Pilot Study

Examining Oncolytic Poliovirus Bioactivity in Tumor Tissue after Intratumoral Administration of PVSRIPO in Women with Invasive Breast Cancer

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		page. Clarification that any subjects who do not have evaluable tumor tissue from both the pre-treatment biopsy and post-treatment surgery will be replaced. (Sections 5.3, 5.4, 5.6, 15) This change is meant to allow replacement of subjects who have an active viral infection while on study, which would impact treatment related analysis and the primary objective of the study.

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AE; adverse event
ANC; absolute neutrophil count
APC; antigen present cells
BCRP; Breast Cancer Research Program
CBC; complete blood count
CBER; Center for Biologics Evaluation and Research
CEA; carcinoembryonic antigen
CMP; complete metabolic panel
CNS; central nervous system
CPC; Cancer Protocol Committee
CRF; case report form
CTC; Common Toxicity Criteria
DAMP; damage-associated molecular pattern
DC; dendritic cell
DCI; Duke Cancer Institute
DoD; Department of Defense
DUHS; Duke University Health System
ECG; electrocardiogram
eCTD; electronic common technical document
eIF; eukaryotic initiation factorELISA; enzyme-linked immunosorbent assay
ER; estrogen receptor
E:T; effector to target ratio
FDA; Food and Drug Administration
FFPE; Formalin-fixed, Paraffin-embedded
H&E; hematoxylin and eosin
HER2; human epidermal growth factor receptor
HIV; human immunodeficiency virus
HRPO; Human Research Protections Office
HRV2; human rhinovirus type 2
HSA; human serum albumin
ICS; Investigational Chemotherapy Services
IHC; immunohistochemistry
IND; investigational new drug
IRB; Institutional Review Board
IRES; internal ribosomal entry site
IT; information technology
IV; intravenous
MMG; mammogram
MRI; magnetic resonance imaging
MOI; multiplicity of Infection
NCI; National Cancer Institute
NHP; non-human primate
OARC; Office of Audit, Risk, and Compliance
OncPV; Oncolytic Poliovirus

OR; operating roomPAMP; pathogen-associated molecular pattern
PD-L1; programmed death ligand 1
PET; positron emission tomography
PFS; progression free survival
PO; by mouth (latin term *per os*)
PR; progesterone receptor
PRTBTC; Preston Robert Tisch Brain Tumor Center
PT; prothrombin time
PTT; partial thromboplastin time
PV1S; poliovirus serotype 1 (SABIN)
PVSRIPO; Polio/Rhinovirus Recombinant
RIO; Research Integrity Office
RNA; ribonucleic acid
SAE; serious adverse event
SGOT; serum glutamic-oxaloacetic transaminase
SOC; standard of care
SOP; standard operating procedure
TCID; tissue culture infectious dose
TIL; tumor infiltrating lymphocytes
TNBC; triple negative breast cancer
ULN: upper limit of normal
WHO; World Health Organization

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

5.1 Purpose

To examine oncolytic poliovirus (OncPV) bioactivity in tumor tissue after intratumoral administration of PVSRIPO (Polio/Rhinovirus Recombinant) in women with invasive breast cancer.

Objectives (Exploratory):
To investigate PVSRIPO-mediated inflammation and immunity in women with stage II-IV invasive breast cancer.

1. **Primary Exploratory Objective**: To describe the change in the amount of tumor infiltrating immune cells in tumor tissue pre- and post-injection of PVSRIPO by H&E (Hemotoxylin and Eosin).
2. **Other Exploratory Objectives**:
    a. To examine tumor tissue pre- and post-injection of PVSRIPO for inflammatory and immune signatures using arrays, CD155 expression by immunohistochemistry (IHC), immune cell infiltrate by IHC and tumor infiltrating immune cells using flow cytometry (post-injection only).
    b. To examine blood for inflammatory and immune signature using arrays, 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. Blood will be collected on day -7 (range day -7 to -10, before polio vaccine booster), day 0 (before PVSRIPO injection), day 2 (range day 1 to 2 after PVSRIPO), day 14 (range day 10 to 20, after PVSRIPO before surgery) and in follow-up at months 1 (± 10 days) and 6 (± 1 month) post-PVSRIPO.

Hypothesis
Administration of oncolytic poliovirus, PVSRIPO, in the tumor causes inflammation, which stimulates innate and adaptive immune activation, in invasive breast cancer.

5.2 Background and Significance

Breast cancer is the most common cancer in women in the United States, with an estimated 246,660 new diagnoses in 2016, and is the second leading cause of cancer death in women, with an estimated 40,450 women in the United States dying from the disease in 2016 (*1*). Breast cancer is a heterogeneous disease comprising several molecular subtypes, based on receptor status, specifically the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2-neu (HER2) receptor. These receptors are both prognostic but also predictive of response to targeted therapy.

Triple Negative Breast Cancer or TNBC (negative for ER, PR, and HER2) represents 15-20% of all breast tumors and has the poorest prognosis, with an earlier age of diagnosis, higher rate of local recurrence and distant metastases, and an elevated 5-yr mortality rate compared to ER positive cancers (Breast Cancer Facts and Figures, 2015-2016, American Cancer Society). Therapies that stimulate durable systemic immunity and selectively eliminate malignant cells will be critical for extending survival and reducing risk of recurrence in women with breast cancer. In several phase I trials targeting the immune

checkpoint PD-1/PD-L1, cancer immunotherapy has been shown to demonstrate some promise in TNBC by showing objective response rates in the metastatic setting.

