4 months after the last dose received.

### 6.4.3 Shipment, Receipt and Storage

Pembrolizumab is planned to be obtained via prescription from a pharmacy and should be stored according to site pharmacy practices in keeping with the PI, with appropriate accountability. Any modifications to this plan will be outlined in the SAM and will not necessitate a protocol amendment.

### 6.4.4 Preparation, Dispensing and Administration

Pembrolizumab will be prepared, dispensed, and administered according to manufacturer's specifications noted in the Preparation and Administration section of the product package insert and per pharmacy practices for this lawfully marketed agent. In brief, the required volume from the vial(s) will be withdrawn and transferred into an IV bag containing 0.9% Sodium Chloride Injection, United States Pharmacopeia (USP) or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion without shaking. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL.

For additional information regarding procurement, distributors, and other resources for pembrolizumab, see the "Guide for Keytruda®". In brief, regarding the diluted solution for infusion:

- May be stored at room temperature for no more than 6 h from the time of dilution. This includes room temperature storage of the diluted solution, and the duration of infusion.

- May be stored under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 h from the time of dilution. If refrigerated, allow the diluted solution to come to room temperature prior to administration.
- Discard after 6 h at room temperature or after 24 h under refrigeration. Do not freeze.
- Diluted solution will be administered intravenously over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter.
- Do not co-administer other drugs through the same infusion line.

The dose of pembrolizumab is keeping with prior investigations in rGBM that were well tolerated [3, 4, 20, 21]: 200 mg administered as an IV infusion over 30 minutes every 3 weeks up to Week 104 or until disease progression or unacceptable toxicity.

#### 6.4.4.1 Disposal and Destruction

Pembrolizumab will be disposed of per institutional guidelines and manufacturer's specifications.

### 6.5 Prior and Concomitant Therapy

At a minimum, the following will be captured for study as well as any prior and concomitant therapies as outlined below and specified in the eCRF Completion Guidelines:

- When administered (start/stop dates)
- Dose(s)
- Route
- Frequency
- Reason for use (ie, indication)

### 6.5.1 Prior Therapy

All prior marketed and experimental tumor treatments along with pertinent information noted above, in addition to reason for failure (ie, progression, tolerability issues) will be recorded in the eCRF. This includes but is not limited to radiotherapy/radiosurgery, chemotherapy (eg, temozolomide, lomustine); carmustine implants/wafers; targeted therapies like bevacizumab or imatinib; devices (tumor treating fields), vaccines, etc. The extent of any prior resection (ie, gross total resection (GTR), subtotal resection (STR), biopsy) at diagnosis and later surgeries will also be noted.

In addition, all medication taken within 30 days of baseline (PVSRIPO infusion) will be documented in the patient's source documents and eCRF. Also refer to the IC/EC (see

Sections 4.1 and 4.2) for any therapies prohibited within the specified timeframe prior to treatment.

### 6.5.2 Other Concomitant Therapy

Concomitant therapies administered during the study must be recorded in the patient's source documents and eCRF. Except for those utilized in management of peritumoral edema or AE, concomitant medications should be maintained at stable doses during the study, if appropriate.

### 6.5.3 Prohibited Medications/Therapies

Except for bevacizumab (7.5 mg/kg q 3 weeks), the following other therapies that are prohibited in patients while "on-study" include but are not limited to:

- Anticancer systemic chemotherapy
- Radiation therapy
- Radiosurgery
- Biologic therapies
- Targeted therapies
- Immunotherapy (other than protocol therapies)
- Investigational therapies
- Live vaccines
- Tumor treating fields

### 6.5.4 Permitted Medications/Medications for Management of Cerebral Edema and irAE

In general, non-cancer treatments considered by the investigator to be necessary for participant's welfare in keeping with community SOC, including prophylactic treatments (ie, anti-emetics) are allowed.

The BSC for control of PTE or CE related sequalae after PVSRIPO infusion and/or combination of PVSRIPO and pembrolizumab, as needed, are allowed, and recommended as follows:

- Preferred ≥ 2 weeks post-PVSRIPO infusion: low dose bevacizumab (7.5 mg/kg q 3 weeks).
    - Treatment with bevacizumab for the shortest course possible is desired over dexamethasone use for control of cerebral edema, when deemed safe to use by the investigator.
    - Treatment with higher doses of bevacizumab for > 2 cycles is not permitted per protocol.

- Within 2 weeks of PVSRIPO infusion or thereafter, at the treating clinician's discretion if rapid response is clinically indicated: low dose dexamethasone (≤ 4 mg/day). As low a dose as possible, for the shortest duration possible is desirable for managing cerebral edema. However, participants should primarily be managed with bevacizumab, thereafter.
- Other supportive care treatments (eg, anti-epileptics for seizure).

#### 6.5.4.1 Steroids

Corticosteroids (dexamethasone recommended) are allowed for treatment of irAEs and/or PTE or CE with guidance that dose and duration be as limited as medically appropriate. Dexamethasone (ideally ≤ 4 mg/day) should be used at the lowest dose required to control symptoms of PTE and mass effect or other inflammatory-related conditions and be discontinued as soon as medically appropriate. Use of corticosteroids should be recorded in the eCRF.

In addition, at discharge following PVSRIPO administration and after each pembrolizumab treatment, participants should have a filled dexamethasone (or equivalent if cannot use dexamethasone) prescription in hand, with instructions regarding what emerging symptoms would warrant immediate consult with the treating clinician to determine if immediate initiation of dexamethasone is warranted and/or to proceed to the clinic for consideration of bevacizumab infusion or other treatments, depending on the nature and severity of the event(s).

Every attempt should be made to use the lowest dose of steroids for the shortest duration and to discontinue/taper steroid use as soon as is feasible.

**Risks Associated with Use:** Use of corticosteroids may lead to cardio-renal, endocrine, and immune system and ophthalmic AEs as well as increased susceptibility to infections, including life-threatening infection. Abrupt withdrawal following prolonged therapy may result in symptoms of the corticosteroid withdrawal syndrome including, myalgia, arthralgia, and malaise. See the dexamethasone PI for additional details.

#### 6.5.4.2 Bevacizumab

A clinically important feature of bevacizumab is the steroid-like improvement of the disrupted blood-brain barrier and reduction in PTE and mass effect when use in rGBM [26-28]. Given this and the success utilizing bevacizumab for the management of PTE or CE and related sequalae in prior studies with pembrolizumab [3] and PVSRIPO [5] and the combination in rGBM patients treated under compassionate use, eligible participants will be allowed to initiate bevacizumab treatment (7.5 mg/kg q 3 weeks) as early as 2 weeks after PVSRIPO infusion, at the time of the first planned pembrolizumab treatment. Bevacizumab use should be reevaluated as soon as

possible but at a minimum, after 3 consecutive doses (9 weeks) and be discontinued as soon as possible at the treating clinician's discretion.

Intermittent, short-term, lower dose bevacizumab use is not anticipated to have an appreciable impact on survival outcomes based on limited anti-tumor activity [26-28]. Given the impact of bevacizumab on enhancing lesions [29], all radiographic response will be confirmed ≥ 8 weeks after discontinuation of bevacizumab to account for the known radiographic pseudoresponse and T2/Fluid-Attenuated Inversion Recovery (FLAIR) will be monitored over time by independent radiographic reviewers blinded to patient outcome and bevacizumab use.

Branded Avastin® (bevacizumab) is preferred but if unavailable, MVASI™ (bevacizumab-awwb) is an acceptable alternative (check with Sponsor before utilizing any others). Bevacizumab will not be provided by the study.

**Risks Associated with Use**: There is a warning and precaution noted in the prescribing information of bevacizumab regarding risks of bleeding/wound healing complications within 28 days following major surgery. For this protocol, investigators will have the discretion to use low-dose bevacizumab at ≥ 2 weeks following the less invasive biopsy/intracranial infusion, if such use is judged by the investigator to be less than the potential risk of sequalae associated with PTE or CE after PVSRIPO treatment alone and/or followed by pembrolizumab. Bevacizumab is also associated with thromboembolic and other events; see the PI.

#### 6.5.4.3 Concomitant Medications for Immune-related Adverse Events

See **Section 7.5.3** for information on irAE and actions to take with pembrolizumab. For the management of any irAE, after the first dose of pembrolizumab, as low a dose as possible of dexamethasone (ideally ≤ 4mg/day), for the shortest duration possible, is desirable.

