A Phase I trial of PVSRIPO for Patients with Unresectable Melanoma

DUKE CANCER INSTITUTE

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Summary of Protocol Amendment Changes

Version Dates	Summary of Major Changes by Section
12/18/2017 to 06/29/2018	• Section 5.3 clarification of regional chemotherapy definition • Table 2 – Injection volume was updated to 0.5 ml for all lesions • Section 9.2 Clarification on biopsy • Section 10.1.4 – PVSRIPO stablility information was updated for consistency • Section 12 - Schedule of Study tests/ procedures and text were updated to include footnotes for Cohort 1 and Cohort 2 activities. Number of research blood draws was reduced. Follow-up was clarified. • Section 12.7.1 – In the definition of Immune-Related Stable Disease, the confirmation at 4 weeks was removed. This was not clinically meaningful and is not part of the study objectives. • Section 13.1.4 – Updated to define SAE reporting procedures • Information on Safety Oversight Committee (SOC) (Section 13.5) was removed. This information is not applicable for an industry-sponsored trial. • Section 14.1 – Updated to reflect monitoring by the Sponsor, Istari, rather than monitoring by the Duke Cancer Institute. • Administrative changes: formatting and cross referencing corrections throughout
06/29/2018 to 10/03/2018	• Administrative change: corrected pagination in footer of document (error starting on page 7 in previous version), updated title page with protocol number and personnel change, updated table of contents with correct pagination, and updated header with new version date. • Section 12: Table 3 – Combined column for Within 6 months with Screening column and clarified with footnote, additional clarifications on biopsies and vital signs were added in footnotes
10/03/2018 to 02/06/2019	• Section 9.1.1 Added description of Cohort 3 where up to 3 injections are in the same lesion with possible enrollment of 3 additional patients. • Figure 5- Incorporated Cohort 3 doing schema • Section 11.1 Inclusion Criterion 8 – added Cohort 3 • Section 12.2 Treatment Period - Cohort 3 information was added into the descriptions of each visit.
02/06/2019 to 05/15/2019	• Section 12: Table 3 and Section 12.2 Treatment Period - Reduced stool sample collection to one time at 10 days

1 TABLE OF CONTENTS

2 LIST OF TABLES

3 LIST OF FIGURES

4 LIST OF ABBREVIATIONS

aPTT	Activated Partial Thromboplastin Time
AE	Adverse Event
AJCC	American Joint Committee on Cancer
CAP	College of American Pathologists
CBC	Complete Blood Count
CED	Convection-enhanced Delivery
CLIA	Clinical Laboratory Improvement Amendments
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CPC	Cancer Protocol Committee
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCI	Duke Cancer Institute
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
DUHS	Duke University Health System
EGFRvIII	Epidermal Growth Factor Receptor (variant III)
eIF	Eukaryotic Initiation Factor
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery
G-CSF	Granulocyte-colony stimulating factor
GBM	Glioblastoma
GCP	Good Clinical Practice
Gd-DTPA	Gadolinium Diethylene Triamine Pentaacetic Acid

GM-CSF	Granulocyte-macrophage colony-stimulating factor
HGG	High Grade Glioma
HRPP	Human Research Protections Program
HRV2	Human Rhinovirus Type 2
HSA	Human Serum Albumin
HSRC	Duke Human Subject Research Compliance Office
ICH	Intracerebral Hemorrhage
ICS	Investigational Chemotherapy Service
IDH	Isocitrate Dehydrogenase
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IND	Investigational New Drug
IRB	Institutional Review Board
IRES	Internal Ribosomal Entry Site
IV (or iv)	Intravenously
KPS	Karnofsky Performance Status
IND	Investigational New Drug
LSQ	Lymphocyte Subset Quantitation
MGMT	O^6-methylguanine-DNA methyltransferase
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NED	No Evidence of Disease
NHP	Non-human Primate
NSICU	Neuro-Surgical Intensive Care Unit
OARC	Office of Audit, Risk, and Compliance
OS	Overall Survival
PD	Progressive Disease

PET	Positron Emission Tomography
PKC	Protein Kinase C
PJP	Pneumocystis jiroveci pneumonia
PPI	Proton Pump Inhibitor
PRTBTC	Preston Robert Tisch Brain Tumor Center
PT	Prothrombin Time
PV	Poliovirus
PV1S	Poliovirus Serotype 1 (Sabin)
PVSRIPO	Polio/Rhinovirus Recombinant
RANO	Response Assessment in Neuro-Oncology
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOC	Safety Oversight Committee
TCID	Tissue Culture Infectious Dose
TERT	Telomerase Reverse Transcriptase
UTR	Untranslated Region
WHO	World Health Organization

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

5.1 PURPOSE

A Phase I study of oncolytic polio/rhinovirus recombinant (PVSRIPO) in patients with unresectable, recurrent, American Joint Commission of Cancer (AJCC) Stage IIIB, IIIC, or IV melanoma at Duke is planned to address the following objectives.

See Table 1 in Section 8 for specific objectives and endpoints.

5.2 BACKGROUND AND SIGNIFICANCE

The American Cancer Society estimates that 87,110 people will be diagnosed with melanoma and 9,730 people will die of melanoma of the skin in 2017[1]. Although melanoma accounts for less than 5% of skin cancer cases, it causes a majority of skin cancer deaths[1]. Melanoma is the most common fatal malignancy among young adults, and results in an average of 17.1 years of productive life lost per death for adults between 20 to 49 years of age[2]. The number of new cases of melanoma in the United States has been increasing for at least 30 years[1].

Despite appropriate initial surgical therapy for melanoma, one-third of patients will develop recurrence [3,4]. Cutaneous and subcutaneous recurrences with or without concurrent systemic disease affects a large portion of patients with recurrence. The site of first relapse in stage III disease was found to be local/in-transit in 28% of cases, regional nodal in 21% of cases, and systemic in 51% of cases in a large review[5]. In patients that recur after resection of stage II disease, 12% of first recurrences were local/in-transit, 7% nodal, and 11% systemic. Local recurrence can be isolated tumor deposits close to the primary lesion known as satellite lesions or in-transit disease, which represents a specific form of recurrence whereby melanoma tumor deposits occur in the dermal or subcutaneous lymphatic vessels. Tumor recurring in this fashion usually appears as multiple nodules throughout the extremity as shown Figure 1. These lesions can also result in disabling effects, causing pain, bleeding, and/or tremendous anxiety and psychological distress. Because this pattern of recurrence represents multifocal involvement of the extremity's lymphatic system, local excision of these "in-transit" lesions is frequently followed by rapid recurrence or, due to extensive disease,

resection is not possible. Furthermore, systemic disease is either present initially or eventually develops in about 50% of patients with satellite or in-transit disease[6].

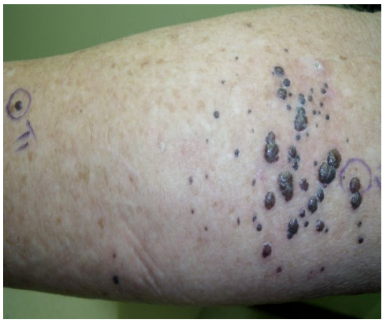

Figure 1. In-transit Melanoma.

5.3 Current Treatment Options for In-Transit Extremity Melanoma

Current treatment options for patients with recurrent melanoma limited to the extremity only include surgical resection, intra-lesional therapy with sclerosing agents or immune adjuvants, isolated limb infusion, hyperthermic isolated limb perfusion (HILP), or amputation. Surgical excision is a reasonable procedure when the lesion(s) are few in number and/or can be encompassed without extensive skin grafting. However, surgical excision of in-transit lesions is often futile, with new lesions appearing before surgical wounds have healed. Intra-lesional therapy with bacillus Calmette-Guerin (BCG) or interferon can provide relief for small lesions that are primarily dermal in location[7]. Regional chemotherapy which involves vascular isolation and perfusion of the extremity with chemotherapy can achieve regional drug concentrations several orders of magnitude higher than could be attained with systemic administration, with minimal systemic toxicity. Regional chemotherapy is associated with complete response rates of 30% but has minimal impact on overall survival[8,9]. Although control of the extremity disease is important, most patients with advanced disease of the extremity will die of systemic disease, and more effective strategies are needed. Regional chemotherapy (isolated limb perfusion or isolated limb infusion) does not treat disease outside the extremity, is limited to patients with extremity disease only (cannot perfuse head neck), and can be toxic. Although regional chemotherapy is still an option, it's becoming increasingly rare due to more effective systemic therapy.

5.4 Current Novel Systemic Therapies for Metastatic Melanoma

Metastatic melanoma has historically been associated with a poor prognosis and survival of less than one year[10,11]. Historic systemic therapies include IL-2 and temozolomide, with associated overall response rates of 16% and 9%, respectively[10,11]. Since 2011, multiple new therapies have become FDA approved for the treatment of metastatic melanoma including unresectable in-transit disease. Examples of novel therapy include mutation based targeted therapies such as vemurafenib, a BRAF inhibitor, or trametinib, a MEK inhibitor [12,13]. For the 50% of melanoma patients with a BRAF mutation, dramatic initial responses can occur in the majority of patients, but resistance develops within 6-9 months [12].

Immune blockade checkpoint inhibitors represent other recently developed novel systemic therapies. Immune blockade therapies appear to have more long-term efficacy, do not appear as susceptible to emergence of rapid resistance like mutation based therapy, and are not restricted to patients harboring specific mutations. Melanoma is a highly immunogenic tumor related, in part, to the high mutational load[14]. Melanoma has thus been the prototype for investigating several forms of immunologic therapy[15] including ipilimumab and pembrolizumab. Ipilimumab, a monoclonal antibody directed against the CTLA-4 protein, promotes immune recognition of self-antigens and thus unleashes cytotoxic activity of endogenous melanoma antigen-specific T lymphocytes[16]. Pembrolizumab is a monoclonal antibody that blocks programmed cell death ligand 1 (PD-1) and therefore counteracts negative regulation of cytotoxic T-lymphocyte activity[17]. As a monotherapy, pembrolizumab had an objective response rate of 45% in treatment naïve patients and twelve month progression free survival (PFS) was 52% in treatment naïve patients [17]. PD-1 based therapy has thus been established as a cornerstone of systemic therapy for patients with advanced melanoma, yet the development of both primary and late resistance remains a problem[18,19]. Dual checkpoint inhibitor therapy can result in higher response rates, but considerable toxicity remains a concern[20]. The combination of ipilimumab and nivolumab (another PD-1 inhibitor) resulted in response rates of 57% and improved overall survival (OS) for patients with metastatic melanoma, however treatment-related adverse events of grade 3 or 4 occurred in 16.3% of the patients in the nivolumab group, 55.0% of those in the nivolumab-plus-ipilimumab group, and 27.3% of those in the ipilimumab group[18]. Thus toxicity with nivolumab (PD-1 inhibitor) alone appears acceptable while the combination of nivolumab plus ipilimumab results in unacceptable toxicity for more than half the patients that receive the combination[19]. While multiple strategies to optimize response are underway including dose reduction in immunomodulatory agents, combination therapies, and predicted response by biomarkers or clinical characteristics, there is opportunity for more directed and specific therapies.

Another strategy for melanoma treatment is oncolytic immunotherapy (proinflammatory effects of viral tumor infection). Specifically, talimogene laherparpvec (T-VEC) is a modified herpes virus that includes insertion and expression of a gene encoding human granulocyte macrophage colony-stimulating factors (GM-CSF) which results in local GM-CSF production to recruit and activate antigen presenting cells[21]. T-VEC was FDA approved for unresectable melanoma based on results of a Phase 3 randomized trial that showed durable response rates of 16.3% in the T-VEC arm compared to 2.1% in the GM-CSF arm [21-23]. While this was an improvement over direct GM-CSF injection, few would advocate subcutaneous GM-CSF as an effective therapy, therefore making a potentially poor control arm therapeutic. Notably, T-VEC was given intralesional with resulting grade 3 or 4 adverse event occurring in ≥ 2% of T-VEC treated patients[22].

Despite important recent advances in the treatment of metastatic melanoma, the ranges of durable response rates are suboptimal and novel combination therapies with higher response rates may come with unacceptable toxicity. Therefore a need to develop therapies with specific, sustained antineoplastic effects remains.