PVSRIPO is a recombinant polio:rhinovirus chimera, engineered to eliminate neuronal competence and is devoid of neuropathogenicity upon inoculation in primates and humans. PVSRIPO specifically targets and destroys tumor cells and initiates innate and adaptive immune events in the host. PVSRIPO has demonstrated promise in a phase I study at Duke University in patients with recurrent WHO Grade 4 malignant glioma and is currently being investigated in a Phase 2 trial in recurrent WHO Grade 4 malignant glioma. A single, intratumoral infusion of PVSRIPO has been shown to result in durable tumor regression in select patients with malignant glioma even in the presence of neutralizing antibodies and an innate antiviral response.

This is a pilot study designed to study PVSRIPO bioactivity in invasive breast cancer following intratumoral injection. The endpoints are exploratory and will guide design of a phase I/II study of PVSRIPO and combinations of PVSRIPO with immune checkpoint blockade (for example PD-1/PD-L1) in women with breast cancer. This pilot study is funded by a grant from the Department of Defense (DoD) entitled “Regional oncolytic poliovirus immunotherapy for breast cancer” (Principal Investigator, Smita Nair, Ph.D.).

Although we plan to target the TNBC population as the intended population for this novel therapeutic, for this feasibility study we will broaden the eligibility criteria to include all tumor subtypes, as there is no biologic rationale to support differing toxicities from different invasive breast cancer subtypes. After safety is confirmed in this feasibility study, we will focus our efforts on the intended TNBC population.

5.3 Design and Procedure

This pilot study is designed to assess PVSRIPO-mediated intratumoral bioactivity, inflammation, and immune events in women with invasive breast cancer. Enrollment target will include six women with invasive breast cancer. Women with stage II-IV invasive breast cancer with at least 1 cm of residual tumor after chemotherapy and scheduled for standard of care surgery will be eligible.

Polio vaccine booster will be administered 1 week, or up to 10 days, prior to PVSRIPO injection. On day 0, after pretreatment biopsy is obtained, $1x10^8$ $TCID_{50}$ (tissue culture infectious dose) of PVSRIPO will be injected into the tumor mass. On day 14 (range 10-20 days), women will undergo standard-of-care (SOC) surgical resection of PVSRIPO-treated tumor. Women will have follow-up at 1-2 days after PVSRIPO injection, 1 month (± 10 days) and 6 months (± 1 month) after PVSRIPO for adverse events, disease status, and whole blood draw for research purposes.

Women will undergo a pre-treatment core needle biopsy with collection of research cores of tumor tissue prior to intratumoral injection of PVSRIPO. Ten to twenty days after PVSRIPO injection, tumor tissue will be collected at the time of SOC surgical tumor resection. The surgically resected tissue will be collected and processed as fresh and formalin-fixed paraffin-embedded (FFPE) tissue. The tissue will be used to evaluate tumor

infiltrating lymphocytes (TILs), CD155 expression, and inflammatory and immune signature using arrays. Any subjects who do not have evaluable tumor tissue from both the pre-treatment biopsy and post-treatment surgery will be replaced.

Additionally, inflammatory and immune analysis will be conducted using blood obtained on day -7 (range day -7 to -10, before polio vaccine booster), day 0 (before PVSRIPO injection), day 2 to 5 (range day 1-2 after PVSRIPO), day 14 (range day 10 to 20 after PVSRIPO, which is before surgery) and in follow-up at months 1 (± 10 days) and 6 (± 1 month) after PVSRIPO. Blood will be analyzed for inflammatory and immune signature using arrays, 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. This pilot study will enable a phase I/II study of PVSRIPO and combinations of PVSRIPO with immune checkpoint blockade (for example PD-1/PD-L1) in women with advanced breast cancer.

5.4 Selection of Subjects

Eligibility Criteria: This pilot study will include women with stage II-IV invasive breast cancer scheduled to undergo surgical resection. Target enrollment is six women with invasive breast cancer over 24 months. Patients will be recruited among patients receiving their treatment for breast cancer at Duke Cancer Institute (DCI). Any subjects who do not have evaluable tumor tissue from both the pre-treatment biopsy and post-treatment surgery will be replaced.

Inclusion Criteria

- Age ≥ 18 years
- Confirmation of invasive breast cancer including any of the following:
    - Triple-negative breast cancer defined as receptor status being estrogen receptor expression ≤ 10%, progesterone receptor expression ≤ 10%, and HER2/Neu expression by IHC 0 or 1+, or 2+ with fluorescence *in situ* hybridization confirming no amplification of HER2 on a pretreatment tumor sample.
    - Hormone positive breast cancer defined as receptor status being estrogen receptor expression > 10%, progesterone receptor expression > 10% prior to initiation of chemotherapy.
    - HER2+ breast cancer defined as HER2/Neu expression by IHC 3+ or fluorescence *in situ* hybridization confirming amplification of HER2 on a pretreatment tumor sample prior to initiation of chemotherapy. HER2+ and hormone positive (ie triple positive) breast cancers are included in this study.
- Stage II-III invasive breast cancer with ≥ 1 cm of residual tumor based on MRI, mammogram, ultrasound, or breast clinical exam as SOC after completion of neoadjuvant chemotherapy, OR Stage IV BC with ≥ 1 cm locally recurrent disease (i.e. chest wall recurrence only)
- ECOG ≤ 1
- Positive serum anti-PV titer prior to biopsy
- Received a boost immunization with trivalent inactivated IPOL™ (Sanofi-Pasteur) at least 1 week prior to administration of the study agent
- Hemoglobin ≥ 9.0 g/dl, ANC ≥ 1,000 cells/µl, platelets ≥ 100,000 cells/µl