Any irAE should ultimately be managed at the clinician's discretion with patient safety as the primary consideration.

Further, although not planned as part of the protocol, for severe cases of peritumoral edema with clinical symptoms requiring treatment that are not managed successfully per the BSC practices noted above or other measures, surgical resection of the lesion infused with PVSRIPO, approximately 4 weeks post PVSRIPO and approximately 2 weeks following the first dose of pembrolizumab, could occur at the discretion of the treating clinician and neurosurgeon.

# 7 Safety and Pharmacovigilance

The Investigator is responsible for the identification and documentation and reporting of AEs and SAEs, as provided in this protocol. During each study visit and other patient contacts (eg, phone contact, telemedicine visit), the Investigator or designated study personnel must assess, through non-suggestive inquiries of the patient or evaluation of study assessments, whether an AE or SAE has occurred. In addition, if the Investigator (or designee) receives a report from other health care providers or legally authorized representatives that is suggestive of an AE/SAE in the study patient, the Investigator must similarly follow up for reporting purposes.

## 7.1 Adverse Events

An AE is an untoward or medical occurrence associated with the use of study treatment (drug, biologic, or device) in clinical investigation participants, which does not necessarily have a causal relationship with the study treatment. An AE can therefore be any unfavorable and unintended symptom, sign, disease or condition, or test abnormality (although diagnosis should always be reported as the AE if known) regardless of the relationship to study treatment. AEs that do not meet the definition for an SAE are considered non-serious AEs. All AEs must be recorded in the patient's source documents and AE case report form.

AEs will be assessed according to the CTCAE v5. 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).

All AEs will be followed until the outcome is determined by the Investigator as one of the following:

- Resolved
- Ongoing
- Chronic/Stable
- Death
- Unknown

Attribution of AEs/SAEs will be determined for each study intervention or concomitant medication/intervention, as applicable (ie, catheter placement, PVSRIPO, pembrolizumab, corticosteroids, bevacizumab).

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)

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

- Non-clinically significant (as assessed by the investigator), isolated, laboratory values, other than those noted in **Section 7.4**; see **Section 7.1.1** for assessing clinical significance of laboratory abnormalities for AE reporting.
- Medical or surgical procedures (eg, endoscopy, appendectomy).
    - Note: The condition that leads to the procedure can be 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/conditions present at baseline that do not worsen.
- Planned hospital admissions or surgical procedures for elective procedures or for an illness or disease that existed before the signing of the ICF or before the participant was enrolled in the study will not be captured as TEAEs/SAEs.
- However, if planned admissions or procedures occur at a time other than what was planned (eg, due to an exacerbation in the preexisting illness or disease), they should be reported as TEAEs/SAEs.

### 7.1.1 Laboratory Based Abnormalities

All protocol-specified laboratory values will be reported as outlined in **Table 1** during the study, along with associated units and reference ranges. Results of unscheduled/SOC laboratory assessments should be reported as an unscheduled visit laboratory assessment only if associated with a TEAE. An investigator should assess the clinical significance for all abnormal laboratory values. In general, if either of the following conditions are met, the abnormal laboratory value may be clinically significant:

- The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
- The abnormality is of a degree that requires additional active management, eg, change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation.

Therefore, a clinically significant lab value is generally one that indicates a new disease process, an exacerbation or worsening of an existing condition, or requires further action(s) to be taken.

A lab result should not be considered in isolation, but in the context of the patient's physical examination and available history (ideally including past and present medical problems, social history, family history, medications, and previous laboratory evaluations).

Isolated non-clinically significant (as assessed by the investigator) laboratory values should not be reported as adverse events.

Laboratory abnormalities considered clinically significant by the investigator should be reported as AEs (or SAEs, as applicable), although the diagnosis should always be reported as the AE if known (eg, treatment emergent fasting hemoglobin A1C values ≥ 7.1% on two consecutive laboratory evaluations would be reported as the AE of diabetes, with the abnormal A1C values supportive of this diagnosis).

### 7.1.2 Non-Treatment Emergent Adverse Events

Any AE occurring after a patient provides informed consent that is related to the non-SOC screening procedures (eg, PV vaccination booster) and biopsy procedure will be assessed, recorded in the patient's source documents and the eCRF with attribution, regardless of causality or seriousness (non-TEAEs).

Any AE occurring from the time of informed consent to start of PVSRIPO infusion that are not considered related to a study required non-SOC screening procedure, should be reported as medical history. For example, if a patient had new onset seasonal allergies requiring medication after signing of ICF but before Screening Period 2, it would be reported as medical history.

### 7.1.3 Treatment Emergent Adverse Events

Treatment emergent AEs will be noted from the time of PVSRIPO infusion through the time the patient comes off study/no longer meets the requirements for the per protocol population (as defined in **Section 8**) and 30 days thereafter. Any AEs related to the catheter placement without PVSRIPO infusion will be summarized separately (eg, patient withdraws due to AE experienced after catheter placement but before PVSRIPO infusion).

## 7.2 Serious Adverse Events

An SAE is any AE meeting the following criteria:

| | |
|---|---|
| Death | An AE that results in death (note: death due to progression is not an AE/SAE but should be reported as noted in the eCRF completion guidelines). |

| | |
|---|---|
| Life-threatening AE | An AE that places the participant, in the view of the Investigator, at immediate risk of death from the AE as it occurred (ie, does not include an AE that had it occurred in a more severe form, might have caused death). |
| Required or prolonged participant hospitalization | An AE that results in an initial participant hospitalization or prolongs an existing hospitalization of the participant. If a participant is hospitalized as part of the clinical use of the study treatment, the period of normal hospitalization will be based on the Investigator's judgement and experience. Hospitalizations longer than this period will be considered prolonged hospitalizations. |
| Persistent or significant disability/incapacity | An AE that results in a substantial disruption of a participant's ability to conduct normal life functions. |
| Congenital anomaly/birth defect | A congenital anomaly/birth defect that occurs in the offspring of a participant exposed to the study drug. |
| Important medical event | An AE that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent 1 of the outcomes listed above. Examples of such "important medical events" include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in participant hospitalization, or the development of drug dependency or drug abuse |

SAEs must be reported by the Investigator to the Sponsor or designee within 24 h of discovery for all patients from the time of consent, while on study and for 30 days after 1) completion of study participation or 2) if patient is rendered off-study and in the follow-up period only. SAEs will be reported in the eCRF with instruction provided in the eCRF Completion Guidelines.

The Investigator 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 Investigator before transmittal to the Sponsor or designee. The Investigator should not wait to for additional information to fully document the related SAE before notification, although additional information may be requested.

Instances of death, 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 Investigator at any time after cessation of study drug(s) and linked by the Investigator 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.

**The SAE/narrative description should include the following, as a minimum:**

- Time to onset following PVSRIPO and/or pembrolizumab infusion, as applicable
- Maximum severity of signs and symptoms
- Treatment(s) undertaken (including dose, route frequency, as applicable)
- Action taken with study drug(s) (eg, permanently discontinued, treatment interruption)
- Concomitant medications at the time of the event(s)
- Time to resolution as the information becomes available

## 7.3 Definition of an Unexpected Adverse Event

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the current IB or PI or is not listed at the specificity or severity that has been observed previously. In addition, reports which add significant information on specificity or severity of a known already documented AE may constitute an unexpected AE.

The Medical Monitor or designee will evaluate expectedness of all SAEs as per the currently available safety information. For all events, medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations and should consult with the Sponsor Medical Monitor or designee if unclear.

## 7.4 Assessment and Definition of Dose Limiting Toxicity

For this study, assessment of treatment emergent DLT will occur after a patient receives PVSRIPO and until 21 days (± 3 days) after the first dose of pembrolizumab.