5.5 PVSRIPO

Oncolytic virotherapy (direct tumor cytotoxicity) has potential to elicit broad sustained, anti-neoplastic effects stemming from combined direct viral cytotoxicity and innate antiviral activation[23]. PVSRIPO is the live attenuated, serotype 1 poliovirus vaccine (SABIN) that was modified by exchange of the entire genetic cognate internal ribosomal entry site (IRES) with the corresponding segment from human rhinovirus type 2[24], thus eliminating its neurovirulence. Since PVSRIPO is a version of the serotype 1 live-attenuated poliovirus vaccine (PV1S), its immunogenic properties and potential for long-term sequelae are expected to be similar. Poliovirus serotype 1 (PV1S) has been safely administered to >10 billion individuals worldwide without untoward long-term immunogenic sequela.

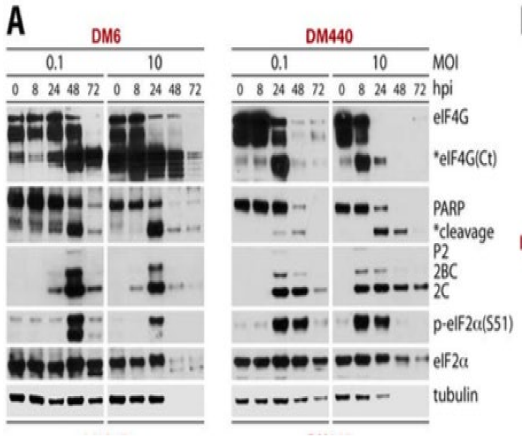

Figure 2: Cytotoxicity of PVSRIPO in Melanoma Cell Lines. Melanoma cell lines DM6 (left) and DM440 (right) were infected with PVSRIPO. Lysates were collected and tested for direct viral cytotoxicity (eIF4G cleavage), host cell demise (PARP cleavage) , and innate antiviral response (p-eIF2α).

PVSRIPO retains translation competence and cytotoxicity in neoplastic cells, which offers ideal conditions for viral IRES-mediated ribosome recruitment due to unhinged protein synthesis control in cancer[25]. To be successful, oncolytic viruses must also have tropism for tumor. The poliovirus receptor (PVR), CD155, is a cell adhesion molecule of the Ig-like superfamily11 expressed during embryonic development [26]. CD155 is broadly expressed in glioblastoma (GBM) and melanoma[27]. Upon binding CD155, viral RNA enters the cytoplasm and begins direct viral cytotoxicity through engagement of anti-viral interferon response[28]. PVSRIPO causes cytopathogenicity in melanoma cell lines as showin in Figure 2[29]. PVSRIPO also induces non-lethal infection of tumor associated macrophages and recruits immune effector responses directed against tumor neoantigens[29].

In a phase 1 trial of intratumoral delivery of PVSRIPO in patients with recurrent GBM, there was one dose limiting toxicity resulting from intracranial hemorrhage at removal of the catheter used to infused PVSRIPO[30]. A recent trial update of all 61 patients treated on the phase 1 trial of PVSRIPO showed a median OS of 12.3 months for the PVSRIPO group versus 10.5 months for the historical controls. The 24 month OS rate of patients infused with $5x10^7$ $TCID_{50}$ of PVSRIPO was 21.1%

compared to 13.5% in the historical control group (data not yet published). Encouraging results with PVSRIPO led the FDA to grant PVSRIPO breakthrough therapy designation as a potential treatment for patients with recurrent GBM in 2016. For more information on the pre-clinical work and clinical work in GBM with PVSRIPO, please refer to the Investigator's Brochure.

The key for a sustained suppression is lasting antigen specific immunity. Strategies that promote antigen presentation in the tumor microenvironment may revive pre-existing antitumor immunity and/or initiate new responses, thereby increasing the anti-tumor immune repertoire. Recent data suggests that the tumor cytotoxicity and interferon dendritic cell activation of PVSRIPO elicit a tumor antigen specific cytotoxic T cell response[29]. Specifically, in addition to direct infection and killing of cancer cells, PVSRIPO's ability to promote anti-tumor responses may result in its ability to infect and activate antigen presenting cells which mediate stimulation of specific cytotoxic T cells *in vitro*. These cytotoxic T cells can recognize tumor-associated antigens such as MART1, a melanoma tumor-associated antigen[29]. The ability of oncolytic virotherapy to target tumor directly and provide specific, sustained anti-tumor responses is worthy of further exploration in solid maligancies such as melanoma.

5.6 Rationale for PVSRIPO in Melanoma

The pathogenesis of GBM and melanoma have many similarities given their shared neuroectodermal origin. CD155 (PVR) is expressed on the majority of melanoma cell lines and PVSRIPO causes cytotoxicity of melanoma cells as shown in Figure 2[29]. There is compelling data in preclinical *in vitro* and *in vivo* models of melanoma demonstrating the potential therapeutic benefit of PVSRIPO. PVSRIPO infection of melanoma cell lines (DM6, DM440) results in cytotoxicity and lysis from an interferon response and cleavage of eIF4G (associated with the viral protease 2A) as shown in Figure 2[29]. *In vitro*, PVSRIPO infection of dendritic cells (DCs) led to DC activation and MART1 (a melanoma associated antigen) specific cytotoxic T cell responses[29]. Furthermore, predictors of response to immunotherapy include an immune active tumor profile with type I interferon emerging as important in CD8 mediated tumor response[31,32]. *In vitro*, PVSRIPO infection of DCs induced sustained type I IFN-dominant responses and cytokine production[29]. Human DC cells exposed to PVSRIPO oncolysate were able to pick up released tumor antigen, present antigen, and prime T cells in an *in vitro* DC-T cell stimulation assay as shown Figure 3[29].

In vivo, single intratumoral injection of PVSRIPO elicits significant anti-tumor efficacy in the murine immunocompetent B16 melanoma model[29]. Importantly, PVSRIPO injection into mice was linked to systemic anti-melanoma antigen-specific cytotoxic T cell lymphocyte (CTL) responses that were evident in lymphocytes isolated from tumor-draining lymph nodes and spleen. Confirming observations in mouse xenotransplantation models, PVSRIPO infusion in the immunocompetent B16 mouse model elicited a series of immune cell invasion steps: 1) neutrophils (peak at 1-2 days post PVSRIPO and rapid disappearance thereafter); 2) DCs (infiltration setting in at day 6 and continuing thereafter); 3) CD4/CD8 T cells (infiltration setting in at day 6-7 and continuing thereafter)[29]. This series of events indicates that, by unleashing a natural innate pro-inflammatory response, PVSRIPO oncolysis turns the immunological 'cold' tumor microenvironment into a hotbed of inflammation and immunogenicity. PVSRIPO infection of tumors, by damaging neoplastic cells and activating IFN responses in antigen-presenting cells, elicits anti-tumor immunity as shown in[29].

These preclinical models provide the rationale for PVSRIPO use in recurrent cutaneous melanoma. The subcutaenous delivery is also attractive as a less invasive approach and the possibility of reduced systemic toxicities. However, if clinical efficacy is demonstrated in the subcutaneous disease model, there is the possibility of injecting or infusing other metastatic lesions (lung, lymph node, brain). Importanly this trial will lead to a phase 1/2 trial of PVSRIPO on combination with immune checkpoint blockade (PD-1/PD-L1). The combination therapy has potential to overcome resistance to monotherapy and generate a more robust immune response[33].

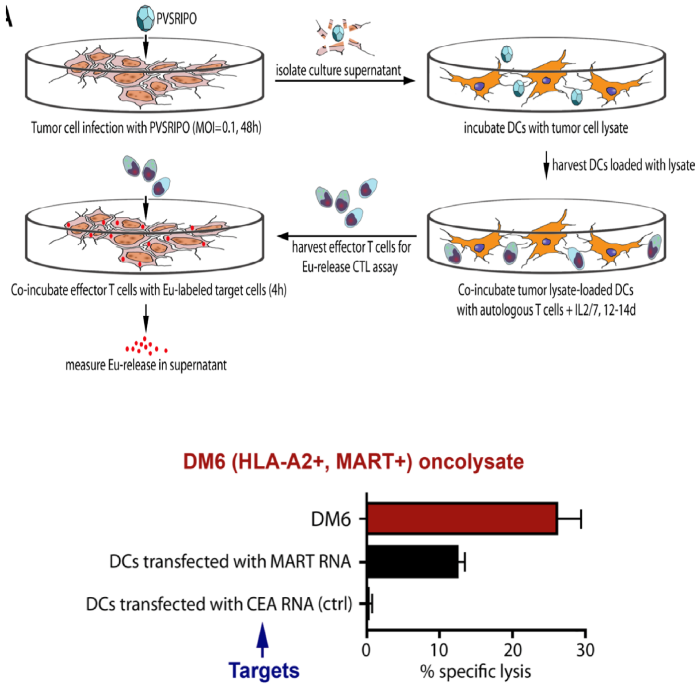

Figure 3**: Human Dendritic Cells (DC) Co-Incubated with DM6 Melanoma Cell Line Oncolysate Stimulate Tumor Antigen-Specific T Cell Responses In Vitro.** Top panel: Schema of assay. Bottom panel: T cells were co-cultured with autologous DCs pulsed with oncolysate from PVSRIPO treated DM6 (melanoma ceel line) cells and the stimulated effector T cells were then harvested and tested in a CTL assay against tumor cells (red bar), tumor antigens (black bars), irrelevant tumor antigens (white bars). Each bar represents average % specific lysis and SD of triplicate samples.

5.7 Design and Procedure

Patients must have histologically proven unresectable, recurrent, melanoma, stage IIIB/C, or stage IV (AJCC staging must be documented in patient's medical record, as determined by CT of the chest, abdomen and pelvis, and/or whole body PET scan, and MRI of the brain within 4 weeks prior to administration of study drug). Patient must have directly injectable disease (at least 1 cutaneous or subcutaneous melanoma lesion at least 10 mm in size (measurable lesion) when 1 dose is planned, at

least 2 measurable lesions when 2 doses are planned, and 3 measurable lesions when 3 doses as planned according to dose escalation). Patients must have at least one measurable lesion that will not be injected. Therefore, the number of injectable lesions may make a subject ineligible for the study depending on when they enroll. Patients with injectable disease must have disease that is palpable or able to be visualized and easily accessible for intratumoral injection. If superficial, the lesion(s) must be documented photographically. Patients must have at least 2, 3, or 4 lesions, depending on when they are enrolled in the study, that qualify as measurable (index) lesions per irRC (Immune related response criteria); except for cutaneous lesions, where sum of diameter for superficial lesions must be at least 10 mm[34] Eligible patients may have received prior therapies including nivolumab, ipilimumab, pembrolizumab, and BRAF/MEK inhibitors. Major exclusion criteria include active severe co-morbidities, autoimmune diseases, or active infections.

Patients can have up to 3 separate lesions injected with PVSRIPO according to the dose escalation/reduction criteria described in Section 9.1.1. Index lesions that are ulcerated or oozing will not be eligible for injection. The PI or designated personnel will select the index lesions for injection. Unresectable disease is required for eligibility, however if the disease becomes resectable, the disease can be surgically resected at any time, at the discretion of the PI.

5.8 Selection of Subjects

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

5.9 Data Analysis and Statistical Considerations

A maximum of 9 patients will be accrued to this trial at an accrual rate of about 1-2 patients per month.

The Primary Objective is to characterize the safety and tolerability of PVSRIPO in melanoma. Common Terminology Criteria for Adverse Events (CTCAE) version 4 will be used to assess adverse events. The DLT criteria are defined in Section 9.1.2

Three patients minimum will be treated with PVSRIPO. See Section 9.1.1 for dose escalation and dose reduction plan. Between each cohort a minimum of three weeks is required before additional enrollment.

Upon completion of the trial, all toxicities will be tabulated by type and grade.

Response to PVSRIPO will be determined with immune-related response criteria (irRC). Full detail of irRC is listed in Table 4 Section 12.7.1.