- Women must have had last dose of chemotherapy at least 2 weeks prior to treatment with PVSRIPO
- Women must have at least 2 weeks minimum (ideal 3-4 weeks) of a wash-out period after any steroid administration (IV, PO, or intraocular)
- Serum creatinine ≤ 2.0 mg/dl, serum SGOT and bilirubin ≤ 2 times ULN (upper limit of normal)
- Women must provide written informed consent prior to enrollment on study, prior to conduct of screening procedures and enrollment on study
- Women of childbearing potential will have a negative serum pregnancy test at screening
- Women of childbearing potential must be willing to avoid pregnancy utilizing approved forms of contraception for the course of the study through 120 days after PVSRIPO injection
- Surgical resection of the tumor is planned and patient is willing to undergo surgical resection of the cancer

Exclusion Criteria

- T1 N0 invasive breast cancer
- Breast cancer with skin necrosis
- Concurrent immune therapy, chemotherapy, or steroid therapy
- Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks prior to polio vaccine booster
- Has a known diagnosis of immunodeficiency
- Has a known additional malignancy that is progressing or requires active treatment
- Has known active central nervous system metastases and/or carcinomatous meningitis
- Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease or a syndrome that requires systemic steroids or immunosuppressive agents
- Has an active infection requiring systemic therapy
- Has known psychiatric or substance abuse disorders that would interfere with the requirements of the trial
- Is pregnant, breastfeeding, or expecting to conceive children within the projected duration of the trial, starting with the screening visit through 120 days after trial treatment
- Has received prior therapy with an anti-PD-1, anti-PDL-1, anti-PDL-2, anti-CD137, or anti-CTLA-4 (or any other antibody or drug specifically targeting T cell co-stimulation or checkpoint pathways)
- Has a known history of Human Immunodeficiency Virus (HIV)
- Has known active Hepatitis B or Hepatitis C
- Active liver disease with elevated transaminases > 2x ULN
- Has received a live vaccine within 30 days prior to PVSRIPO treatment
    - Inactivated vaccines are acceptable and are not an exclusion criterion

5.5 Duration of Study

This pilot study will accrue 6 women with invasive breast cancer. Follow-up will include immunological assessments at 1-2 days post-PVSRIPO injection, 1 month and 6 months post-PVSRIPO for blood draws.

5.6 Data Analysis and Statistical Considerations

Six women with invasive breast cancer will participate in this clinical pilot study, in which we will explore PVSRIPO bioactivity. Any subjects who do not have evaluable tumor tissue from both the pre-treatment biopsy and post-treatment surgery will be replaced. Descriptive statistics will be used to summarize inflammatory and immune signatures in tumor and blood, tumor infiltrating immune cell analysis, changes in immune cell composition in blood, and changes in T cell activation in blood. As such, the correlative studies and the pilot clinical study design are part of Aim 1 of the funded DoD grant application entitled “Regional oncolytic poliovirus immunotherapy for breast cancer” (Principal Investigator, Smita Nair, Ph.D.).