Peritumoral and CE occur frequently in rGBM and may be secondary to the disease process itself, surgical procedures, necrosis from previous radiation, or inflammation due to immune infiltration of the brain or destruction of tumor cells, which may manifest as neurologic dysfunction. Given that transient PTE has been observed following PVSRIPO use, the primary focus for DLT will be neurologic events meeting the criteria for a DLT defined as follows:

1. ≥ Grade 3 neurologic events related to PTE or CE within the first 3 weeks of the first pembrolizumab administration that:
    a. Do not resolve to ≤ Grade 1 with the management practices outlined herein (≤ 4mg/dexamethasone or 7.5 mg/kg bevacizumab) within 3 weeks of initiating such treatments,

    and/or

    b. Require surgical debulking of the PVSRIPO infused tumor within 3 weeks of the first pembrolizumab administration

**To assess for any other potential DLT, CTCAE v5.0 will be utilized, and the following will also be considered DLT if treatment related:**

1. Any of the following SAEs:
    a. Treatment-related death
    b. Treatment-related life-threatening event
    c. A medically significant condition (defined as an event that compromises patient safety or may require medical or surgical intervention to prevent one of the outcomes above).
    d. Requires inpatient hospitalization or prolongation of existing hospitalization
    e. Results in persistent or significant incapacity or substantial disruption to conduct normal life functions
2. Any Grade 4 non-hematologic toxicity
3. Any Grade 3 non-laboratory-based toxicity that is not reversible to its pre-PVSRIPO treatment baseline within 2 weeks of onset with the following **exceptions**:
    a. Grade 3 fatigue lasting ≤ 3 days or Grade 3 fatigue in a participant with Grade 1 fatigue at baseline
    b. Grade 3 diarrhea, nausea, or vomiting without use of anti-emetics or anti-diarrheals per standard of care
    c. Grade 3 adrenal insufficiency due to suboptimal steroid replacement therapy
    d. Grade 3 rash without use of corticosteroids or anti-inflammatory agents per standard of care
4. Grade 3 or Grade 4 non-hematological laboratory values only if meets one of the serious criteria outlined in 1 above or:
    a. The abnormality persists for > 1 week
    b. The abnormality results in a drug-induced liver injury

    **Note:** Treatment-emergent, non-clinically significant laboratory abnormalities that are treatable with routine measures, or reversible (≤ 1 week of onset) laboratory abnormalities in and of themselves (including liver function tests, uric acid, etc.), are not considered DLT.

    **Exceptions (these laboratory values are DLT):**

    - Grade 4 thrombocytopenia of any duration
    - Grade 3 thrombocytopenia associated with bleeding requiring a platelet transfusion
5. Febrile neutropenia meeting following criteria:
    a. Grade 3: ANC < 1000/µl with a single temperature of > 38.3° C (101° F) or a sustained temperature of ≥ 38° C (100.4 degrees F) for ≥ 1 hour
    b. Grade 4: ANC < 1000/µl with a single temperature of > 38.3° C (101° F) or a sustained temperature of ≥ 38° C (100.4° F) for ≥ 1 h, with life-threatening consequences and urgent intervention indicated

## 7.5 Adverse Events of Special Interest

### 7.5.1 Cerebral Spinal Fluid Leakage or Aseptic Meningitis

Based on communications with the FDA agreed upon in IND 014735, Serial Submission 0197, in the event 2 or more patients enrolled under the current inclusion/exclusion criteria relating to catheter placement, present with evidence of cerebral spinal fluid leakage or aseptic meningitis, enrollment of patients under these criteria will be paused for further evaluation. The previous criteria (ie, catheter tip placed ≥ 1 cm from the ventricle and corpus callosum) will be used for continued enrollment until this evaluation is complete. These events will not be considered DLT for the purpose of evaluating PVSRIPO followed by pembrolizumab use unless further evaluation suggests an association.

### 7.5.2 Cerebral or Peritumoral Edema

Peritumoral edema (and possibly the signs and symptoms thereof) is considered expected with PVSRIPO therapy in rGBM based on the mechanism of action, whereby a targeted anti-tumor inflammatory immune response is elicited in and around the tumor. For the recommended best practices to manage signs symptoms related to PTE or CE, see **Section 6.5.4** (eg, low dose bevacizumab [7.5 mg/kg q 3 weeks] or dexamethasone [≤ 4 mg/day], along with initiation of other supportive care treatments, as required [eg, antiepileptics]).

Peritumoral or CE may also 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. The timing of onset of such events should be considered in relation to PVSRIPO or pembrolizumab infusion when considering attribution of such events to these therapies (see PVSRIPO IB for guidance).

Peritumoral or CE may present as a constellation of general signs and symptoms such as altered mental status, cognitive disturbance, confusion, somnolence, lethargy, fatigue, unresponsiveness, encephalopathy, or coma or can be more focal, depending on the patient and tumor. The individual signs and symptoms may include but are not limited to: seizures, dysphasia, paresthesia, headaches, focal neurologic deficit, ataxia, dystonia, facial muscle weakness, hemiparesis, pyramidal tract syndrome, hemianopia, visual field cut, blurred vision, diplopia, and urinary incontinence. Participants will be monitored throughout the course of the study and upon noting any signs or symptoms of PTE or CE, may receive an antiangiogenic agent (bevacizumab), steroids, receive an osmotic diuretic, and/or have 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 patient and the patient's family.

SAEs considered related the local inflammatory effect on the tumor (ie, peritumoral edema) should be reported in a way that distinguishes it from a more generalized brain edema (eg, "edema cerebral (peritumoral)". In cases where the edema is more generalized, reporting as "edema cerebral" would be more appropriate. In each case, the appropriate severity grade of the PTE or CE and the resulting symptoms/specific dysfunction should be noted in the edema-specific AE/SAE collection forms (and narrative description for SAE related to PTE or CE) by severity grade along with other relevant information (eg, start and stop date of each sign/symptom and any other information considered relevant by the investigator). Any signs or symptoms considered to be out of proportion to the extent of the PTE may be reported as additional SAEs, if indicated.

Except in cases of imminent life-threatening emergency, investigators should consult with the Sponsor Medical Monitor or designee is recommended prior to implementing anti-tumor treatments beyond the protocol-recommended supportive care options.

Delays and or discontinuation of pembrolizumab treatment should occur as outlined in **Sections 5.3** and **7.5.3** for events related to PTE and CE.

### 7.5.3 Adverse Events of Immunologic Etiology Associated with Pembrolizumab

Adverse events of an immunologic etiology have been associated with pembrolizumab exposure. These irAE may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Existing clinical trial data have shown that most irAEs related to pembrolizumab were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids, and/or other supportive care. See **Section 6.5.4.3** on medication use for treatment of irAE.

Depending on the severity of the irAE, pembrolizumab will be temporarily withheld or permanently discontinued. In general, for an irAE requiring dose interruptions, low-dose dexamethasone (≤ 4mg/day) should be initiated and tapered when the irAE is ≤ Grade 1.

For a life-threatening irAE, IV dexamethasone should be initiated first followed by oral steroids. Other immunosuppressive treatment should also be initiated if the irAEs cannot be controlled by corticosteroids alone.

The next doses(s) of pembrolizumab will be withheld until the irAE has been reduced to ≤ Grade 1 and dexamethasone dose has been tapered to ≤ 4mg/day and/or discontinued.

Pembrolizumab should be permanently discontinued if the irAE does not resolve within 12 weeks of last dose or dexamethasone cannot be reduced to ≤ 4 mg/day within 12 weeks.

- With regard to events ≥ Grade 2 related to PTE or CE (eg, hemiparesis, seizure), pembrolizumab treatment should be held until those events resolve to ≤ Grade 1. Pembrolizumab should be permanently discontinued for Grade 4 PTE or CE and/or events ≥ Grade 3 related to PTE or CE.

**Refer to the most recent Keytruda® (pembrolizumab) package insert and "Guide for Keytruda®" for a list of adverse reactions for which pembrolizumab should be interrupted or permanently discontinued along with recommended management. This may include but not be limited to the following:**

- Immune-mediated pneumonitis: Withhold for moderate, and permanently discontinue for severe, life-threatening or recurrent moderate pneumonitis.
- Immune-mediated colitis: Withhold for moderate or severe, and permanently discontinue for life-threatening colitis.
- Immune-mediated hepatitis: Monitor for changes in hepatic function. Based on severity of liver enzyme elevations, withhold or discontinue pembrolizumab. Consider corticosteroid therapy.
- Immune-mediated endocrinopathies:
    - Adrenal insufficiency: Withhold for moderate and withhold or permanently discontinue for severe or life-threatening adrenal insufficiency.
    - Hypophysitis: Withhold for moderate and withhold or permanently discontinue for severe or life-threatening hypophysitis.
    - Thyroid disorders: Monitor for changes in thyroid function. Withhold or permanently discontinue for severe or life-threatening hyperthyroidism.
    - Type 1 diabetes mellitus: Monitor for hyperglycemia. Withhold pembrolizumab in cases of severe hyperglycemia.
- Immune-mediated nephritis: Monitor for changes in renal function. Withhold for moderate, and permanently discontinue for severe or life-threatening nephritis.
- Immune-mediated skin adverse reactions including Stevens- Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN): Withhold for severe and permanently discontinue for life-threatening skin reactions.
- Other immune-mediated adverse reactions: In organ transplant recipients, consider the benefit of treatment with pembrolizumab versus the risk of possible organ rejection.