6 STUDY SCHEMA

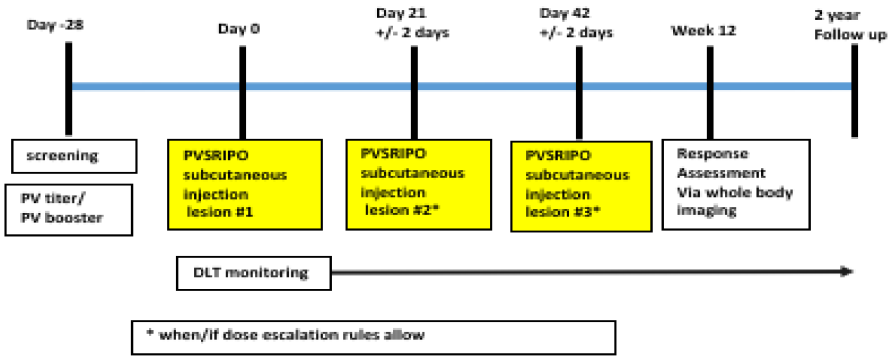

Figure 4: Study Schema

7 BACKGROUND AND SIGNIFICANCE

7.1 STUDY DISEASE

Melanoma is the most deadly form of skin cancer and has been increasing in incidence for 30 years[1] Despite important recent advances in the treatment of metastatic melanoma, reviewed thoroughly in Section 5.2, the ranges of durable response rates are suboptimal and novel combination therapies with higher response rates may come with unacceptable toxicity. Therefore, a need to develop therapies with specific, sustained, antineoplastic effects remains.

7.2 STUDY AGENT: PVSRIPO

7.2.1 PVSRIPO Overview

PVSRIPO is based on the prototype neurovirulent poliovirus (PV) serotype 1 PV1S vaccine modified by exchange of its internal ribosomal entry site (IRES) with its counterpart from human rhinovirus type 2 (HRV2), generating PVSRIPO. PVSRIPO is characterized by loss of the inherent neurovirulence of PV[36-38]. For more details on the make-up, mechanism of action, and formulation of PVSRIPO, please refer to the Investigator's Brochure.

7.2.2 PVSRIPO Tumor Tropism

Every aspect of PVSRIPO oncolytic immunotherapy is dominated by its relationship with its host cell receptor, CD155[39-42]. CD155 is broadly expressed in neuroectodermal malignancies, e.g. malignant glioma and melanoma.

7.2.3 PVSRIPO Pre-Clinical Experience

In animal tumor models including the B16 model, oncolytic PVs elicit efficient anti-neoplastic effects resulting in tumor regression and, eventually, destruction[29]. There is histologic evidence for direct, virus-mediated tumor cell killing and indirect, host-mediated inflammatory responses directed against melanoma. Preclinical data in melanoma is reviewed in Section 5.6.

PVSRIPO was subjected to extensive dose-range finding, toxicology, biodistribution, shedding and neutralizing antibody tests with intrathalamic inoculation of up to 5 x 10^9 $TCID_{50}$ of PVSRIPO in *M. Fascicularis* [43]. These revealed: (i) absence of morbidity and mortality; (ii) absence of neuropathological signs consistent with virus-induced CNS damage; (iii) absence of virus dissemination from the brain or viremia; (iv) absence of extraneural replication; (v) absence of shedding with saliva, urine or stool; (vi) presence of a neutralizing antibody response. For more information on pre-clinical studies of PVSRIPO, please refer to the Investigator's Brochure.

7.2.4 PVSRIPO Clinical Experience

PVSRIPO has been tested in a phase 1 study in subjects with GBM and has generated a durable clinical response in approximately 20% of patients. These responses occurred despite prior disease progression following surgical resection, cranial radiation therapy, chemotherapy (temozolomide, TMZ), and (sometimes) bevacizumab. Twenty-one patients were enrolled into the PVSRIPO dose-finding portion of this phase 1 study. The dose was rapidly escalated from $1x10^8$ $TCID_{50}$ (dose level 1) to $1x10^{10}$ $TCID_{50}$ (dose level 5) after which, the dose was reduced to 5 x

10^7 $TCID_{50}$ due to long-term steroid or bevacizumab therapy to control inflammatory reaction secondary to PVSRIPO-mediated immune response. For more information about the clinical findings of PVSRIPO, refer to the Investigator's Brochure for PVSRIPO.

Based on the original dose finding and safety study in patients with recurrent GBM (ClinicalTrials.gov Identifier: NCT01491893; Duke IRB Pro00031169), this study will start at a dose of $1x10^8$ $TCID_{50}$ (dose level 1) in melanoma patients. See Section 9.7 for rationale for starting dose.

7.3 STUDY PURPOSE/RATIONALE

Local oncolytic immunotherapy is a promising biologic approach to treatment that not only induces viral mediated tumor destruction, but also harnesses a complex immune response that can serve in disease control for the injected tumor and possibly distant lesions. The effects of local oncolytic immunotherapy may help to induce inflammation and generate systemic immunity. The data gained from this trial of PVSRIPO may support a future trial where the inflammation generated by PVSRIPO in combination with a checkpoint inhibitor (eg anti- PD-1 therapy) could generate a more robust immune response. The genetically engineered oncolytic PV (PVSRIPO) has tropism for melanoma cells by virtue of expression of CD155 in tumor cells. To facilitate concentration of the therapeutic agent at the tumor site, while minimizing systemic exposure, PVSRIPO will be subcutaneously injected directly into the tumor. The main toxicity attributable to PVSRIPO in brain tumors has been post-treatment peri-tumoral inflammation that has required prolonged steroid therapy and/or low dose bevacizumab to control edema. Because of the subcutaneous injection without the confinement of the skull, there should not be any complications due to peri-tumoral edema in this study.

8 OBJECTIVES AND ENDPOINTS

Table 1: Objectives

	Objective	Endpoint	Analysis
Primary	To characterize the safety and tolerability of PVSRIPO in AJCC Stage IIIB, IIIC, or IV melanoma.	The proportion of patients with DLTs by cohort (see Section 9.1.2 for definition of DLT)	See Section 15.4
Exploratory	To describe the response rates of PVSRIPO-injected versus non-injected lesion(s).	Measurement of cutaneous lesions every 3 weeks	See Section 15.5
Exploratory	To describe the number of CD8 positive T cells present in the tumor biopsies before and after injection of PVSRIPO.	Number of CD8 positive T cells by IHC on pre treatment and post treatment biopsy	See Sections 12.8 and 15.5
Exploratory	Determine the pathologic response in tumor biopsies after PVSRIPO.	The change in tumor pathology from baseline to after PVSRIPO injection	See Sections 12.8 and 15.5
Exploratory	Determine changes in the tumor microenvironment from biopsies after PVSRIPO.	The change in inflammatory cells and markers after PVSRIPO and detection of viral replication in injected versus non injected lesions.	See Sections 12.8 and 15.5
Exploratory	Describe how systemic immune cell populations change after treatment with PVSRIPO.	Change relative to basline in type and function of T cells via flow cytometry	See Sections 12.8 and 15.5

9 INVESTIGATIONAL PLAN

9.1 STUDY DESIGN

Within this phase 1 study, patients will have one to three lesions of melanoma injected with PVSRIPO, depending on when they begin the first injection.

9.1.1 Dose Reduction/Escalation

A modified 3+3 phase 1 trial design as shown in Figure 5 will be used in this study. Patients in cohort 0 will receive PVSRIPO at a dose of $1x10^8$ $TCID_{50}$ on day 0 into one index lesion (as described in Section 9.1.4). A minimum of three patients will have one lesion injected with PVSRIPO at $1x10^8$ $TCID_{50}$. If 0 or 1 of 3 have a DLT (defined in Section 9.1.2) during the DLT period (21 days after injection), three patients will be enrolled to cohort 1, which consists of PVSRIPO at a dose of $1x10^8$ $TCID_{50}$ on day 0 into one index lesion and a second injection at the same dose ($1x10^8$ $TCID_{50}$) into a separate lesion on day 21 (±2 days). If 2 or more patients have a DLT in cohort 0, the dose will decrease to $5x10^7$ $TCID_{50}$ and 3 patients will have 1 lesion injected on day 0 and this reduced dose will be cohort 1. Additional cohorts will be based on the number of toxicities as detailed in Figure 5. We will treat a maximum of 9 patients. A second injection of a second lesion may not be given in an individual patient if that patient experiences a DLT following their first injection. If an individual patient in cohort 1 or 2 has a DLT, they will not be replaced; rather the trial will continue as detailed in Figure 5. We will enroll in 3 patient cohorts. There must be a minimum of 3 weeks after the last dose in the third patient of the cohort before additional patients can enroll. If 2 of 3 patients in cohort 1 have a DLT, we will temporarily suspend accrual, review DLTs, and re-assess before proceesing to enroll additional patients. If the trial resumes, will proceed to a lower second dose per outline below in Cohort 2, or possibly stop the trial. If 2 of the first 3 patients in cohort 2 have a DLT, we will pause and re-assess before treating a third patient in cohort 2.

Cohort 3 will also treat 3 patients with 3 injections but into the same lesion; each injection will be 3 weeks apart. The proposed dose for each injection will be 1x10^8 TCID. We will also include a dose reduction plan (schema below). If the patient has a DLT after any dose, they will not be able to get additional doses. The trial now will enroll a minimum of 6 patients and a maximum of 12 patients.

In the study calendar, patients cohort 3 will follow the same schedule as cohort 2 patients with the exception that the 3 injections will be into the same lesion.

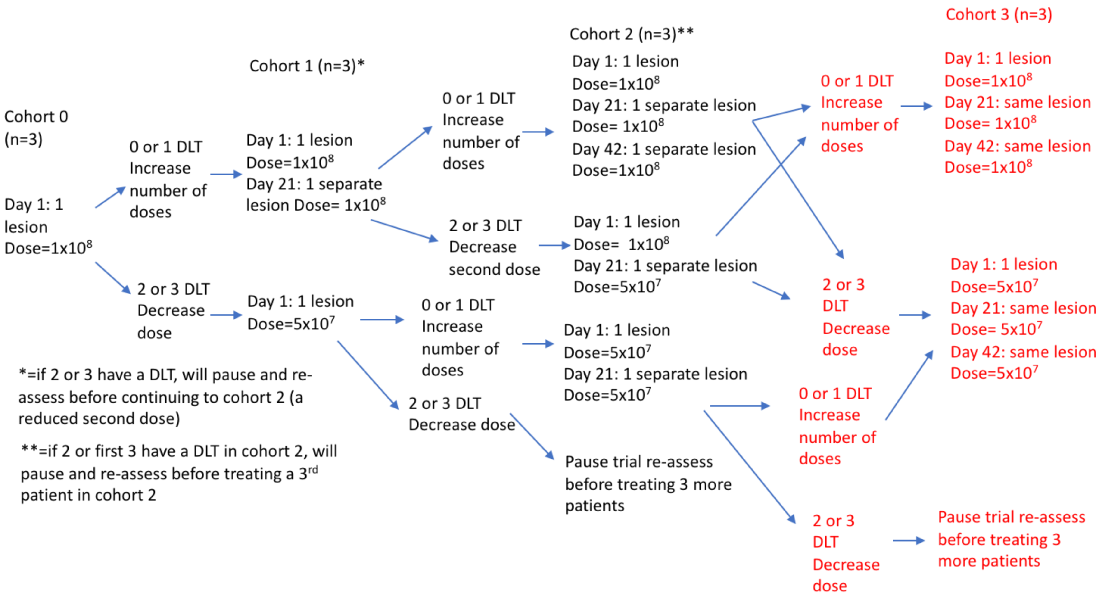

Figure 5: Dose Reduction/Expansion Flow Based on DLTs Experienced by Melanoma Patients Injected with PVSRIPO.

9.1.2 Definition of Dose-Limiting Toxicity

Toxicities will be graded according to the NCI CTCAE version 4 criteria. Any possible DLTs will be identified from each PVSRIPO injection until the end of a 21-day observation period, during which the patient will be evaluated in clinic on day 21 (± 4 days). DLTs will be defined as any of the below events that do not resolve to pre-treatment baseline or ≤ Grade 1 within 21 days or any toxicity indicated below that resolves within the 21-day period, but then recurs as Grade 4 or higher during that same 21-day period.