6 STUDY SCHEMA

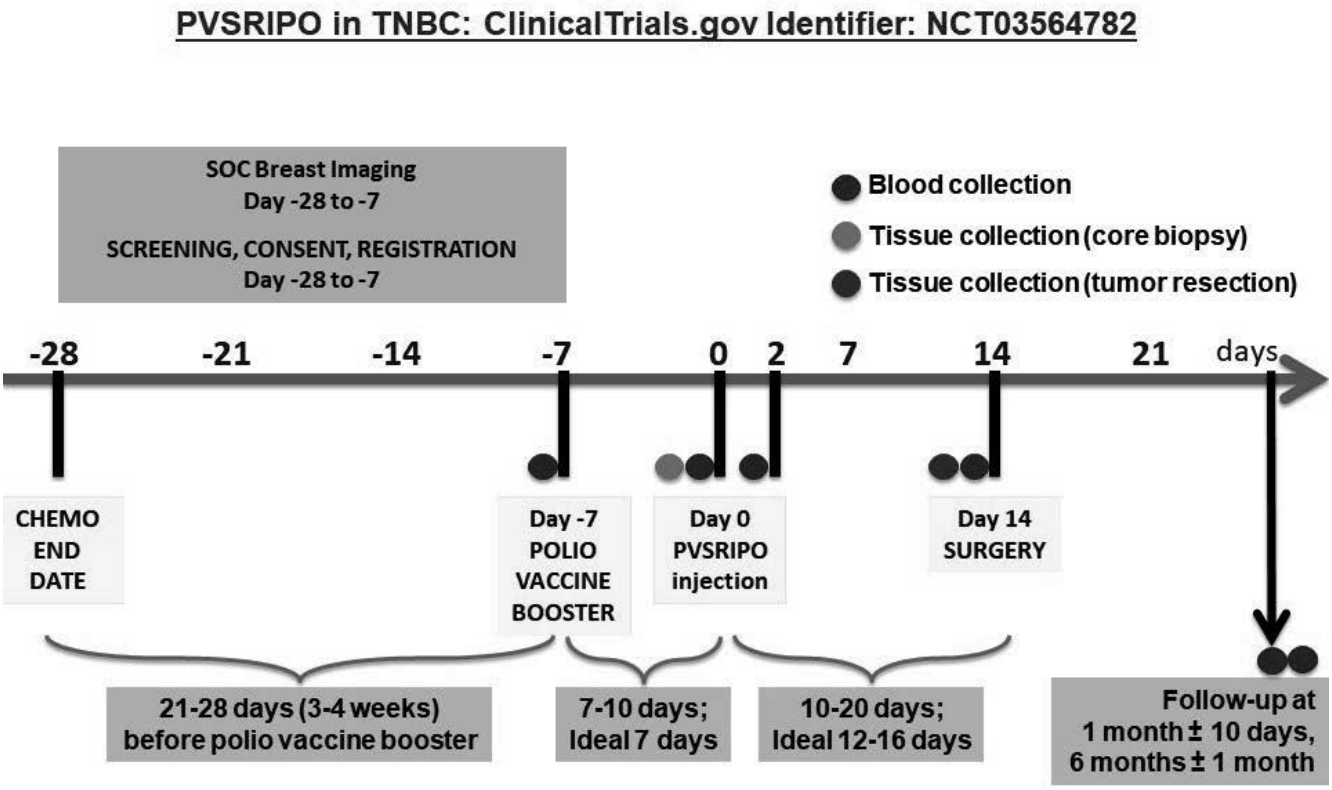

Figure 1. Study Schema PVSRIPO for Invasive Breast Cancer.

7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

Breast cancer is the most common cancer in women, with an estimated 246,660 new diagnoses in 2016, and is the second leading cause of cancer death in women, with an estimated 40,450 women in the United States dying from the disease in 2016 (*1*). Breast cancer is a heterogeneous disease comprising several molecular subtypes, based on receptor status, specifically the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2-neu (HER2) receptor. These receptors are both prognostic but also predictive of response to targeted therapy. Although often curable when localized to the breast and lymph nodes, on the whole, breast cancer remains incurable for most women in the metastatic setting (*2*). An estimated 20% to 30% of women diagnosed with invasive breast cancer will have a recurrence and may eventually die of their disease (Department of Defense (DoD) Breast Cancer Research Program (BCRP), "The Breast Cancer Landscape").

Triple Negative Breast Cancer (TNBC) refers to tumors lacking the expression of estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) and portends a poor prognosis compared with tumors expressing these receptors. These tumors account for about 15% of all breast cancers and have been shown to be more aggressive than other tumor subtypes. These women are diagnosed at an earlier age and have a higher risk of local and distant recurrence (*3*). The higher risk of distant metastases results in an elevated 5-year mortality rate compared to ER positive cancers (*4*). In addition, the paucity of targeted treatment available for TNBC likely contributes to the lower survival rate. Despite the risk of increased local recurrence and distant metastases, standard criteria are used to select the approach to local therapy as more extensive surgeries have not been shown to improve survival (*3*). At the time of distant recurrence, there are no standard of care approaches for systemic chemotherapy. Common chemotherapies utilized in the neoadjuvant, adjuvant, and metastatic setting for invasive breast cancers include anthracyclines, cyclophosphamide, taxanes, capecitabine, eribulin, vinorelbine, and platinums. In addition to these chemotherapies, common anti-HER2 treatments used in HER2+ breast cancer include trastuzumab, pertuzumab, T-DM1, and lapatinib.

Immunotherapy has been demonstrated to mediate regression of large bulky tumors in the stage IV setting in multiple clinical settings, resulting in improved overall and disease free survival in melanoma and non-small cell lung cancer, as well as promising objective response rates in head and neck, gastric, and urothelial cancers (*5*-*10*). The presence of tumor infiltrating lymphocytes (TILs) correlates with a favorable prognosis in various malignancies including TNBC (*11*). Several phase I/II clinical trials targeting the immune checkpoint PD-1/PD-L1 pathways in metastatic TNBC have shown promising results with overall response rates of 18-20% (*9, 12*).

This is a pilot study designed to examine PVSRIPO bioactivity in breast cancer following intratumoral injection. The endpoints are exploratory and will guide a phase I/ II study of PVSRIPO and combinations of PVSRIPO with immune checkpoint blockade in women with breast cancer. Although we plan to target the TNBC population as the intended population

for this novel therapeutic, for this feasibility study we will broaden the eligibility criteria to include all tumor subtypes, as there is no biologic rationale to support differing toxicities from different invasive breast cancer subtypes. After safety is confirmed in this feasibility study we will focus our efforts on the intended TNBC population.