In addition, pembrolizumab can cause severe or life-threatening infusion-related reactions, including hypersensitivity and anaphylaxis. During infusion, monitor patients for signs and symptoms of infusion-related reactions including rigors, chills, wheezing, pruritus, flushing, rash, hypotension, hypoxemia, and fever. For severe (Grade 3) or life-threatening (Grade 4) infusion-related reactions, stop infusion and permanently discontinue pembrolizumab. Follow the recommend management practices noted above, in the current pembrolizumab package insert, or in keeping with current medical practices when managing reactions noted during or after pembrolizumab infusion.

### 7.6 Notification for Serious or Unexpected Adverse Events

The investigator, or responsible person per local requirements, must comply with the applicable local regulatory requirements related to the reports of SAEs to regulatory authorities and the IRB/ IEC.

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/PVSRIPO followed by pembrolizumab use. The Investigator (or Sponsor where required) must report any SUSARs to the IRB/IEC according to the Institution's regulations.

The Sponsor is responsible for the ongoing evaluation of the study drug. Any adverse experience associated with the use of the study drug that is both serious and unexpected must be reported. In accordance with the US Code of Federal Regulations, Title 21 CFR Part 312.32, and the International Conference on Harmonization (ICH) Guidelines for Clinical Safety Data Management Definitions and Standards for Expedited Reporting, the Sponsor must submit written documentation in the form of an IND Safety Report or SUSAR reports, respectively. All events qualifying as IND Safety Reports/SUSARs will be reported to the IRB/IECs. IND Safety Reports/SUSARs are required to be reported within 7 calendar days for life-threatening events and those resulting in death or 15 calendar days for all others. These timeframes begin with the first notification of the IND Safety Reports/SUSARs to the drug safety group from the Investigator.

The Sponsor should submit to the regulatory authority all safety updates and periodic reports, as required by applicable regulatory requirements.

# 8 Statistical Analyses

This single-arm study is designed to evaluate the efficacy, safety and tolerability of PVSRIPO when followed by pembrolizumab treatment in patients with rGBM. Summary statistics (eg, n, %, CI) will be employed to describe the key findings (ORR, DOR, incidence and severity of DLT, Grade 3 or higher AE, etc.).

Adverse events considered related to screening or other pre-infusion procedures that are not standard of care (eg, PV booster, biopsy prior to infusion, catheter placement without PVSRIPO infusion) will be summarized separately from TEAE (after PVSRIPO infusion), as will data from patients receiving only PVSRIPO and not at least one dose of pembrolizumab. The number of doses of pembrolizumab received and other baseline characteristics will also be explored as variables for safety and efficacy (eg, age, number of prior recurrences, baseline NANO and KPS, extent of prior resection, prior bevacizumab and chemotherapy use, tumor genetic profile).

All patients enrolled and treated with PVSRIPO will be summarized in all analyses. In addition, a per-protocol analysis will also explore the impact of excluding patients meeting pre-defined criteria from radiographic and survival analyses as outlined in **Section 8.4.6**. For comparisons of efficacy or safety relative to PVSRIPO alone or pembrolizumab alone from other data sources, both the overall and per-protocol populations may be considered.

In general, patients should follow the protocol treatment plan outlined and will be considered on study if there are no major protocol deviations including prohibited tumor-treatments, until the time of confirmed disease progression, or until the investigator and/or patient feel it is in the patient's best interest to stop study treatments or study participation.

The SAP, which will be finalized before conducting the analyses, will present details about planned statistical summary methods and analyses. A brief summary of the SAP is provided below by section. The SAP will govern in the event the protocol and the SAP differ.

## 8.1 Sample Size Considerations

In an ongoing analysis of the Phase 1 study of PVSRIPO in rGBM, an ORR of 11% was demonstrated (7/61 patients achieved a CR or PR; data on file, Istari). With 30 subjects treated in this study, there is > 75% power to demonstrate a 20% or greater improvement in ORR (ORR > 31%, two-sided P<0.05).

## 8.2 Analyzed Population Sets

All patients enrolled and treated with PVSRIPO will be summarized in all analyses. For any informal summary comparisons of safety or efficacy relative to PVSRIPO alone or pembrolizumab alone from other data sources, both the overall and per-protocol populations may be considered.

A screening analysis set will include all patients who signed the ICF and include screen failures who failed up until PVSRIPO infusion (ie, considered a screen failure if biopsy at time of catheter placement does not confirm rGBM). The safety and efficacy analysis sets are described in **Sections 8.4.5** and **8.4.6**, respectively.

## 8.3 Handling of Missing Data

Missing or implausible data noted during routine interim monitoring visits, via programmed or manual edit checks withing the eCRF, and during database review and cleaning will be brought to the attention of the Investigator 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. It will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

## 8.4 Statistical Analysis Plan

A brief description of the planned statistical analysis is provided below. The final analyses will be described in greater detail in the SAP, prior to conduct of the specified analyses.

### 8.4.1 Disposition of Patients

A number of tabulated summaries will be created to adequately describe the disposition of patients prior to study enrollment and treatment, as outlined in the SAP.

### 8.4.2 Protocol Deviations

All the protocol deviations will be provided in listing format. Number and percentage of patients not meeting IC/EC will be tabulated for the safety data set.

### 8.4.3 Demographics, Disease Characteristics and Medical History

All demographic and medical history information will be summarized using the safety data set.

### 8.4.4 Demographics

Key demographic variables will be summarized, as outlined in the SAP.

#### 8.4.4.1 Disease Characteristics

The following disease characteristics relating but not limited to the following will be tabulated and may be explored as variables for safety/response:

- Tumor mutational status, by site standard means of analysis, at diagnosis and recurrence/baseline including but not limited to the following should be recorded if available or may be assessed by central pathology laboratory on archival tissue/tissue obtained at baseline biopsy:
    - PV receptor (CD155)
    - EGFRvIII and wild-type EGFR status
    - MGMT promoter methylation status
    - IDH 1 and 2
    - TERT
    - PD-1/PD-L1

**Tumor treatment history:**

- Initial diagnosis; GBM or transformation from a lower grade to a higher grade, along with tumor characteristics (size, location, etc.)
- Prior MRI transmitted to Sponsor or designee, if required
- Age at diagnosis
- Time from initial diagnosis and initial and subsequent therapies, including PVSRIPO
- Extent of prior surgical resection
- All prior therapies (approved/investigational)
    - Prior tumor treatments will be recorded in the relevant sections of the disease specific medical history

#### 8.4.4.2 Medical History

Medical history will be tabulated by number (%) of patients in the safety data set for each system organ class and preferred term.

#### 8.4.4.3 Prior and Concomitant Medications or Therapies

Prior medications are defined as medications taken or therapies received by patients prior to PVSRIPO infusion. Concomitant medications are defined as medications taken or therapies received by patients during the study after first dose of PVSRIPO. Medications will be coded using the World Health Organization (WHO) Drug Dictionary version. Summaries will be performed on the Safety set.

The number (%) of patients receiving or taking prior and concomitant medications or therapies will be summarized based on the WHO Anatomical Therapeutic Chemical (WHO ATC) Level 1 and generic name. In addition, by-patient listings will be provided for the following:

- Prior investigational agents
- Secondary therapies (during the study)

#### 8.4.4.4 Unscheduled Visits

Data collected at unscheduled visits will be included and analyzed for both safety and efficacy analyses in the same fashion as the data collected at scheduled visits and will be reported in the eCRF, except as otherwise noted.

Descriptive statistics (mean, standard deviation, median, minimum, maximum and quartiles) by nominal visit or time point for safety endpoints such as laboratory measurements and vital signs will include only data from scheduled visits per protocol.

### 8.4.5 Safety Analysis

The full safety analysis set will include all patients who were administered at least one dose of the PVSRIPO. The safety of study-required pre-infusion procedures including catheter placement, will also be summarized.