DLTs will include:

- Any Grade 4 or higher non-hematologic toxicities probably, possibly, or definitely related to PVSRIPO; with the exception of vitiligo
- Any Grade 4 or higher hematologic toxicities probably, possibly, or definitely related to PVSRIPO.

9.1.3 PVSRIPO Injection

The entire volume of the agent will be pre-loaded into a 1 mL syringe by the investigational pharmacist and connected to a 25 G needle. PVSRIPO at each time point will be given in 1 standard dose (either $1x10^8$ $TCID_{50}$, or $5x10^7$ $TCID_{50}$ according to Section 9.1.1). The injection volume will be 0.5 mL.

Table 2: Treatment Schedule Overview

Treatment Timepoint	Max. Injection Volume	Dose	Injection Criteria

First: Study Day 0	0.5 mL	$1x10^8$ $TCID_{50}$ or $5x10^7$ $TCID_{50}$	• inject largest lesion first
Second, if applicable: Study day 21 plus or minus 2 days	0.5 mL	$1x10^8$ $TCID_{50}$ or $5x10^7$ $TCID_{50}$	• Inject next largest lesion different from injection 1 • may inject a new lesion(s) (lesion that has emerged since initial treatment)
Third, if applicable: Study day 42 plus or minus 2 days	0.5 mL	$1x10^8$ $TCID_{50}$ or $5x10^7$ $TCID_{50}$	• Inject next largest lesion different from injection 1and injection 2 • may inject a new lesion(s) (lesion that has emerged since initial treatment) first

Melanoma index lesions present in patients in different quantities and sizes (from 10 mm and larger but rarely no larger than 5 cm in diameter). To facilitate logistics of drug preparation, we will have 1 volume of the same dose (either $1x10^8$ $TCID_{50}$, or $5x10^7$ $TCID_{50}$ according to Section 9.1.1) . A volume of 0.5 mL will be given to all lesions, the priority of lesions is shown in Table 2. A maximum total of $1x10^8$ $TCID_{50}$ will be given per lesion. When lesions are clustered together (sum equals 10 mm), we will inject them as a single lesion with a total of 0.5 mL.

9.1.4 Administration

The following steps will be followed for the administration of PVSRIPO:

Pre-Injection

1. The lesion and surrounding areas will be cleaned with an alcohol swab and let dry.

2. The injection site may be treated with a topical or local anesthetic agent, if necessary, at the discretion of the administering physician. The anesthetic agent will be injected around the periphery of the lesion, not directly into the lesion.

Injection

1. PVSRIPO will be injected intralesionally into a cutaneous or subcutaneous lesion that is visible and/or palpable (Figure 6) PVSRIPO will be injected along multiple tracks, using a single insertion point, as far as the radial reach of the needle allows within the lesion to achieve even and complete dispersion. Multiple insertion points may be used if a lesion is larger than the radial reach of the needle.

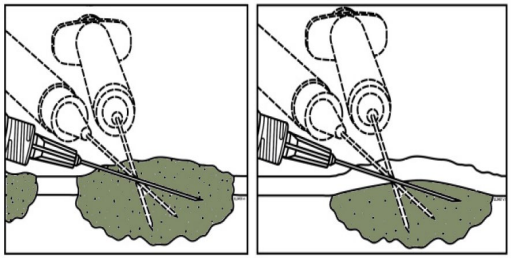

Figure 6: Injection of Cutaneous (left) or Subcutaneous (right) Lesions.

2. PVSRIPO will be injected evenly and completely within the lesion by pulling the needle back without exiting the lesion. The needle will be redirected as many times as necessary while injecting the remainder of the dose of PVSRIPO.

3. The needle will be withdrawn from the lesion slowly to avoid leakage of PVSRIPO at the insertion point.

Post-Injection

1. Pressure will be applied to the injection site(s) with sterile gauze for at least 30 seconds.

2. The injection site(s) and the surrounding area will be swabbed with alcohol.

3. Following a glove change, the injected lesion will be covered with an absorbent pad and dry occlusive dressing.

4. The exterior of the occlusive dressing will be wiped with alcohol.

5. Patients will be advised to:

- Keep the injection site(s) covered for at least the first week after each treatment visit or longer if the injection site is weeping or oozing.
- Replace the dressing if it falls off.

Up to 10 index lesions can be selected for response determination and up to 3 index lesions may be injected over the course of the study. See Section 12.7.1 for definition of index and non index lesions.

9.1.5 Acute Reaction

Any reaction symptoms determined to be an acute reaction to the study drug will be managed by the administering physician.

If a subject experiences any of the following treatment-related toxicities, further PVSRIPO administration should be delayed until the toxicity has resolved to at least CTCAE grade 1 or has returned to baseline:

- grade 2 or greater immune-mediated adverse events, with the exception of vitiligo
- grade 2 or greater allergic reactions
- any other grade 3 or greater hematologic or non-hematologic toxicity

9.2 BIOPSY SAMPLING AND ANALYSES

Biopsy material will be obtained from tumor tissue prior to first virus administration. This tissue material may be subjected to routine histology to confirm tumor recurrence by the study pathologist, Angelica Selim or her designate. If the patient already had documented recurrence by fine needle aspiration or previous biopsy within 4 weeks prior to PVSRIPO injection, no additional routine histology to confirm tumor will be necessary until after PVSRIPO therapy. Molecular genetic tests, as described in Section 12.8 will also be conducted on extracts of tumor cells from the protocol-specified biopsy prior to PVSRIPO injection, if there is sufficient tissue sample remaining after the standard clinical pathologic testing. All tests will be performed on fresh, frozen, or fixed tissue.

Patients may undergo biopsies of injected lesions up to 28 days prior to injection but no less than 7 days before injection, day 10, and day 84 following each injection. Given that each patient has different sizes and numbers of lesions not all biopsies may be possible; only larger lesions can be biopsied 3 times. The priority for biopsies will be 1) pre-treatment; 2) day 84 biopsy; 3) day 10 biopsy. Additionaly, biopsies may be performed to assess pathologic response. Surgical excisions will also include examination of pathologic response. Non injected lesions may also be biopsied pre-treatment, day 10, and day 84, if tumor burden is sufficient. More than 1 biopsy can be performed on injected or non-injected lesions at the timepoints at the discretion of the PI. For example 2 or more non-injected lesions can be biopsied, or more than 1 biopsy (for large lesions) can be done.

9.3 SAFETY CONSIDERATIONS

- Biopsy Complications: 3-5 mm punch biopsy is generally a non-hazardous procedure; however, possible hazards may include: 1) reaction to anesthetic (numbing medicine), 2) excessive bleeding, 3) bruising, 4) infection and 5) excessive scarring.
- Poliomyelitis: PVSRIPO has been tested in non-human primates according to the World Health Organization (WHO) standardized monkey neurovirulence tests. These tests revealed the absence of neuropathogenic properties, evident as failure to induce symptoms of poliomyelitis in non-human primates after intracerebral inoculation of virus. However, PVSRIPO is a replication-competent viral agent that, in principle, retains the potential to cause motor neuron damage. Possible complications include transient or permanent mono- or paraparesis, paralysis, and life-threatening respiratory insufficiency.
- Virus Recombination and Mutation: PVSRIPO retains the ability to alter its genome during replication upon administration to patients. Various mechanisms are known to lead to genetic adaptation, including spontaneous mutagenesis and recombination that may give rise to viral progeny with changed properties. Empirical studies in tissue culture and laboratory animals demonstrated that prolonged passaging in GBM cells does not select for genetic changes in viral progeny. However, the occurrence of such events cannot be categorically excluded in patients receiving intracerebral PVSRIPO. Genetic changes may cause an altered phenotype of PVSRIPO, including adaptation to improved virus replication in the normal CNS.

- Long-Term Sequelae of Virus Injection: PVSRIPO does not encode foreign genetic material; PVs are unable to insert viral genetic material in the host genome and PVs are incapable of inducing latent or chronic CNS infection. Therefore, PVSRIPO is unable to induce long-term expression of foreign polypeptides or long-term persistence. Thus, there are no long-term neurologic consequences to intracerebral PVSRIPO administration in study subjects. For these reasons, no specific measures to analyze the potential for persistence of virus replication in the CNS of long-term survivors are indicated.
- Gastrointestinal Infection and Virus Excretion: After oral uptake, poliovirus replicates in the gastrointestinal tract and is excreted by infected individuals within stool. Gastrointestinal viral replication usually is asymptomatic, but may cause mild symptoms of gastrointestinal discomfort. Tests of PVSRIPO in non-human primates suggest that excretion of virus does not occur after intracerebral administration, implying the absence of gastrointestinal replication. However, PVSRIPO excretion within stool cannot be categorically excluded in patients enrolled in this study. Therefore, treatment entails collection of stool specimens to assess virus excretion during the study.
- Risk of Phlebotomy: Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the site of the needle puncture, light headedness, fainting and very rarely, local infection, which may be severe. These risks are reduced by the fact that the blood will be drawn by a qualified physician, nurse or phlebotomist (a professional trained to draw blood).
- Risks to Household Contacts of Study Subjects: Primate toxicology studies showed that intracerebral infusion of PVSRIPO does not lead to extraneural dissemination or replication and, hence, is not associated with virus shedding. Therefore, and because PV transmission occurs via the fecal-oral route, the likelihood of unintended exposure of patient household contacts is exceedingly low. If accidental exposure occurred, it would equal the risk of exposure to any type 1 Sabin vaccine virus or vaccine virus derivative. Thus, in essence, exposure with PVSRIPO is equal to oral immunization with a safe version of type 1 Sabin. Since type 1 Sabin vaccine virus or vaccine virus derivatives have to be considered part of the human environment, exposure to PVSRIPO would not represent an added risk beyond the possibility for exposure that already exists. Study subjects will be advised of the risks of exposure to unvaccinated household contacts. We will also keep the injected lesion covered for 1 week or longer if the tumor becomes weeping or oozing.
- Healthcare providers: Healthcare providers who are immunocompromised or pregnant should not prepare or administer PVSRIPO and should not come into direct contact with the PVSRIPO injection sites, dressings, or body fluids of treated patients. Wear personal protective equipment (protective gown or laboratory coat, safety glasses or face shield, and gloves) while preparing or administering PVSRIPO. Avoid accidental exposure to PVSRIPO, especially contact with skin, eyes, and mucous membranes. Cover any exposed wounds before handling. In the event of an accidental occupational exposure (e.g.,through a splash to the eyes or mucous membranes), flush with clean water for at least 15 minutes. In the event of exposure to broken skin or needlestick, clean the affected area thoroughly with soap and water and/or a disinfectant.
- Unknown Risks: The overall risk classification of this research is unknown.

9.4 CONCOMITANT MEDICATIONS

9.4.1 Steroids

Corticosteroids should be used at the lowest dose to control symptoms of edema or reactions to PVSRIPO. Use of corticosteroids should be recorded in the electronic database. If the subject requires corticosteroid dosing of >10 mg prednisone daily (or equivalent) for related toxicities, PVSRIPO dosing must be withheld until the corticosteroid dose has decreased to <10 mg prednisone daily (or equivalent).

9.4.2 Growth Factors

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

9.4.3 Anti-emetics

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

9.4.4 Proton Pump Inhibitors

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

9.4.5 Febrile Neutropenia

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

9.4.6 Pneumocystis Jiroveci Pneumonia (PJP) Prophylaxis

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

9.4.7 Epidermal Procedures

If an epidermal procedure is required for a reason other than tumor progression, these procedures should be documented, but will not constitute criteria for declaring the patient "off study".