7.2 Study Agent

PVSRIPO is the live attenuated, oral (Sabin) serotype 1 poliovirus vaccine containing a heterologous internal ribosomal entry site (IRES) derived from the human rhinovirus type 2 (HRV2). Poliovirus receptor is mediated by the poliovirus receptor, CD155, an oncofetal cell adhesion molecule, which is widely expressed ectopically in malignancy, e.g. invasive breast cancer. Tumor specificity is mediated by selective expression of CD155 in invasive breast cancer. Translation is repressed at the heterologous HRV2 IRES in normal breast tissue and a supportive environment for PVSRIPO translation, growth, and cytotoxicity is mediated by universally active protein kinase C (PKC)-Ras-Erk signaling to translation machinery in cancers. PVSRIPO is administered intratumorally. Since PVSRIPO is a version of the serotype 1 live-attenuated (Sabin) poliovirus vaccine (PV1), its immunogenic properties and potential for long-term sequelae are expected to be similar. Poliovirus serotype 1 (SABIN) (PV1S) has been safely administered to >10 billion individuals worldwide without untoward long-term immunogenic sequelae. The pre-clinical data described below was included in the funded DoD grant application to provide rationale for this pilot study.

7.2.1 Pre-Clinical Experience

For details about the pre-clinical experience with PVSRIPO, please refer to the Investigator's Brochure for PVSRIPO.

PVSRIPO tumor tropism: PVSRIPO has natural cancer tropism, due to widespread ectopic expression of the poliovirus receptor (PVR) CD155 in solid cancers, where it may serve as a tumor immune evasion strategy (*13*-*17*). Despite neuronal incompetence, PVSRIPO is highly cytotoxic in neoplastic cells (*17*). PVSRIPO's oncolytic efficacy depends on ectopic CD155 expression and unhinged MAPK-signals to translation machinery. Both properties define most malignancies, including breast cancer. Efficient tumor tropism and rapid, violent cytotoxicity distinguish PVSRIPO from other oncolytic viruses. PVSRIPO uniquely has oncolytic efficacy despite pre-existing neutralizing antibodies and viral cell killing proceeds in the presence of an active innate antiviral response (*18, 19*).

Figure 2A depicts CD155 expression on breast cancer cell lines and primary breast tumor tissue (*20*) (de-identified primary tissue was used and the sub-type of primary tumor tissue was not determined). Note that CD155 is not expressed on normal breast epithelial cultures (S2N). PVSRIPO exhibits anti-tumor activity in breast cancer models of brain metastases and leptomeningeal disease, which are notoriously refractory to therapy (*20*) (**Figure 2B**). **Figure 3** depicts CD155 expression on primary triple negative breast tumor tissue.

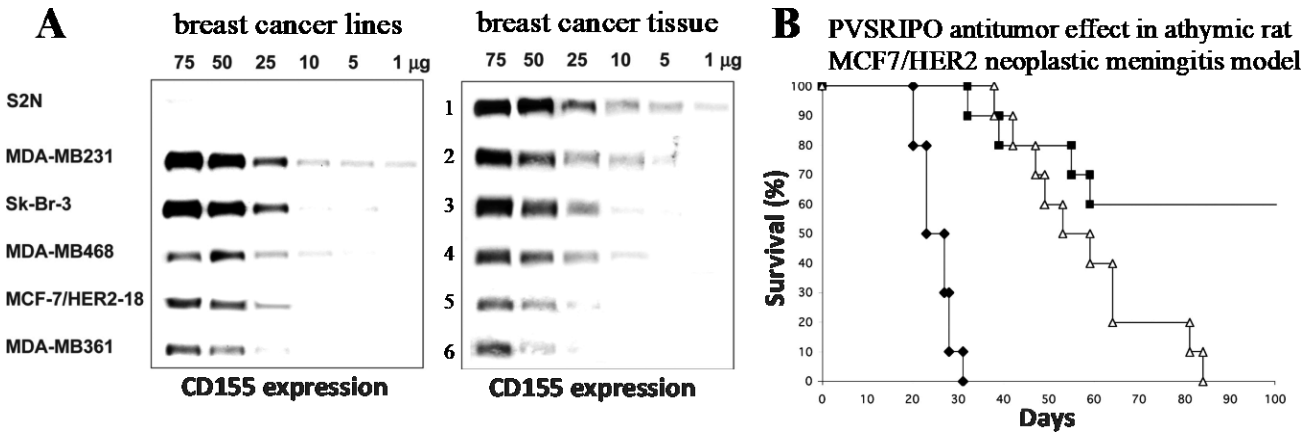

Figure 2. Breast Cancer Susceptibility to PVSRIPO. A. CD155 expression in breast cancer cell lines and primary breast cancer tissues by western blot. B. PVSRIPO efficacy *in vivo*. Rats (n=10) with intrathecal xenografts were treated once with PVSRIPO at $1x10^7$ pfu (open triangles) or $1x10^9$ pfu (closed squares), 3 days after tumor implant. UV-inactivated PVSRIPO was used as control (closed diamonds).