Separate summaries will be prepared for the primary safety analysis set, which is patients who received PVSRIPO and ≥ 1 dose of pembrolizumab. Patients who received PVSRIPO alone will be summarized separately (secondary safety analysis).

Summaries of TEAE will begin at the time of PVSRIPO infusion. Adverse events considered related to screening or other pre-infusion procedures that are not SOC (eg, PV booster, biopsy prior to infusion, catheter placement without PVSRIPO infusion) will be summarized separately (screening data set).

All safety endpoints for patients receiving PVSRIPO and ≥ 1 dose of pembrolizumab may be summarized overall and by the number of pembrolizumab treatments received while on study (eg, 1, 2 to 3, 4 to 6, etc.).

Adverse events will be summarized by highest grade per patient and by attribution according to CTCAE v5.0, using the latest version of MedDRA preferred term as event category and MedDRA primary system organ class body term as Body System category. Unless otherwise stated, adverse events will be displayed in terms of frequency tables by treatment group by decreasing frequency. Each patient will be counted only once within each preferred term or system organ class. If a patient experiences more than one AE (or laboratory value) within a preferred term or system organ class for the same recording period, only the AE with the strongest relationship or the worst severity, as appropriate, will be included in the summaries of relationship and severity.

Events meeting the criteria for a DLT or SAE, leading to discontinuation, resulting in death, etc., will be reported in additional summaries. Changes from baseline over time and abnormal laboratory values, vital signs and NANO and KPS will also be reported.

Changes in toxicity grade, seriousness or outcome of AEs are recorded as separate entries in the eCRF with associated end and start dates (start date equals end date of previous entry). Such entries reporting the same event in such immediately consecutive periods will be considered as one event in the analysis in case of an improvement in toxicity grade. These events will be kept as separate records in the database to maintain the full detailed history of the events. The start date of the initial record in the sequence is taken as start date of the entire event. Duration of the AE will be adjusted accordingly in the analysis.

### 8.4.6 Efficacy Analysis

The full efficacy analysis set will include all patients who were administered at least one dose of PVSRIPO but patients receiving PVSRIPO alone will be analyzed separately from those who received PVSRIPO and ≥ 1 dose of pembrolizumab, which will be considered the primary efficacy analysis set.

All efficacy endpoints for patients receiving PVSRIPO and ≥ 1 dose of pembrolizumab will be summarized overall and in subgroups by the number of pembrolizumab treatments received while on study (eg, 1, 2 to 3, 4 to 6, etc.), for those with pembrolizumab interruptions, with treatment-related irAE, etc.

In addition, a per-protocol analysis will also occur to explore the impact of excluding patients from radiographic and survival primary analyses who:

- Underwent surgical resection of target lesions for control of PTE or CE or other reasons following-PVSRIPO infusion
- Received > 4 mg/day dexamethasone or equivalent prior to or for > 14 days after the first pembrolizumab treatment
- Received ≥ 3 consecutive treatments with bevacizumab at doses exceeding 7.5 mg/kg
- Received other non-protocol specified anti-tumor treatments
- Did not receive ≥ 1 dose of pembrolizumab (these patients will be replaced)
- Had major deviations, classified as follows:
    - Did not fulfill eligibility criteria
    - Met criteria for withholding or discontinuation of study treatments but continued treatment
    - Received incorrect study drug or dose of study drug.
    - Timing of study drug administration outside of protocol-specified windows
    - Received prohibited concomitant medication
    - Protocol-required procedure not adhered to
    - Other

The per-protocol analysis set will be identified prior to database lock.

There will be 2 patient strata at enrollment: the first, based on receipt of any prior anti-angiogenic or anti-VEGF targeted agents (eg, bevacizumab, cediranib, aflibercept, vandetanib, cabozantinib, sunitinib, etc.) and the second, based on prior chemotherapy use. The size of the stratum for VEGF targeted agents will be capped at ≤ 50% of the total population enrolled (ie, do not want > 50% of the patients enrolled to have failed prior VEGF targeted therapies). The size of the stratum for prior chemotherapy use will be ≤ 50% of the total population enrolled, regardless of MGMT promoter methylation status (ie, want > 50% of the patients receiving the combination to have failed prior chemotherapy use). Enrollment and eligibility criteria will be monitored to meet these goals. Data will be analyzed together and separately, by these strata.

Responses may also be summarized in by subgroup analyses including but not limited to baseline NANO score and KPS, prior bevacizumab and chemotherapy or other tumor treatment use/failure, extent of surgery at diagnosis (GTR, STR), MGMT promoter methylation status and/or other tumor genetic factors at diagnosis and baseline, lesion cross-sectional area at baseline, time from initial diagnosis to baseline, number of prior progressions, age, and sex.

For any informal summary comparisons of safety or efficacy relative to PVSRIPO, pembrolizumab or other ICI alone or in combination from other data sources, both the overall and per-protocol populations may be considered.

#### 8.4.6.1 Objective Response Rate and Disease Control Rate

Given PVSRIPO's pattern of response and mechanism of action (direct tumor cell killing followed by adaptive anti-tumor immune response), 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. This is anticipated to occur to a similar or possibly greater extent when followed by pembrolizumab treatment.

In the Phase 1 and Phase 2 rGBM PVSRIPO clinical trials, an initial increase in the extent of FLAIR abnormalities was observed after PVSRIPO, which resolves over time. 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; see **Figure** 4. Depending on the size of the tumor, the inflammatory changes could be observed up to 12 months post-PVSRIPO infusion.

This immunoprogressive appearance can resolve and patients may go on to have an ORR and achieve long-term survival [5]. However, the time to response is delayed, as noted for other immune-therapies [27]; the median time to response in the Phase 1 study based on an ongoing analysis was 15.4 months (range 5.7 to 29.7 months; data on file, Istari). In addition, areas of new enhancement in the vicinity of the target lesion and in previously non-enhancing T2/FLAIR and response tracking, where previously non-enhancing/infiltrative disease enhances and subsequently resolves, has been noted with PVSRIPO in the Phase 1 and Phase 2 rGBM studies within the first 12 months post-infusion and is being independently assessed by central radiographic reviewers (data on file, Istari; see example in **Figure 5**). This immunoprogressive type of radiographic appearance has been described with other immunotherapeutics [1, 30-32].

Given this and the potential use of low dose bevacizumab for managing mass effect related to PTE, adjustments to define radiographic response, disease control and progression are required, as has been noted with other oncolytic viruses [30]. Therefore, the number and percent of patients meeting criteria for a confirmed response (CR or PR) and disease control (confirmed CR, confirmed PR or SD for ≥ 6 months), as well as confirmed progression will be based on the criteria outlined in **Table 3**. In addition, an exploratory analysis will evaluate response via iRANO criteria.

**Table 3 Criteria for Determination of Response/Progression with PVSRIPO Followed by Pembrolizumab**

| Response | Criteria |
|---|---|
| **1. Complete** | • Disappearance of all enhancing disease and meets criteria in # 4 below |
| **2. Partial** | • ≥ 50% reduction of biperpendicular diameter of enhancing disease and meets criteria in # 4 below |
| **3. Stable Disease** | • Does not qualify for CR or PR above AND,<br>• No increase of biperpendicular diameter of total enhancing disease > 25%, AND<br>• Meets criteria in # 4 below |
| **4. Must be met for all responses above:** | • Response persists for ≥ 4 weeks<br>• Response persists for ≥ 8 weeks post-discontinuation of bevacizumab (once confirmed off bevacizumab, response duration will be reported as when 1st observed when response 1st noted)<br>• No greater than physiologic steroids (≤ 4 mg/day dexamethasone or equivalent) for CR; stable/improved steroids for PR and SD.<br>• Stable/improved clinically[1] |
| **Immunoprogression** | • Increase in enhancing lesion size (with or without new lesions/enhancement into T2/FLAIR), within 12 mos of PVSRIPO infusion that responds radiographically to steroids (≤ 4 mg/day dexamethasone or equivalent) or bevacizumab (≤ 7.5 mg/kg q3 weeks) or a combination of both within 3 months of noting appearance of radiographic progression)<br>AND either:<br>• Absence of significant clinical decline<br>OR<br>• If significant clinical decline is present, it is responsive to post-infusion interventions including steroids (≤ 4 mg/day dexamethasone or equivalent) or bevacizumab (≤ 7.5 mg/kg q3 weeks) or a combination of both within 3 months of radiographic progressive-type appearance (ie, ≥ 25% increase of biperpendicular diameter of enhancing disease/new lesions/worsened T2/FLAIR) |
| **Progression** | • Does not meet criteria for immunoprogression<br>AND<br>• ≥ 25% increase of biperpendicular diameter of enhancing disease from the smaller of baseline or post-infusion nadir (enhancing at baseline plus any new enhancement) and/or worsened T2/FLAIR, confirmed on a subsequent scan performed at least 3 months later (unless clinical deterioration prevents confirmatory scan). If confirmed, progression date is the date that progression was first observed. |