9.5 STUDY DRUG BLINDING

Not applicable

9.6 RANDOMIZATION

Not applicable

9.7 RATIONALE FOR SELECTION OF DOSE, REGIMEN, AND TREATMENT DURATION

The dose of PVSRIPO for this trial was selected based on IND-directed toxicity studies and on experience from a phase 1 dose-escalation study in adults with recurrent WHO Grade IV malignant

glioma. Dose-range finding and toxicology studies in non-human primates (NHP) documented the absence of viral encephalomyelitis, poliomyelitis and meningitis with intracerebral injection of PVSRIPO up to a dose of 5 x 10^9 $TCID_{50}$ [43] In the adult WHO Grade IV malignant glioma study, 4 patients were dosed at the maximum intended dose of 10^{10} $TCID_{50}$, injected intracerebrally, without signs of viral encephalomyelitis, poliomyelitis or meningitis. In the phase 1 GBM clinical trial, the starting dose was 1 x 10^8 $TCID_{50}$, which is 1/10th of the highest non-toxic dose in NHPs from the definitive, IND-directed toxicology study and 1/50th of the highest non-toxic dose in NHPs from the dose-range finding toxicology study. For more information on these pre-clinical studies, please refer to the Investigator's Brochure. Based on the original dose finding and safety study in patients with recurrent GBM (ClinicalTrials.gov Identifier: NCT01491893; Duke IRB Pro00031169), this study will start at a dose of 1x10^8 $TCID_{50}$ (dose level 1) in melanoma patients.
1x10^8 $TCID_{50}$ (dose level 1 from the GBM trial) was chosen for the following reasons.

1. In the dose escalation study conducted in patients with GBM, patient 1 received dose level 1, 1x10^8 $TCID_{50}$. This patient responded favorably to PVSRIPO therapy and was disease-free 60+ months after receiving a single intratumoral injection of PVSRIPO.
2. Brain inflammation in the area of the tumor or immediately adjacent was the main toxicity associated with intratumoral infusion of PVSRIPO and the primary reason for dose reduction in the phase 1 brain tumor study. There were no signs of encephalitis, systemic toxicity or systemic inflammatory processes. Given that melanoma lesions are not in physiologically confined areas, eg the brain in the cranium, the effects of inflammation due to PVSRIPO are not of significant clinical impact.
3. A higher dose is warranted in this setting given that there may be some systemic leakage upon injection, therefore requiring an overall higher dose to ensure that a substantial amount of the PVSRIPO remains in the tumor. We will therefore start with dose level 1 (1x10^8 $TCID_{50}$) from the GBM study, to ensure that we elicit a response in tumor (both tumor killing and immune consequences) post-PVSRIPO in this Phase 1 study.

9.8 Rationale for Correlative Studies

While tumor-selective PVSRIPO propagation is an important mediator of cytotoxicity, significant intra- and peritumoral inflammation likely ensues. This indicates that an immunogenic response is likely being generated, certainly against the virus itself and most likely against the tumor as well. While the exact host immune response to PVSRIPO oncolysis is currently unknown, but is being investigated, host innate antiviral defenses are likely to trigger a broad immune effector cascade that needs to be examined in patients receiving PVSRIPO therapy. Blood will therefore be collected for immune function studies before and at periodic intervals following treatment with PVSRIPO. The accessibility of tumor tissue in the cutaneous melanoma also allows for correlative studies directly on tumor tissue and the tumor microenvironment. This tumor tissue may be extracted as biopsies or as complete tumor resection, if available.

9.9 Definition of Evaluable Subjects, On Study, and End of Study

Any patient that receives at least one PVSRIPO injection will be evaluable for toxicity and efficacy analyses. Any patient that does not receive PVSRIPO injection will be withdrawn from the study and replaced.

9.10 Early Study Termination

This study can be terminated at any time for any reason by the PI or the sponsor. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 12.5, which describes procedures and process for prematurely withdrawn patients.

10 STUDY DRUG

10.1 PVSRIPO

10.1.1 Names, Classification, and Mechanism of Action

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

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

10.1.2 Packaging and Labeling

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

10.1.3 Supply, Receipt, and Storage

The study agent and vehicle will be supplied to the Investigational Pharmacy by the sponsor or their representative. The study agent will be shipped via approved methods in the appropriate packaging on dry ice. Storage of the study agent at -70°C has been empirically determined to not compromise biological and biophysical properties for at least 5 years. The agent will be stored long-term in an ultra-low temperature freezer (less than or equal to -70°C). Once thawed, it is a clear colorless liquid with no evidence of particulates or foreign matter.

10.1.4 Dispensing , Preparation, Administration

For aliquot preparation, the agent will be thawed slowly on ice (4°C) and kept at that temperature. Thawed vials of PVSRIPO are stable at 4°C for 48 hours. PVSRIPO contained in the clinically intended delivery apparatus (i.e., ready for injection) is stable at room temperature for 18 hours,

but should be used as soon as possible.. The manufacturer will provide the study agent's potency (as tissue culture infectious doses, TCID) and will also supply the appropriate vehicle for aliquot preparation. Aliquot preparation will occur in the ICS. All handling of the study agent will occur in a biosafety cabinet or a similarly contained environment.

Any materials in contact with the study agent, e.g. pipettes, vials, etc., will be disposed of as biological waste. The final desired aliquot of the study agent will be prepared at the intended volume (0.5 ml) and drawn into the intended delivery device (1 ml syringe) using a needle. The disposable needle will be a safer hypodermic needle with a safety sheath to cover the needle using a one-handed technique after the agent is drawn into the syringe. The sheathed needle is then removed from the syringe containing the study agent aliquoted at the desired dose, and the syringe will be capped and connected to a 25 G needle. The capped needle and syringe will be transported to the study site in a 'ziplock' bag placed in a portable cooler while maintaining a temperature of 4°C.

10.1.5 Compliance and Accountability

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

10.1.6 Disposal and Destruction

All materials coming in contact with the study agent, the syringe delivered from the Investigational Pharmacy, tubing, dressings or coverings used to protect the injected lesion site, will be disposed of as biological waste in the treatment room. Disposal of any unused or partially used study agent will be the responsibility of the Investigational Pharmacy. Used sharps will be disposed in a biohazard sharps container and incinerated in final disposal. All personnel caring for the patient will be wearing appropriate safety devices, e.g. disposable gloves, gown, and face protection, which will be disposed of as biological waste in the treatment room. Appropriate hand hygiene is required before and after any handling of the study agent.

10.1.7 Spill Procedure

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

10.1.8 Exposure Follow-Up

Any virus exposures will be reported to the Employee Health Exposure Hotline (919-684-8115), and Sponsor followed by submission of the Report of Occupational Injury or Illness form. There is no specific preventative treatment. Based on the circumstances of exposure, employee health may choose to monitor for infection by submitting stool for enterovirus culture at 7, 14, and 21 days

after an exposure event.

11 SUBJECT ELIGIBILITY

11.1 INCLUSION CRITERIA

1. Positive serum anti-poliovirus titer prior to biopsy.
2. The patient must have received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week prior to administration of the study agent.
3. Patient must have histologically proven unresectable, recurrent, melanoma, stage IIIB, IIIC, or stage IV (AJCC staging must be documented in patient's medical record, as determined by CT of the chest, abdomen and pelvis, and/or whole body PET scan, and MRI of the brain within 4 weeks prior to administration of study drug)
4. Patients with BRAF mutations, must have failed at least 2 lines of therapy, specifically one BRAF targeted therapy and at least one anti-PD-1 based therapy. For BRAF wild type, patients must have failed at least one anti-PD-1 based therapy.
5. Patient must be ≥ 18 years of age.
6. Patient must have an ECOG/Zubrod status of 0-1.
7. Patient's disease must be bi-dimensionally measurable by caliper or radiological method as defined in the irRC criteria.
8. At least 1 injectable cutaneous, subcutaneous or nodal melanoma lesions ≥ 10 mm in longest diameter or, multiple injectable melanoma lesions which in aggregate have a longest diameter of ≥ 10 mm (Cohorts 0, 3 and possibly 1). For cohorts where 2 or 3 injections are planned (Cohorts 1 and 2), the patient must have at least 2 injectable melanoma lesions (when 2 doses are planned) or 3 injectable melanoma lesions when 3 doses are planned in different lesions.
9. At least one measurable lesion that will not be injected.
10. Serum lactate dehydrogenase (LDH) levels less than 1.5 x upper limit of normal (ULN)
11. Patient must have adequate bone marrow, liver and renal function as assessed by the following:
    a. Hemoglobin > 9.0 g/dl
    b. White blood count (WBC) of > 2000 m3
    c. Absolute neutrophil count (ANC) > 1,000/mm3
    d. Platelet count > 75,000/mm3
    e. Total bilirubin < 2.0 x ULN
    f. ALT and AST < 2.5 x the ULN
    g. Creatinine < 2.0 x ULN
12. Patients must have a life expectancy of > 6 months.
13. Patient must provide a signed and dated written informed consent prior to registration and any study-related procedures.
14. Ability to read and understand English and the ability to complete paper and electronic survey assessments.

11.2 EXCLUSION CRITERIA

1. Females who are pregnant or breast-feeding.
2. Adults of reproductive potential not employing an effective method of birth control.
3. Patients with severe, active co-morbidity, defined as follows:
   a) Patients with an active infection requiring treatment or having an unexplained febrile illness (Tmax > 99.5°F/37.5°C)
   b) Patients with impaired cardiac function or clinically significant cardiac disease, such as congestive heart failure requiring treatment (New York Heart Association Class ≥ 2), uncontrolled hypertension or clinically significant arrhythmia; QTcF > 470 msec on ECG if performed or congenital long QT syndrome; acute myocardial infarction or unstable angina pectoris < 3 months prior to study
   c) Patients with known lung (FEV1 < 50%) disease or uncontrolled diabetes mellitus (HgbA1c>7)
   d) Patients with albumin allergy.
   e) Autoimmune disease: History of or current active autoimmune diseases, [e.g. including but not limited to inflammatory bowel diseases [IBD], rheumatoid arthritis, autoimmune thyroiditis, autoimmune hepatitis, systemic sclerosis (scleroderma and variants), systemic lupus erythematosus, autoimmune vasculitis, autoimmune neuropathies (such as Guillain-Barre syndrome)]. Vitiligo and adequately controlled endocrine deficiencies such as hypothyroidism are not exclusionary.
   f) Known immunosuppressive disease, human immunodeficiency virus (HIV) infection, or chronic Hepatitis B or C
4. Patients with a previous history of neurological complications due to PV infection
5. Patients who have not recovered from the toxic effects of prior chemo- and/or radiation therapy. Guidelines for this recovery period are dependent upon the specific therapeutic agent being used. Toxicities must have resolved to CTCAE grade 1 or less with the following excpetions (alopecia, fatigue, vitiligo)
6. Patients with undetectable anti-tetanus toxoid IgG
7. Patients with known history of agammaglobulinemia
8. Patients on greater than 10 mg per day of prednisone within the 2 weeks prior to admission for PVSRIPO injection
9. Patients with worsening steroid myopathy (history of gradual progression of bilateral proximal muscle weakness, and atrophy of proximal muscle groups)
10. Patients with prior, unrelated malignancy requiring current active treatment with the exception of cervical carcinoma *in situ* and adequately treated basal cell or squamous cell carcinoma of the skin
11. Clinically active cerebral or bone metastases
12. Greater than 3 visceral metastases (this does not include nodal metastases associated with visceral organs)
13. Prior allogeneic stem cell transplantation
14. Concomitant therapy with any of the following: IL-2, interferon, or other non-study immunotherapy regimens; cytotoxic chemotherapy; immunosuppressive agents; other investigation therapies; or chronic use of systemic corticosteroids (used in the management of

cancer or non-cancer-related illnesses). However, during the course of the study, use of corticosteroids is allowed if used for treating irAEs, adrenal insufficiencies, or if administered at doses of prednisone 10 mg daily or equivalent.

15. Active clinically serious infection > CTCAE Grade 2.
16. Antineoplastic therapy, radiotherapy, or any other investigational drug within 15 days prior to first study drug administration.