	CD155		
#	+++	++	+/-
1		100%	
2		50%	40%
3		50%	40%
4	95%	5%	
5		80%	20%
6		80%	20%
7	95%	5%	
8	80%	10%	10%
9	80%	10%	10%
10	20%	80%	
11	90%	10%	
12	80%		10%
13		95%	
14		30%	60%
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16	10%	80%	10%
17	80%	10%	10%
18	30%	70%	
19		95%	

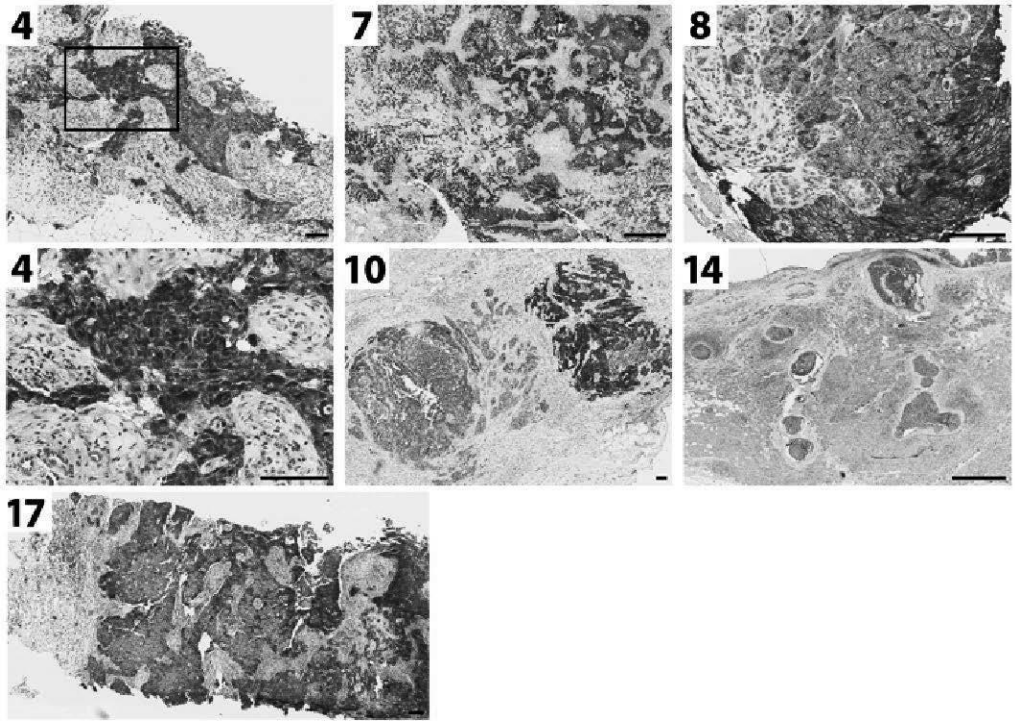

Figure 3. Immunohistochemistry for the PV Receptor (CD155) in 19 Triple-Negative Breast Cancer Cases. For methods and detailed information on the assay, expression scoring, etc. see Chandramohan *et al*. (*21*). The table summarizes expression scores in the 19 cases. The numbered panels show IHC results for the corresponding cases in the table. Size bars represent 1 mm (7, 14) or 100 mm (4, 8, 10, 17).

Molecular mechanisms of PVSRIPO tumor specificity: PVSRIPO targets constitutively active Raf-Erk1/2 signal transduction to translation machinery via Erk1/2 to downstream

MAPK-interacting kinases and to eIF4G to generate conditions that favor 5' methyl-7- guanidine cap-independent, IRES-mediated translation (*22*-*24*). The Raf-Erk1/2 pathway is a central gene regulatory node in malignancy that is recalcitrant to small-molecule inhibitors, due to drug resistance mechanisms that restore Erk1/2 activity, for example to trastuzumab in breast cancer (*25*). PVSRIPO oncolysis *depends* on active Raf-Erk1/2 signals thus exploiting an essential mitogenic mechanism in cancer. PVSRIPO causes potent cytotoxicity, with irreversible, lethal effects on infected host cells as early as 90 min post PVSRIPO infection (*26*). The TNBC cell line MDA-MB231 and the triple-negative inflammatory breast cancer (IBC) cell line SUM149, are susceptible to PVSRIPO-mediated lysis and express CD155 (**Figure 4**). Lethal PVSRIPO-mediated cytotoxicity results in the loss of intact eIF4G and the generation of the eIF4G cleavage product (**Figure 4A**) (*27*).