Investigator reported results may be verified by independent central radiographic reviewers blinded to patient outcome and possibly bevacizumab use. Response will be summarized at

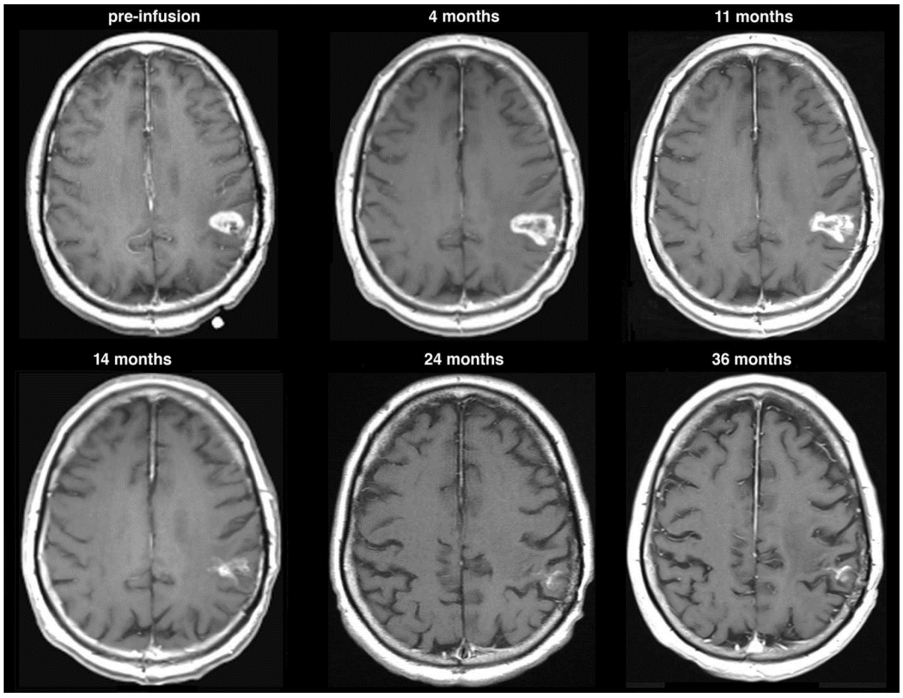
[1] Not inclusive of clinical deterioration clearly due to an intercurrent illness or condition not directly related to the patient's tumor.

regular intervals including those achieving a response by 6, 12 and 24 months post-PVSRIPO infusion.

Given that the NANO scale has been shown to provide a more detailed and objective measure of neurologic function for predicting the prognosis of glioblastoma patients especially at the time of progression [33], the NANO scale will be utilized to explore clinical neurologic function as a correlate of response, especially with regard to immunoprogression and/or confirmed progression, along with KPS.

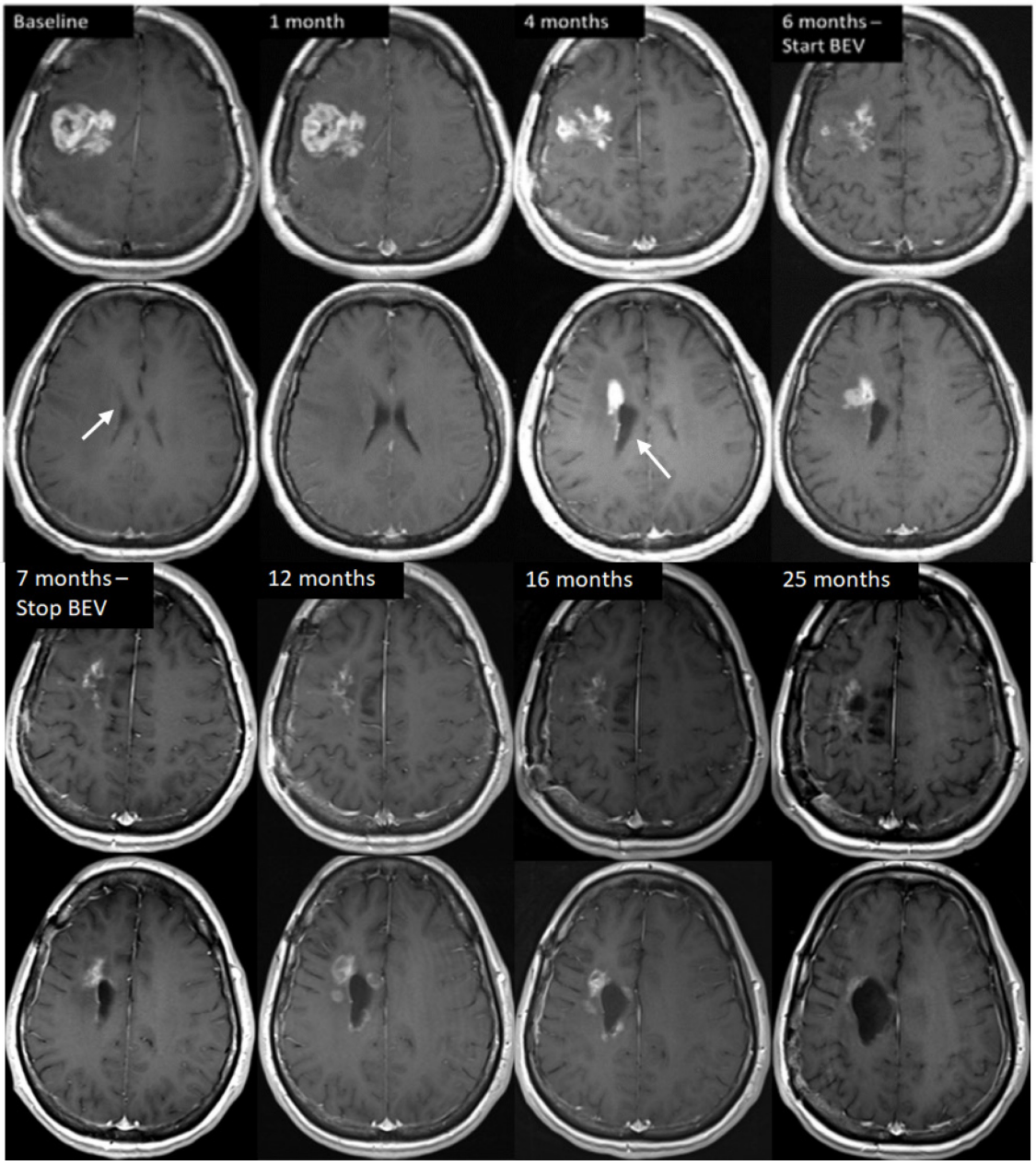
Final criteria for determining response and progression will be outlined in the SAP and the SAP will prevail in the event differences are noted.

**Figure 4 Example of Initial Increase in Lesion Size with PVSRIPO Treatment Followed by Retraction**




In **Figure 4** above, note the ≥ 25% increase in infused lesion size (at months 4 and 11) after PVSRIPO treatment followed by retraction, beginning around 14 months post-infusion.

**Figure 5 Example of New Enhancement/Disease Tracking after PVSRIPO Treatment, Which Resolves over Time**




In **Figure 5**, note the appearance of new enhancement over time, including into previously non-enhancing T2/FLAIR, that resolves over time. Also note the reduction in mass effect, beginning at month 1 post-infusion, which is more prominent at month 4 (2nd row). The ventricle, nearly closed at baseline due to tumor mass effect on the ventricular space (white arrow), is open at 4 months (2nd white arrow), despite new enhancement into previously non-enhancing disease. This disease tracking/areas of new enhancement around the previously non-enhancing infiltrative disease, continues through month 16 post-PVSRIPO infusion but the reduction in mass effect on the ventricle is noted through 25 months. This observation supports the notion that the new

enhancement is not new active disease, which would be expected to exert pressure on and close the ventricle, but rather is indicative of immunotherapeutic response and disease tracking.