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Table 3: Schedule of Study Tests/Procedures

	Screening: Within 28 days prior to PVSRIPO	**Study**													
Day		**0**	**2**	**10 (± 2 days)**	**21 (± 2 days)**	**23[8]**	**31[8] (±3 days)**	**42 (±3 days)**	**44[8](±3 days)**	**52[8](±3 days)**	**63 (±5 days)**	**84 (±7 days)**	**105[8](±7 days)**	**126[8] (±7 days)**	**then q2-3 months for 2 yrs [13]**
General Evaluations															
Informed Consent	x														
Physical Exam[17]	x			x	x [1]		x	x [1]		x	x	x	x	x	X
Lesion Measurements	x [6]	x [1]			x [1]			x[1]				x	x	x	X
Performance Status		x [1]	x	x	x [1]	x [1]	x	x[1]	x	x	x	x	x	x	X
Adverse Events		Continuous [11]													
Laboratory Evaluations															
EKG	x														
PV Booster	X[16]														
Tetanus toxoid titer	X[16]														
PV titer (blood)	X[16]	X [9]		x			x								
LSQ Titer	X[16]														
PV titer (stool)				x											
CBC w/diff	x	x [1]		x	x [1]		x	x [1]		x		x	x	x	
PT, aPTT	x														
LDH	x											x			x

	Screening: Within 28 days prior to PVSRIPO	**Study**													
Day		**0**	**2**	**10 (± 2 days)**	**21 (± 2 days)**	**23[8]**	**31[8] (±3 days)**	**42 (±3 days)**	**44[8](±3 days)**	**52[8](±3 days)**	**63 (±5 days)**	**84 (±7 days)**	**105[8](±7 days)**	**126[8] (±7 days)**	**then q2-3 months for 2 yrs [13]**
CMP	x	x [1]		x	x [1]		x	x [1]		x		x	x	x	
Serum pregnancy	x	x [1]			x [1]			x [1]							
Treatment															
PVSRIPO injection		X			X[7]			X[7]							
Disease Evaluations															
irRC skin lesion		X [1]			X[1]			X[1]				x	x	x	x
PET/CT [12]	x											x			x [10]
Brain MRI	x											X[10]			x [10]
Correlative aspects															
whole blood (35 ml) immune analysis[18]	X[14a, 16]	X([14b]	x	x			x					x			X [10][14c]
tumor biopsy lesion 1	x [2] [3] [4]			x								x [3]			
tumor biopsy lesion 2[7]				X[7]			X[7]						X[7]		
tumor biopsy lesion 3[7]							X[7]			X[7]				X[7]	
tumor biopsy non injected [15]	X [4]			x			x			x		X	X[7]	X[7]	
tumor resection												x [5]	X [5]	X [5]	

1=done before pvsripo is given, but can be within 48 hours of PVSRIPO

2= if not yet histologically confirmed, will need histologic confirmation, biopsy for correlative studies should be done up to 21 days before and must be done no less than 7 days before injection

3=correlative and histology can be done on same biopsies

4=histology can be done on lesion planning to inject or not inject

5=tumor resection can be done at discretion of PI, the resection can be used for histologic and correlative aspects

6=only need to measure 1 to 4 lesions to qualify depending on dose escalation cohort

7= when applicable per dose escalation guidelines

8=these visits and all study procedures for that visit will only occur if the patient received a second and/or third PVRSIPO injection per dose escalation cohort

9=after booster, but before PVSRIPO preferably just before PVSRIPO at same time as immune blood draw

10=at discretion of treating physician

11=collection of adverse events will continue for 90 days post last dose of study drug

12= if patient has limited metastatic disease, irRC reads will also come from PET/CT scans, if patients do not have any metastatic lesions, irRC will only be performed on measurements of skin lesions

13= patients without progressive disease will be followed up to 2 years with procedures in this column; for patients with progressive disease, chart review only will occur every 3 months at the time of progression and they may be contacted by the study team

14a =needs to be done just BEFORE booster

14b = JUST BEFORE PVSRIPO injection

14c =at follow up visit 1, around 6 months after PVSRIPO injection #1

15 = if patient has sufficient disease

16= does not need to be repeated during 28 day screening period, if performed within 6 months prior to PVSRIPO injection

17= Physical exam includes vital signs

18=whole blood collection for immune analysis can be done at discrestion of PI

12.1 SCREENING EXAMINATION

The screening examination will take place within 28 days before PVSRIPO injection. An informed consen must be signed by the patient or their legal guardian(s) before any screening procedure takes place. Within 4 weeks before receiving the PVSRIPO injection, the patient must have received a PV booster. The patient must also have whole blood drawn for LSQ and tetanus titer, and PV titer to document immunity.

Pre-treatment evaluations within 28 days before PVSRIPO injection to determine eligibility will include the following, unless otherwise indicated:

- History and physical exam, including a full assessment, performance status (ECOG), and measurement of lesions that determine eligibility (1-3 depending on dose escalation)
- Informed Consent
- Laboratory Evaluations:
  - CBC with differential
  - CMP
  - PT, aPTT
  - LDH
  - Beta-HCG, if appropriate
  - EKG
  - Whole blood (10 mL) for PV titer (pre vaccine boost), LSQ, and anti-tetanus toxoid titer
- Whole blood (35 mL) for immunologic analysis before polio vaccine boost, Baseline PET/CT or CT c/a/p plus brain MRI
- Biopsy of the lesion that will be injected (can be up to 21 days before must be no less than 7 days before injection) and planned non injected lesion if patient has sufficient disease.

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

12.2 TREATMENT PERIOD

Day 0

- CBC w/diff, CMP, serum pregnancy before PVSRIPO (no more than 48 hours prior)
- PV titer before PVSRIPO
- Whole blood (35 ml) for immune analysis before PVSRIPO
- irRC skin lesion before PVSRIPO
- Collection of Adverse Events
- Lesion measurements before PVSRIPO
- Performance status before PVSRIPO
- PVSRIPO injection (lesion #1)
- Monitor for DLT (dose limiting toxicities) until day 21

Day 2

- Performance status (ECOG)
- Collection of Adverse Events
- Blood for immunologic assay (35 mL)

Day 10 (± 2 days)

- physical exam
- Performance status (ECOG)
- PV titer blood
- PV titer stool
- CBC w diff/CMP
- Collection of Adverse Events
- Whole blood (35 mL) for immunologic analysis
- Tumor biopsy (lesion #1 and a non-injected lesion)
- Tumor biopsy (of intended lesion #2), if applicable

Day 21(± 2 days)

- physical exam
- Performance status (ECOG)
- Serum pregnancy test, CBC/CMP, (no more than 48 hours prior)
- irRC skin lesion before PVSRIPO
- Collection of Adverse Events
- Lesion measurements
- PVSRIPO injection (#2)
- Begin DLT assessment for injection #2 for 21 days. End DLT assessment for injection #1

Day 23 (~2 (±2) days after #2 injection, only for patients in Cohort 1, 2, and 3 who receive a second PVSRIPO injection)

- Performance status
- Physical exam
- Collection of Adverse Events

Day 31 (~10 (±3) days after second PVSRIPO injection, only for patients in Cohort 1, 2, and 3 who receive a second PVSRIPO injection)

- Tumor biopsy (#2 and non injected)
- Tumor biopsy (site of injection #3), if applicable
- 35 ml Whole blood for immune analysis
- Physical exam
- Performance status
- PV titer blood
- CBC w/diff, CMP
- Collection of Adverse Events

Day 42 (± 3 days)

- physical exam
- Performance status (ECOG)
- Lesion measurements
- irRC skin lesion before PVSRIPO

- Collection of Adverse Events
- CBC w/diff, CMP,serum pregnancy before PVSRIPO (no more than 48 hours prior)
- PVSRIPO injection (#3)
- Begin DLT assessment for injection #3 for 21 days. End DLT assessment for injection #2

Day 44 (~2 (±2) days after #3 injection, only for patients in Cohort 2 and Cohort 3 who receive 3 PVSRIPO injections)

- Physical exam
- Performance Status (ECOG)
- Collection of Adverse Events

Day 52 (~10 (±3) days after third PVSRIPO injection, only for patients in Cohort 2 and Cohort 3 who receive a third PVSRIPO injection)

- Tumor biopsy (#3 and non-injected)
- Physical exam
- Performance status
- CBC w/diff, CMP
- Collection of Adverse Events

Day 63 (± 5 days)

- physical exam
- Performance status (ECOG)
- Collection of Adverse Events
- End DLT assessment for injection #3

Day 84 (± 7 days)

- physical exam
- Lesion measurements
- Performance Status (ECOG)
- irRC
- CBC w/diff,/CMP
- LDH
- Collection of Adverse Events
- PET/CT and brain MRI
- Whole blood (35 mL) for immunologic analysis, at the discretion of the PI
- Tumor biopsy of relevant lesions (injected index lesion No.1 and non-injected tumor)
- Surgical resection at discretion of PI

Day 105 (± 7 days)

- physical exam,
- Lesion measurements
- irRC
- CBC w/diff, CMP
- CMP

- Collection of Adverse Events
- Tumor biopsy of relevant regions (injection site No. 2 and non-injected tumor)
- Surgical resection, at the discretion of the PI

Day 126(±7 days, for cohort 2 and cohort 3 only)

- Physical Exam
- Performance status
- Lesion measurements
- CBC w/diff, CMP
- irRC
- Collection of Adverse Events
- Tumor biopsy of relevant lesions (injection site No. 3 and non-injected tumor)

PRN Every 2-3 months after last study visit (last study visit is Day 84 (1 dose), 105 (2 doses), or 126 (3 doses)) up to 2 years for subjects who do not progress

- physical exam
- Lesion Measurements
- LDH
- Whole blood (35 mL) for immunologic analysis (At the discretion of the PI, this blood sample, at least 2 years post-study drug infusion, may be obtained.) at 6 months from PVSRIPO injection #1
- Surgical resection, at discretion of PI
- Collection of Adverse Events will continue for 90 days post last dose of study drug or for 30 days after a patient is off study. For injection #3 AE collection is study day is +7 days of Day 132

If progression occurs, then subjects may be contacted by the study team approximately every 3 months up to 2 years after the last study visit.

While patients are on study, their melanoma may not be treated with any modality other than PVSRIPO. Patients will be considered off study upon treatment of the tumor with another modality. When subjects are considered off study, this indicates that subjects will no longer be obligated to undergo study-related tests and procedures, but the data described below will still be collected from these subjects as feasible.

Due to the mechanism of action of PVSRIPO, subjects may experience transient growth in existing tumors or the appearance of new tumors prior to maximal clinical benefit of PVSRIPO. Subjects who experience growth in existing tumors or the appearance of new tumors will be allowed to remain on therapy until the 12th week after therapy unless, in the opinion of the investigator, immediate surgical resection or any other treatment for melanoma is warranted.

12.3 FOLLOW-UP PERIOD

Subjects will be followed for collection of the additional data if possible, but it is not mandatory and will not be considered a deviation if the data cannot be obtained. Follow up can be obtained by a phone call or

in person visit. Subjects' medical records will be reviewed for the remainder of their life, in order to follow survival, as well as subjects' imaging. Subjects will also be followed for progression and final results of subsequent treatments. Patients without progressive disease will be followed up to 2 years with procedures in the study calendar; for patients with progressive disease, chart review only will occur every 3 months at the time of progression and they may be contacted by the study team.

12.4 End of Study

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

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

12.5 Early Withdrawal of Subject(s)

12.5.1 Criteria for Early Withdrawal

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

- Progressive disease as documented by PET/CT or physical examination at any time
- Treatment with non-protocol therapy
- Pregnancy
- Upon request of the subject
- If, in the investigator's medical judgment, further participation would be injurious to the subject's health or wellbeing
- Development of intolerable symptoms
- Protocol deviation
- Non-compliance of the subject
- Administrative issues

12.5.2 Follow-Up Requirements for Early Withdrawal

Subjects who receieved at least 1 injection of PVSRIPO should be seen in clinic or contacted at a minimum of every 3 months for 1 year, if possible.

12.5.3 Replacement of Early Withdrawal(s)

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

12.6 Study Assessments

12.6.1 Medical History

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

12.6.2 Physical Exam

Standard physical exam and performance status assessment will be conducted and documented per institutional guidelines.

12.6.3 Radiographic Review

A PET/CT will be obtained prior to PVSRIPO injection to evaluate and quantify disease. Patients with greater than 3 visceral metastases will be excluded. A PET/CT will also be obtained 3 months after the first injection of PVSRIPO to document tumor response.

12.6.4 Laboratory Evaluations

The timing of laboratory assessments that will be obtained during the course of the study is given above in Table 3. A list of each evaluation is below.