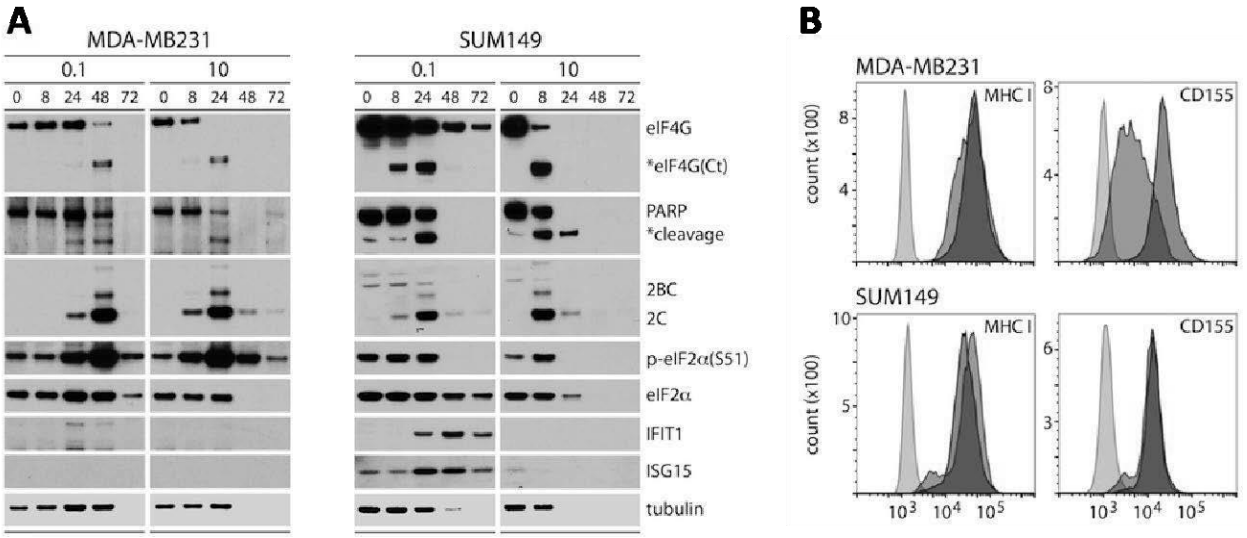

Figure 4. PVSRIPO Cytotoxicity in Breast Cancer Cell Lines. Breast cancer cell lines, MDA-MB231 and SUM149, were infected at multiplicity of Infection (MOIs) of 0.1 or 10 with PVSRIPO. **A.** Lysates were collected at the denoted intervals and tested by immunoblot for markers of viral cytotoxicity (eIF4G cleavage), host cell demise (PARP cleavage), viral replication (viral proteins P2/2BC/2C), and antiviral responses (p-eIF2α(S51)/IFIT1/ISG15). Loss of cellular proteins (e.g. tubulin) and viral protein at later time points correlates with lysis of infected cells. **B.** Cells were infected at an MOI of 0.1 and harvested at 48 h post infection (hpi) for analysis of MHC class I and CD155 expression by flow cytometry (light grey, uninfected cells stained with isotype control; dark grey, uninfected cells stained for MHC or CD155; and red, infected cells stained for MHC or CD155).

PVSRIPO activates dendritic cells: PVSRIPO oncolysis releases tumor antigens and generates DAMPs and PAMPs (damage-associated and pathogen-associated molecular patterns) that stimulates immune cells (*28*). Poliovirus RNA is a classic PAMP that engages cytoplasmic pattern recognition receptors and downstream responses (Mda5-Stat1-IFNα/β) in immune cells. Poliovirus naturally targets antigen presenting cells (APCs), such as macrophages, monocytes, and dendritic cells (DCs), via CD155. To delineate the effects of PVSRIPO on DCs, we examined markers of cytotoxicity and activation status on antigen presentation. DCs infected with PVSRIPO at MOI of 1, 10, and 100 did not exhibit lytic

cytoxicity as compared to lytic cytotoxicity in cancer cells infected with PVSRIPO (**Figure 5A**). Viral propagation was detected, however, in sharp contrast to cancer cells, did not yield progeny beyond background levels (**Figure 5A**) (*29*). An increase in Stat1 phosphorylation (which leads to increased Stat1 total protein) followed by the upregulation of interferon (IFN)-stimulated genes ISG15, IFIT1, and MDA5 protein, signify the activation of type-I IFN responses (**Figure 5B**) (*27*). Inefficient cleavage of eIF4G and PARP and decreased 2C expression following infection, suggests severe limitations on viral replication and cytotoxicity. This is in sharp contrast to what we observe in tumor cells treated with PVSRIPO (**Figure 4**). In animal tumor models, there is histologic evidence for virus-mediated tumor cell killing and indirect host-mediated inflammatory responses focused against tumor (*24, 30-33*).