#### 8.4.6.2 Duration of Response and Durable Radiographic Response

The DOR is defined as time from first confirmed response until confirmed PD or death, whichever comes first.

A DRR is defined as a radiographic response that persists for ≥ 6 months.

The DOR and DRR for those meeting the criteria for confirmed response (CR or PR) with and without those achieving SD will be calculated. Endpoints as evaluated by iRANO and inclusive of SD will be utilized to describe these endpoints for overall DCR.

#### 8.4.6.3 Progression Free Survival

If calculable, PFS will be based on the time of first PVSRIPO infusion to death or unequivocal confirmed progression, based on the definition of progression noted in **Table 3**.

#### 8.4.6.4 Landmark Survival

Landmark Survival: the proportion of patients alive at ≥ 6, ≥12, ≥18 and ≥ 24 months from time of first PVSRIPO infusion.

#### 8.4.6.5 Overall Survival

Overall Survival is defined as time from first PVSRIPO infusion to death from any cause, or last follow-up if patient is alive. Kaplan-Meier estimates (product-limit estimates) will be presented with a summary of associated statistics including the median survival time with two-sided 95% CIs. The survival rates will be estimated throughout the study through a maximum of 24 months, with corresponding two-sided 95% CIs.

## 9 Ethical Considerations

### 9.1 Institutional Review Board or Independent Ethics Committee

The protocol, the ICF and patient assent documents (as applicable), relevant supporting information and all types of patient recruitment or advertisement information for this study must be approved by the appropriate IRB/IEC before the study is initiated. Copies of the Letter of Approval from the IRB/IEC, which contains specific identification of the documents approved, must be received by the Sponsor prior to shipment of study drug supplies to the investigational site. Any amendments to the protocol, as well as a change of Principal Investigator, must also be

approved by the IRB/IEC and documentation of this approval must be provided to the Sponsor prior to implementing changes in the study. The IRB/IEC utilized must comply with current ICH GCP. The IRB/IEC must supply to the Sponsor or their designee, upon request, a list of the IRB/IEC members involved in the vote and a statement to confirm that the IRB/IEC is organized and operates according to ICH GCP and applicable laws and regulations.

Records of IRB/IEC review and approval of all documents pertaining to the study must be kept on file by the Principal Investigator and are subject to inspection by a regulatory authority at any time. IRB/IEC re-approval is required every 6 months or annually as required by local regulation. Where required, the Principal Investigator is to notify the Sponsor, in writing, of the approval to continue the study.

The Investigator's responsibilities regarding the IRB are as follows:

- Obtain IRB/IEC approval of the protocol, ICF, and any advertisements for patient recruitment prior to their use.
- Obtain IRB/IEC approval for any protocol amendments and revisions to the ICF before implementing the changes.
- Provide the IRB/IEC with any required information before and during the study.
- Submit progress reports to the IRB/IEC, as required, during the conduct of the study; request re-review and approval of the study, as needed; provide copies of all the IRB/IEC re-approvals and relevant communication to the Sponsor.

Notify the IRB/IEC within 10 days of all serious and unexpected AEs related to the study investigational product that are reported to the Investigator by the Sponsor if required. The Investigator is responsible for updating the IRB/IEC on the progress of the study and of any changes made to the protocol at least once a year or at regular intervals as deemed appropriate. The Investigator must also keep the IRB/IEC informed of any AEs, according to the IRB/IEC policy.

## 9.2 Ethical Conduct of the Study

The Investigator(s) will conduct the study in accordance with this protocol, the Declaration of Helsinki, ICH, GCP and all other applicable laws and regulations. The Investigator(s) and the Sponsor will sign the protocol and study contract, to confirm agreement. The Investigator(s) will not implement any amendment (deviation or changes of the protocol) without agreement by the Sponsor and approval of the associated IRB, IEC or equivalent, except where necessary to eliminate immediate hazard(s) to study participants, or when change(s) involve only logistical or administrative aspects of the study.

## 9.3 Data and Safety Monitoring Board (DSMB)

A DSMB will be engaged to assess the safety of the interventions during the study. The DSMB will provide recommendations about stopping or continuing enrollment in the study. Evaluations will be conducted by the DSMB after the initial patients undergo DLT assessment during the safety lead in period and for the evaluation of SUSAR throughout the study, as specified in the DSMB charter. The charter will prevail in the event any differences are noted in protocol and charter. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection, recruitment, and retention of participants and their management.

## 9.4 Protocol Revisions and/or Deviations

The protocol may be revised for any number of reasons (eg, to expand the study, to modify the planned treatment or follow-up in consideration of safety or efficacy from this study or other sources). All changes to the prior version of the protocol will be outlined in a summary section for all amendments. The amended protocol (along with any amended ICF, if applicable) must be approved by the designated IRB/IEC or other institutional local regulating authorities, as required, prior to implementation of the changes, except in the case where immediate changes are required to prevent harm to current or future patients.

A list of protocol deviations will be maintained by the Sponsor or their designee(s). Deviations should be reported to the relevant IRB per IRB guidelines, and those required for reporting per ICH and/or Sponsor or their designee's guidelines/Standard Operating Procedures (SOP) will be included in the clinical study report. Such deviations may include but are not limited to:

- Patients that are dosed on the study despite not satisfying the inclusion and exclusion criteria
- Patients that develop withdrawal criteria whilst on the study but are not withdrawn
- Patients that receive the wrong treatment or an incorrect dose
- Patients that receive an excluded concomitant medication
- Deviation from ICH GCP

## 9.5 Patient Information and Consent

The Investigator is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation in layman's terms regarding the nature of the study, along with the aims, methods, objectives, and any potential risks. The ICF must be appropriately signed and dated by the patient or the patient's legally authorized representative and the person obtaining the consent (if required by the IRB) prior to conducting/obtaining any

study-related assessments including the discontinuation of any medications prohibited for the study.

Each patient must be provided with a statement that the investigation involves research and that the IRB/IEC has approved solicitation of patients to participate; a fair explanation of the procedures to be followed and their purposes, including identification of any procedures which are experimental; a description in lay language of any possible side effects; a description of any attendant discomforts and risks reasonably to be expected; a description of any benefits reasonably to be expected; a disclosure of any appropriate alternative procedures that might be advantageous for the patient; an offer to answer any inquiries concerning the procedures, and instruction that the person is free to withdraw consent and discontinue participation in the project or activity at any time without prejudice to the patient. Payment to research participants for participation in the study is considered compensation for the investment of time and for incurred costs, and not a payment nor an incentive. The method and manner of compensation should comply with applicable regulatory requirements. All information concerning payment, including the schedule of payments, must be set forth in the ICF, including a disclosure that the Investigator is being paid to perform the stated research. A patient (or the patient's legally authorized representative) must give written consent to participate in the study. This ICF must be dated and retained by the Principal Investigator as part of the study records. A copy shall be given to the person signing the form. The informed consent process must be documented in the patient's source documents.

The original and any amended signed and ICF must be retained at the study site; and a copy must be given to the patient or patient's legally authorized representative(s).

The ICF shall also contain the patient's authorization for the use and disclosure of his/her protected health information (PHI) in connection with the study. The authorization shall include at a minimum a clear description of the following: the duration of the authorization, the patient's right of access to the PHI (or any suspension thereof during the course of the study), type of information to be used/disclosed in the study, the names or classes of parties that may use or disclose the PHI, the purpose of the use/disclosure of PHI, the extent of the patient's right to revoke the authorization, the extent to which participation in the study is conditioned on signing the authorization, and the potential for re-disclosure of PHI.

The Investigator agrees that the Sponsor and its employees or agents will have the right to review and audit pertinent medical records and source documents relating to this clinical study (see **Sections 10.1** and **10.2**). As part of the informed consent process, the ICF will include a statement from each patient participating in the study permitting the release of his/her medical

records as necessary for inspection by authorized personnel of the Sponsor, FDA or other regulatory authorities, IRB, IEC, or equivalent and the staff managing the clinical study.