- CBC w/differential
- CMP
- PT, aPTT (one prior to beginning trial)
- Beta-HCG, if applicable, within 48 hours prior to receiving PVSRIPO

12.7 Tumor Assessments

Response will be determined according to immune related response criteria[35]. Tumor measurement data in combination with photographic records of lesions obtained prior to intervention will be used to establish baseline measurements.

Using a standard centimeter calibrated caliper, the longest axis and the perpendicular axis of the tumor will be measured and recorded in metric notation.

Primary source documentation of disease burden will consist of digital photographs taken at baseline prior to treatment, Day 21, Day 42, Day 63, and Day 84 after each PVSRIPO injection. Photographs should adequately document the number and size of recorded lesions (a millimeter-scale ruler should be placed in field of image to provide scale).

12.7.1 Definition of Overall Response Using irRC

Baseline Measurement: Sum of products of the 2 largest perpendicular diameters (SPD) of all index lesions. The sum of the products of the two largest perpendicular diameters (SPD) of all index lesions will be calculated and reported.

Baseline Documentation of "Non-index Lesions": All other lesions (or sites of disease), including any measurable lesions that were not chosen as index lesions will be identified as non-index lesions. Non-index lesions should be recorded and assessed qualitatively over the course of therapy.

Definitions:

Index lesions: Up to 5 cutaneous lesions and up to 5 metastases (no more than 2 visceral organs) can be chosen. The distribution of these index lesions should be representative of the subject's overall disease status. Index lesions should be selected on the basis of their size (lesions with longest bi-dimensionally perpendicular diameters) and suitability for accurate repeated measurements by imaging techniques (CT, MRI or ultrasound) and/or other method such as clinical exam. The sum of the products of the two largest of perpendicular diameters (SPD) of all index lesions will be calculated and reported.

Measureable Disease: Measurability is defined by the ability to measure a lesion bi-dimensionally with surface area determined by multiplying the longest diameter by the diameter perpendicular to the longest diameter as defined below. An individual lesion measure is therefore provided by the product of a tumor's longest diameter and the diameter perpendicular to that. All measurements will be determined using a ruler or calipers and reported in metric notation (mm) and will be recorded bi-dimensionally.

Measurable Lesions: Measurable lesions are defined at baseline as lesions that can be accurately and serially measured in at least 2 dimensions and for which the longest diameter is:

• ≥ 10 mm as measured by CT scan, MRI, or ultrasound for nodal/soft tissue disease (including lymph nodes)

• ≥ 10 mm caliper measurement by clinical exam for superficial cutaneous or subcutaneous melanoma lesion as measured by caliper

• multiple superficial melanoma lesions which in aggregate have a total diameter of ≥ 10 mm

Nonmeasurable Lesions: All other lesions, including small lesions (longest diameter < 10 mm by CT/MRI/ultrasound for nodal/soft tissue disease [including lymph nodes] or < 10 mm caliper measurement by clinical exam for superficial cutaneous melanoma lesion) and other truly nonmeasurable lesions are considered nonmeasurable and characterized as non-index lesions. This will include any measurable lesions beyond the maximum number of 5 lesions that were not chosen as index lesions.

Follow-up "Index Lesions": At each subsequent tumor assessment, the SPD of the index lesions are added together to provide the total tumor burden.

Follow-up "Non-index Lesions": Non-index disease measurements are not required and these lesions should be followed as "present", "absent", or in rare cases "unequivocal progression".

Follow-up "New Lesions": At each subsequent tumor assessment, if new measurable lesions have appeared they should be added to SPD of the index lesions to provide the total tumor burden. Any nonmeasurable new lesions should be followed as non-index lesions and described as "present", "absent", or in rare cases "unequivocal progression".

Definition of Overall Response Using irRC

Overall response using irRC will be based on these criteria:

Immune-Related Complete Response (irCR): Complete disappearance of all tumor lesions (index and non-index together with no new measurable/unmeasurable lesions) for at least 4 weeks from the date of documentation of complete response.

Immune-Related Partial Response (irPR): The sum of the products of the two largest perpendicular diameters of all index lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the sum of the products of the two largest perpendicular diameters of all index lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irSPD). A decrease, relative to baseline of 50% or greater is considered an immune Partial Response (irPR). The decrease must be confirmed 4 weeks later.

Immune-Related Stable Disease (irSD): irSD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease.

Immune-Related Progressive Disease (irPD): It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute progressive disease:

- At least a 25% increase in the sum of the products of all index lesions and new measurable lesions (irSPD) over the nadir SPD calculated for the index lesions. PD must be confirmed 4 weeks later.

Table 4: Immune-Related Response Criteria Definitions

Index Lesion Definition	Non-Index Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Percent change in tumor burden (including measurable new lesions when present)	Overall irRC Response
Complete Response	Complete Response	No	No	-100%	irCR
Partial Response	Any	Any	Any	≥ -50%	irPR
				<-50% to <+25%	irSD
				≥+25%	irPD
Stable Disease	Any	Any	Any	<-50% to <+25%	irSD
				≥+25%	irPD
Progressive Disease	Any	Any	Any	≥+25%	irPD

12.8 CORRELATIVE ASSESSMENTS

- 35 mL of whole blood for tests of anti-tumor immune responses prior to start of therapy (pre polio boost and just before PVSRIPO injection #1), day 2, and day 10 after PVSRIPO injection #1, then at day 31, day 105, and 6 months after PVSRIPO injection #1. Immune monitoring samples may be analyzed by Dr. Smita Nair, Dr. Jing Li, or their designate in the Department of Immunology at Duke University. Peripheral blood mononuclear cells (PBMCs) will also be collected pre-treatment and at selected times post treatment. Analysis will take place in the laboratory of Smita Nair, PhD. To examine blood for inflammatory and immune signature using arrays and immune cell composition (antigen presenting cells, B cells and T cells), T cell activation by flow cytometry and B cell activation by ELISA and peptide arrays on day -7 (prior to polio vaccine booster), day -2 (prior to PVSRIPO), day 2, 10, and day 84 (after PVSRIPO), and in follow-up (if available).

- Tumor biopsy will be performed before treatment begins. If not already done , this biopsy will be for pathologic confirmation of melanoma. Tumor biopsy will take place prior to PVSRIPO injection (no less than 7 days prior), days 10 after each injection, day 84 after injection and additional biopsies, at the discretion of the PI. If surgical resection is performed, tumor from the specimen will be used after standard histologic assessment and may be used for any of the aforementioned correlative assessments with tissue. The pre-treatment and day 84 biopsy must include pathologic examination for presence of tumor. Additional tumor biopsies will examine histologic response.If additional tissue remains, it may be used for RNA immune signatures and possibly calculation of mutational burden.
- Tumor biopsy studies will include:

  1.Detect presence of PVSRIPO activity in both injected and non-injected lesions. Viral replication will be assessed in the laboratory of Matthias Gromeier using the following techniques: IHC and/or immunoblot detection of non-capsid viral protein (only produced during viral replication; not present in the virus inoculum), plaque assay detection of infectious PVSRIPO, and qRT-PCR for PVSRIPO negative strand RNA (only produced during viral replication; not present in the inoculum). Viral replication in non-injected lesions will be particularly important. If non-injected lesions demonstrate a clinical response, knowing if viral replication is occurring in non-injected lesions will help delineate if response is related to direct viral cytotoxicity or broader immune response.

  2.Exam markers of lymphoid subsets, immune correlates and CD155 (poliovirus receptor) expression. Tumor biopsy studies will include a panel of use of a multiplex IHC machine. The panel includes: CD4, CD8, PD-1, FoxP3, PD-L1, PVR (CD155), and TIGIT. These will be performed in a CLIA certified lab. CD155 expression will be important to veriify PVRSIPO tumor tropism. T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is a candidate for novel immune checkpoints; CD155 is a ligand for TIGIT. The other markers will help elucidate the type of inflammation and changes in immune cell subtypes.

  3.Histologic examination of tumors. In our previous experience with in-transit melanoma, treated lesions to regional chemotherapy can flatten with remaining pigment. Although

pigment may remain, no viable tumor remains. Thus we will examine lesions at 84 days post injection for confirmation or absence of viable tumor cells.

4.RNA for interferon immune signatures. Given the importance of type I interferon production, we will examine interferon signatures in tumor specimens. Only if enough tissue remains will we do this analsysis

5.DNA isolation for calculation of mutational burden. Response to immune checkpoint inhibitors seems to correlate with higher mutational load, though studies have been inconsistent. In limited biopsies from 15 patients with recurrent GBM, a lower mutational load was associated with a clinical benefit after PVSRIPO treatment (unpublished). Only if enough tissue remains will we do this analysis. This is not a defined exploratory analysis of this trial but if tissue remains we may do this analysis at a later time point.

13 SAFETY MONITORING AND REPORTING

13.1 ADVERSE EVENTS

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

An adverse event (AE) is any untoward medical occurrence in a subject receiving study drug and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of PVSRIPO, whether or not related to use of either drug. Abnormal laboratory findings without clinical significance (based on the PI's judgment) should not be recorded as AEs. Laboratory value changes that require therapy or adjustment in prior therapy, or changes to the protocol treatment regimen are considered adverse events.

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

- Medical or surgical procedures (e.g., endoscopy, appendectomy). The condition that leads to the procedure is, however, an AE.
- Situations where an untoward medical occurrence did not occur (e.g., social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing diseases or conditions present or detected at the start of the study that do not worsen.

From the time the subject signs the informed consent form through the End of Study visit (as defined in Section 12.4), all AEs must be recorded in the subject's medical record and adverse events case report form.

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

Attribution of AEs will be indicated as follows:

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

13.1.1 AEs related to study protocol.

See Section 9.3

13.1.2 Reporting of AEs

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

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

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

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

13.1.3 Serious Adverse Events

An AE is considered "serious" if it has one of the following outcomes:

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

13.1.4 Reporting of SAEs

All serious and unexpected adverse events should be reported immediately to the PI or his/her designee. Only adverse events that the PI determines to be serious, unanticipated, and related or possibly/probably (i.e. more likely than not) related to the research must be reported to the IRB. Those adverse events will be submitted to the IRB according the following guidelines:

- Report within 24 hours of learning about any subject's death that was unanticipated and more likely related to the research than unrelated;
- Report within 5 business days of learning about any serious, unanticipated, and related or possibly/probably related AE;
- Report within 10 business days of learning about any other unanticipated problem or event that was more likely related to the research than unrelated. The Sponsor must report to the FDA, in an IND safety report, any suspected adverse reaction that is both serious and unexpected. Before submitting this report, the Sponsor needs to ensure that the event meets all three of the definitions contained in the requirement:
    - Suspected adverse reaction (i.e. there is a reasonable possibility that the drug caused the adverse event)
    - Serious
    - Unexpected

SAEs must be reported by the PI within 24 hours of discovery if the event occurs during the clinical study or within 28 days of receiving the study drug, whether or not the SAE is considered to be related to the study drug. Applicable forms provided by the Sponsor or designee must be transmitted via secure email to the Sponsor **within 24 hours of discovery at pv_istari@klserv.com.**

The PI should not wait to receive additional information to document fully the event before notification of an SAE, although additional information may be requested. Where applicable, information from relevant laboratory results, hospital case records, and autopsy reports should be obtained.

Instances of death, congenital abnormality, or an event that is of such clinical concern as to influence the overall assessment of safety, if brought to the attention of the PI at any time after cessation of study drug administration and linked by the PI to this study, should be reported to the Sponsor.

All SAEs must be reported to the IRB by the PI in accordance with the IRB regulations.

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report. The Sponsor is required to report to the FDA all IND Safety reports in writing within 15 days (7 days for unexpected fatal or life-threatening suspected adverse reaction). All other adverse events will be reported to the FDA in the Annual Report.