In the United States (US), per US law, the release of medical records and the review of the contents will comply with the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

### 9.6 Personal Data Protection and Confidentiality

Anonymity of patients participating in this study will be maintained. Only the patient study number should appear on any study documents submitted to the Sponsor. Every effort will be made to maintain the confidentiality of documents that identify the patient by name (eg, signed ICF, laboratory reports, clinic charts), except to the extent necessary to allow auditing by the FDA, or other regulatory authorities.

## 10 Quality Control and Quality Assurance

### 10.1 Audits and Inspections

At its discretion, Istari Oncology (or designee) may conduct a quality assurance audit of this study. If such an audit occurs, the Investigator agrees to allow the auditor direct access to all relevant documents and the schedule his/her time and the time of his/her staff to permit meetings with the auditor to discuss findings and relevant issues.

In addition, regulatory agencies may conduct a regulatory inspection of this study. If such an inspection by a regulatory authority is announced, the Investigator will immediately inform Istari (or designee). If such an inspection occurs, the Investigator agrees to allow the inspector direct access to all relevant documents and to schedule his/her time and the time of his/her staff to permit meetings with the inspector to discuss findings and any relevant issues.

The main purposes of an audit or inspection are to confirm that the rights and well-being of the participants enrolled (ie, signing informed consent and undergoing study procedures) have been protected, that enrolled participants are appropriate for the study, and that all data relevant for the evaluation of the investigational product have been process and reported in compliance with the planned arrangements, the protocol, the investigational site, and IRB/IEC SOPs, ICH GCP and applicable regulatory requirements.

## 10.2 Study Monitoring

This study will be monitored by Istari, or its designee, in accordance with ICH GCP and applicable regulations. By signing this protocol, the investigator agrees to periodic, on-site and/or remote monitoring of all appropriate study documentation.

The progress of the study will be monitored by periodic on-site and/or remote visits and frequent communications between the Sponsor or designee(s) and the Investigator (either by phone, email, or post).

During these contacts, the monitor will perform the following activities per the Study Monitoring Plan:

- Check and assess the progress of the study
- Review study data collected
- Conduct source document verification
- Identify any issues and address their resolution

The objectives of monitoring procedures are to verify that data are authentic, accurate, and complete; that the safety and rights of participants are being protected; and that the study is conducted in accordance with the currently approved protocol (and any amendments), ICH GCP, and all applicable regulatory requirements.

### 10.2.1 Source Document

Source documentation consists of, but is not limited to, in-patient hospital charts, clinic notes, out-patient records, original tests results, laboratory data, worksheets, investigational product accountability records, consent forms, etc. Source documents must be available for review and inspection during on-site and/or remote monitoring of the study by Istari Oncology, their designees, IRB/IEC and/or appropriate regulatory authorities.

### 10.2.2 Case Report Form

Patient data will be collected in an eCRF via electronic data capture (EDC). The EDC system will be Part 11 compliant and will have a documented audit trail for all changes made to the eCRF.

The Investigator or designee must enter all required patient data using the specified data collection method defined by Istari in a reasonable timeframe after data are obtained (eg, within 48 h; exception is SAE reporting; see **Section 7.2**). The Investigator must sign and date a declaration on the eCRF attesting to their responsibility for the quality of all data entered and that

the data represents a complete and accurate record of each patient's participation in the study. Details on use of the EDC are provided in the eCRF Completion Guidelines.

### 10.3 Quality Laboratory Standards

Other than research samples, clinical laboratory tests are conducted as per SOC and standards are in keeping with the qualified individual site laboratory quality standards and procedures. Research laboratory samples and procedures may be monitored and/or audited in keeping with pre-specified monitoring/audit plans by the Sponsor or their designee. Laboratory normal ranges should be provided to the Sponsor or their designee for all laboratories utilized as part of the SOC assessments in this study.

### 10.4 Data Management

Data management will be performed in accordance with the SOP of Istari or its designee. Patient data will be collected in an eCRF. Data will be reviewed and validated. Data clarifications will be requested of the Investigator or his/her designee, and the database will be edited appropriately prior to database hard lock. Selected variables will be coded. The database will be authorized for release when all data management quality control procedures are completed.

## 11 Study Administration

### 11.1 Participating Sites/Study Centers

Participating centers will be evaluated by the Sponsor or their designee by completion of formal site feasibility and site-qualification assessment, including but not limited to review for acceptable facilities, laboratories, adequate operating procedures, staff and investigator qualifications, and patient population. These may be abbreviated if the center has participated in a clinical study with the Sponsor or their designee and/or in a related indication with the investigational product within a 12-month period.

The Investigator and essential support staff will review current ICH GCP guidelines and all aspects of protocol application and study management with the Sponsor or their designee. It is the responsibility of the Investigator to ensure training of ancillary study staff.

### 11.2 Required Documents Prior to Study Initiation

Required documentation on file with the site and/or Sponsor or their designee prior to authorizing shipment of investigational protocol to the site will be in keeping with ICH GCP

guidelines/standards and the Sponsor or their designee's SOP. Review by the Sponsor/their designee is required before the trial formally begins recruitment at each site.

### 11.3 Clinical Trial Supplies

Clinical trials supplies should not be discarded or destroyed without proper accountability and authorization by the Sponsor or their designee. Any supplies provided to the site for the conduct of the trial may be returned to the Sponsor or their designee or may be authorized for destruction or shipment to 3rd parties, at their request and cost.

### 11.4 Investigator Site File

The investigational site will maintain a specific study file. This file is patient to inspection as described in **Section 10** of this protocol. This file may contain, but is not limited to the following:

- Protocol and protocol amendments
- IB
- IND Safety Reports
- IRB/IEC approval
- IRB/IEC approved sample ICF
- IRB/IEC (or privacy board) waiver of authorization (if applicable)
- IRB/IEC correspondence
- IRB/IEC membership list
- Signed Form FDA 1572 with corresponding curriculum vitae
- Communications (letters, memos, meeting notes, printed emails)
- Site signature log
- Delegation of authority log
- Study drug accountability records (receipt and dispensing)
- Laboratory normal ranges and certification
- Laboratory director's curriculum vitae
- SAE reports (as applicable)
- Patient identification log
- Patient screening / enrollment log
- Informed consent log
- Data captured in electronic data
- Investigator(s) financial disclosure statement

## 11.5 Study Completion

Upon completion of the study (including early discontinuation by the investigator, Sponsor, local IRB/IEC, or other regulatory authorities (eg, FDA, European Medicines Agency [EMA]), the following activities, when applicable, must be conducted by the monitor in conjunction with the investigator, as appropriate:

- Return of all study data to Istari (or designee)
- Data clarifications and/or resolutions
- Accounting, reconciliation, and final disposition of used and unused study drug
- Review of site study records for completeness

In addition, Istari reserves the right to temporarily suspend or prematurely discontinue this study either at a single site or at all sites at any time and for any reason. If such action is taken, Istari will discuss this with the investigator (including the reasons for taking such action) at that time. Istari will promptly inform all other investigators and/or institutions conducting the study if the study is suspended or terminated for safety reasons and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IRB/IEC promptly and provide the reason for the suspension or termination.

## 11.6 Final Report

Istari or their designee will be responsible for generating a final clinical study report. Istari acknowledges the importance of public disclosure/publication of information collected or generated by the Institution and Principal Investigator. All data generated from this study shall be held in strict confidence along with all information furnished by Istari. Independent analyses and/or publication of these data by the Investigator or any member of his/her staff are not permitted without prior written consent of Istari, in keeping with the executed clinical trial agreement. The draft of the publication and the lead authors will be determined by a Sponsor/Investigator publication committee or similar.

## 11.7 Retention of Study Records

Essential documents as described above should be retained for one of the following time periods:

- At least 2 years after the last marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region; or
- At least 2 years have elapsed since the formal discontinuation of clinical development of the study drug

These documents should be retained for a longer period however if required by applicable regulatory requirements or by the executed clinical trial agreement with the Sponsor. It is the responsibility of Istari to inform the investigator/institution as to when these documents no longer need to be retained. The executed clinical trial agreement will govern if language differs from that contained herein.

## 11.8 Confidentiality and Publication

The Investigator and other study site personnel will keep confidential any information provided by Istari (including this protocol) related to this study and all data and records generated in the course of conducting the study, and will not use the information, data, or records for any purpose other than conducting the study, in keeping with all provisions related to such in the executed clinical trial agreement.

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**-END OF PROTOCOL-**