13.2 Emergency Unblinding of Investigational Treatment

Not applicable

13.3 Other Reportable Information

Not applicable

13.4 Special Warnings and Precautions

Not applicable

13.5 Safety Oversight Committee (SOC)

Not applicable

13.6 External Data and Safety Monitoring Board (DSMB)

Due to a potential conflict of interest, an external DSMB-Plus was created for the phase 1 PVSRIPO clinical trial performed at Duke University Medical Center. The current study will be monitored by a DSMB. The DSMB will be responsible for safeguarding the interests of trial subjects and assessing the safety of the interventions during the trial. The DSMB will provide recommendations about stopping or continuing enrollment in the trial. To contribute to enhancing the integrity of the trial, the DSMB may also formulate recommendations relating to the selection, recruitment, and retention of subjects and their management. Additional details regarding the responsibility of the DSMB and its chair may be found in the charter document.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

This clinical research study will be monitored both internally by the PI and Sponsor (Istari) monitor or their designee, according to their respective SOPs. All monitor(s) will be qualified, trained on the study protocol and will be familiar with all study procedures. The procedures and plan outlined herein, are applicable to monitoring conducted only by Istari or their designee(s).. In terms of internal review the PI will continuously monitor and tabulate adverse events. Appropriate reporting to the IRB of record will be made. If an unexpected frequency of Grade III or IV events occur, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled;

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

This Monitoring Plan also serves as SOP for the Sponsor (Istari)/Sponsor monitors or their designee(s) and is applicable to the Investigators and site study team**.** All Sponsor/Sponsor monitors obligations may be delegated to a qualified third party via a transfer of obligations (i.e., contract CRA or clinical research organization (CRO)) and the plan/procedure may be modified if obligations are transferred.

DEFINITIONS & ABBREVIATIONS:

a) MONITORING

The act of overseeing the progress of a clinical study, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, SOPs, Good Clinical Practice (GCP), and the applicable regulatory requirement(s).

b) Good Clinical Practice (GCP):

A standard for the design, conduct, performance, monitoring, recording, analyses, and reporting of clinical studies to ensure that the data and reported results are credible and accurate, and that the rights, integrity, and confidentiality of study subjects are protected.

c) Monitoring Report:

Is a written report listing the monitor's findings from a monitoring visit (MV). This report will be sent to the PI and study team after each MV.

d) INVESTIGATOR SITE FILE (ISF)

The repository for the essential documents for the conduct of a clinical trial. These documents individually and collectively permit evaluation of the conduct of the study and the quality of the data produced. These documents demonstrate the compliance of the Sponsor-Investigator and of the monitor with standards of GCP and with all applicable regulatory requirements.

e) SOP (Standard Operating Procedure)

This is a document that describes the agreed procedures of a routine process.

SITE MONITORING SCHEDULE

Initiation, interim/routine and close out monitoring visits are planned by the Sponsor or their designee and will be conducted throughout the study. The site initiation visit will be conducted as soon as:

- All the necessary approvals have been obtained

- Staff recruited
- Investigational product has been delivered to site (is about to be delivered to site)
- CRF and source documents are ready
- Laboratory is ready to start receiving/processing/storing study samples, as applicable.

The first routine monitoring visit is anticipated to occur when the first participant is enrolled and within 60 days of enrollment. It is anticipated that routine/interim monitoring visits will occur after enrollment of every 3-4 subjects (within 60-90 days of enrollment) for an anticipated total of 2 to 3 interim monitoring visits during the enrollment period and again approximately every 6 to 12 months during the follow-up phase once all subjects are enrolled.

However, monitoring frequency will be continually assessed by the study management team, the Sponsor or their designee and the monitor. For example, the monitoring frequency may need to increase if recruitment is faster than predicted, at times of data entry deadlines (such as interim analysis or prior to a DSMB-plus scheduled safety report review), if there is an unexpected frequency of serious and/or unexpected toxicities based on the investigator's brochure, or "for cause", if critical issues are noted at previous MVs or for other issues. The monitor can also conduct a MV upon the request of the Sponsor (Istari), Site/Center Leadership, an Investigator, an IRB or DSMB-plus. The monitoring team, in cooperation with the requestor and Sponsor, will evaluate the MV request and determine whether the MV is appropriate and/or timely.

The close out visit will be scheduled after all subjects have completed the last follow-up visit and/or discontinued, all data are entered into the eCRF and all queries are resolved.

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

14.2 AUDITS

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

Audits are designed to protect the right and well-being of human research subjects. Audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse

events, type of study, or vulnerable populations), phase 1 studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

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

14.3 Data Management and Processing

14.3.1 Case Report Forms (CRFs)

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

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

14.3.2 Data Management Procedures and Data Verification

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

Completeness of entered data will be checked automatically by the eCRF system and users will be alerted to the presence of data inconsistencies. Additionally, the data management team and the statistical team will cross-reference the data to verify accuracy. Missing or implausible data will be brought to the attention of the PI requiring an appropriate response (i.e., confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

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

14.3.3 Study Closure

Following completion of the studies, the study PI will be responsible for ensuring the following activities:

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

15 STATISTICAL METHODS AND DATA ANALYSIS

All statistical analysis will be performed under the direction of the statistician designated in key personnel. Any data analysis carried out independently by the investigator must be approved by the statistician and Sponsor or their designee before publication or presentation.

15.1 Analysis Sets

All patients who receive at least one injection of PVSRIPO will be included in safety analyses.

15.2 Patient Demographics and Other Baseline Characteristics

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

15.3 Treatments

The number of patients that receive PVSRIPO injection will be totaled.

15.4 Primary Objective and Method of Analysis

A maximum of 9 patients will be treated on this study at an accrual rate of about 1-2 patients per month. The Primary Objective is to determine the safety profile of PVSRIPO in Stage IIIB, IIIC, and IV recurrent melanoma patients as determined by DLTs.

Three patients minimum will be injected with PVSRIPO. Please refer to Section 9.1.1 for dose escalation and reduction criteria.

Upon completion of the trial, all toxicities will be tabulated by type and grade according to dose cohort.

15.5 Exploratory Objectives

One exploratory objective is to describe the response rates of injected versus non-injected lesions from PVSRIPO. This will be done by comparing cutaneous lesion measurements, descriptive measurements only will be obtained. We will estimate 95% confidence intervals for response rates.

Other Exploratory objectives of this study are to describe the number of CD8 positive T cells present on biopsy before and after injection of PVSRIPO, to determine the change in pathologic response of biopsies from baseline to 84 days, to determine changes in the tumor microenvironment and immune cell populations before and after injection of PVSRIPO. Immunotherapy can trigger an inflammatory immune response that is observed by presence of tumor infiltrating lymphocytes and therefore tumor biopsies at baseline, day 10, and day 84 will also be examined for tumor infiltrates.

Another exploratory objective is the assessment of immunologic changes stemming from the administration of PVSRIPO. Peripheral blood will be collected at defined intervals for correlative immune monitoring studies in serum and in peripheral blood monocytes. These include (i) analyses of innate and inflammatory immune events (HMGB1, inflammatory cytokines, NK and NKT-cells and

regulatory immune subsets (Tregs & MDSCs); (ii) analyses of adaptive immune responses (lineage, maturation, induction and activation/functional status of tumor antigen-specific T-cells).

15.6 Interim Analysis

If any patient experiences an inflammatory reaction that does not improve within 116 days of PVSRIPO injection or commencing steroid treatment, the inflammatory reaction will be considered "irreversible." If a patient experiences an "irreversible" inflammatory reaction, further enrollment of patients on the PVSRIPO trial will be temporarily interrupted and data on all previously treated patients will be carefully reviewed to determine if the study needs modification. Options may include dose reductions in future patients (next PVSRIPO dose would be 5 x 10^7 $TCID_{50}$, then 1x10^7 $TCID_{50}$), modification of the approach to treating a patient with an inflammatory reaction, study termination, or accrual continuation without modification.

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

15.7 Sample Size Calculation

We have chosen to have 9 patients participate in this pilot study.

16 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

16.1 Regulatory and Ethical Compliance

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

16.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee

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

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

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

16.3 Informed Consent

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

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

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

16.4 Study Documentation

Study documentation includes, but is not limited to, source documents, case report forms (CRFs), monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated "Regulatory Binder," which includes, but is not limited to, approved protocol versions, approved informed consent forms, FDA Form 1572s, and CAP and CLIA laboratory certifications.

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

16.5 Privacy, Confidentiality, and Data Storage

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

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

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

Upon completion of the study, research records will be archived and handled per DUHS Human Research Protections Program (HRPP) and Sponsor policies. Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

16.6 Data and Safety Monitoring

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

16.7 Protocol Amendments

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

16.8 Records Retention

The Principal Investigator will maintain study-related records for the longer of a period of:

- at least two years after the date on which a New Drug Application is approved by the FDA
- at least two years after formal withdrawal of the IND associated with this protocol
- at least six years after study completion (Duke policy)

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18 APPENDICES

18.1 Staging Reference AJCC 7TH EDITION – 2009

T Classification	***Thickness***	***Ulceration Status***
Tis	N/A	N/A
T1	≤ 1.0mm	a: w/o ulceration and mitosis < 1 / mm^2 b: with ulceration and mitosis ≥ 1 / mm^2
T2	1.01-2.0mm	a: w/o ulceration b: with ulceration
T3	2.01-4.0mm	a: w/o ulceration b: with ulceration
T4	> 4.0mm	a: w/o ulceration b: with ulceration
N Classification	***# of Metastatic Nodes***	***Nodal Metastatic Mass***
N0	No evidence of lymph node metastasis	
N1	1 node	a: micrometastasis b: macrometastasis
N2	2-3 nodes	a: micrometastasis b: macrometastasis c: In transit metastases/satellites without metastatic nodes
N3	4 or more metastatic nodes, or matted nodes, or in-transit metastases/satellites and metastatic nodes	
M Classification	***Site***	***Serum LDH***
M0	No evidence of metastasis to distant tissues or organs	
M1a	Distant skin, subcutaneous or nodal metastases	Normal
M1b	Lung metastases	Normal
M1c	All other visceral metastases Or any distant metastases	Normal Elevated

18.2 ECOG/Zubrod Performance Status Scale

0 Asymptomatic and fully active.

1 Symptomatic; fully ambulatory; restricted in physical strenuous activity.

2 Symptomatic; ambulatory; capable of self-care; more than 50% of waking hours are spent out of bed.

3 Symptomatic; limited self-care; spends more than 50% of time in bed, but not bedridden.

18.3 Selected Regional Clinical Limb Toxicity (CTCAE 4.03)

Table 5: Selected Regional Clinical Limb Toxicity (CTCAE 4.03)

Grade	Adverse Event: Erythroderma
2	Erythema covering >90% BSA without associated symptoms; limiting instrumental ADL
3	Erythema covering >90% BSA with associated symptoms (e.g., pruritus or tenderness); limiting self care ADL
4	Erythema covering >90% BSA with associated fluid or electrolyte abnormalities; ICU care or burn unit indicated
5	Death
Grade	**Adverse Event: Skin Ulceration**
1	Combined area of ulcers < 1 cm; nonblanchable erythema of intact skin with associated warmth or edema
2	Combined area of ulcers 1 - 2 cm; partial thickness skin loss involving skin or subcutaneous fat
3	Combined area of ulcers >2 cm; full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to fascia
4	Any size ulcer with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures with or without full thickness skin loss
5	Death

Grade	Adverse Event: Peripheral Motor Neuropathy
1	Asymptomatic; clinical or diagnostic observations only; intervention not indicated
2	Moderate symptoms; limiting instrumental ADL
3	Severe symptoms; limiting self care ADL; assistive device indicated
4	Life-threatening consequences; urgent intervention indicated such as fsciotomy
5	Death
Grade	**Adverse Event: Peripheral Sensory Neuropathy**
1	Asymptomatic; loss of deep tendon reflexes or paresthesia
2	Moderate symptoms; limiting instrumental ADL
3	Severe symptoms; limiting self care ADL
4	Life-threatening consequences; urgent intervention indicated such as fasciotomy
5	Death
Grade	**Adverse Event: Myositis**
1	Mild pain
2	Moderate pain associated with weakness; pain limiting instrumental ADL
3	Pain associated with severe weakness; limiting self care ADL
Grade	**Adverse Event: Pain in Extremity**
1	Mild pain
2	Moderate pain; limiting instrumental ADL
3	Severe pain; limiting self care ADL