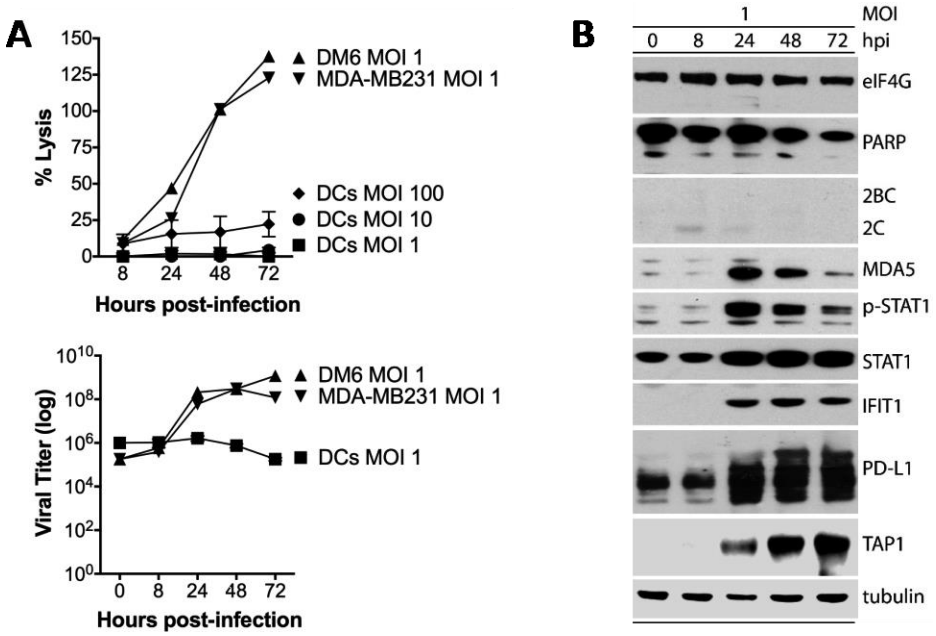

Figure 5. Infection and Activation of Dendritic Cells (DCs) by PVSRIPO. Human monocyte-derived DCs were infected with PVSRIPO at MOI of 1, 10, and 100. **A.** LDH-release assay (top) and viral progeny (bottom) analysis was conducted using infected DCs and compared to infected DM6/MDA-MB231 cells. **B.** Western blot analysis was conducted at the designated time points post infection (PI). PVSRIPO viral protein (2C) detection signifies productive infection. An increase in Stat1 phosphorylation (which leads to increased Stat1 total protein) followed by the upregulation of IFN-stimulated genes ISG15, IFIT1, and MDA5 protein, signify activation of IFN responses. Inefficient cleavage of eIF4G and PARP and decreased 2C expression following infection, suggests severe limitations on viral replication and cytotoxicity.

To examine if presence of PAMPs and DAMPs in PVSRIPO oncolysate activates DCs, tumor lysates were collected after exposing tumor cells to mock- or PVSRIPO-infection (MOI = 0.1; 48h) and treating human monocyte-derived DCs with the resulting oncolysate (**Figure 6**) (*27*). To control for the effects of infectious PVSRIPO particles present in the oncolysate on DCs (~5x10^8 pfu/mL), virus was removed using a 100kD filter. This approach removes all infectious virus but does not remove IFN/dsRNA/HMGB1 or other conceivable PAMPs/DAMPs. Exposure to oncolysate increased DC co-stimulatory molecule expression

and other markers of DC activation (**Figure 6B**). This effect was mitigated by 100kD filtration, suggesting that PVSRIPO infection may actively participate in DC activation (*27*).

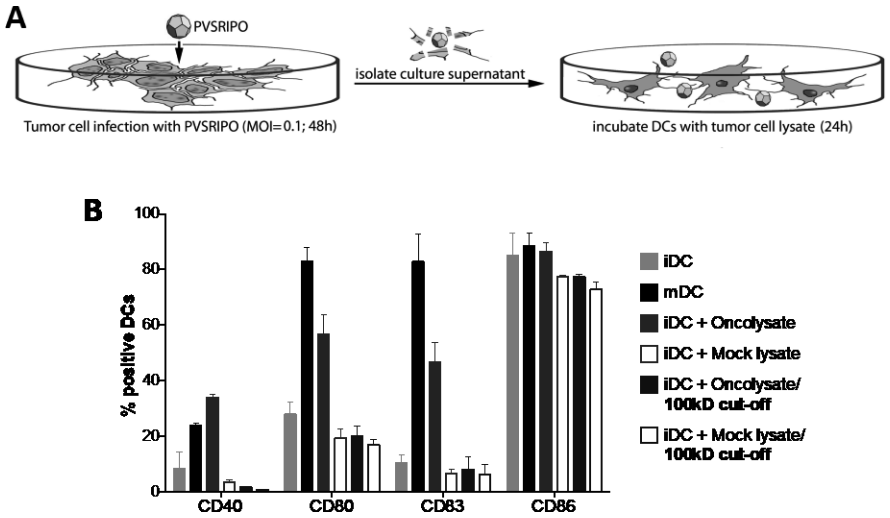

Figure 6. PVSRIPO-Induced Oncolysate Causes DC Activation in a Virus Dependent Fashion. A. Outline of *in vitro* human DC activation experiment. **B.** PVSRIPO-induced tumor lysate (PVSRIPO oncolysate) activates human DCs. DCs were generated (*34*) from HLA-A2+ donor cells. Supernatant from untreated or PVSRIPO-infected (MOI = 0.1; 48hpi) tumor cells were either unfiltered or filtered through a 100 kD filter. DCs were treated with the resulting supernatants for 24 hours. Standard immature DCs (**iDCs**) or DCs matured in cytokine cocktail (**mDCs**) were used as controls. Flow cytometry was used to assess DC activation/maturation phenotype and viability. This experiment was repeated twice and representative data is shown.

7.2.2 Clinical Experience

Clinical efficacy of OncPV therapy against recurrent WHO Grade 4 malignant glioma
PVSRIPO has been tested in a phase I study in subjects with recurrent WHO Grade 4 malignant glioma 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. Fifteen patients were enrolled into the PVSRIPO dose-escalation portion of this phase I study. Dose was rapidly escalated from $1x10^8$ $TCID_{50}$ (dose level 1) to $1x10^{10}$ $TCID_{50}$ (dose level 5) after which dose was reduced 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 malignant glioma (ClinicalTrials.gov Identifier: NCT01491893; Duke IRB Pro00031169), we will use $1x10^8$ $TCID_{50}$ (dose level 1) in women with invasive breast cancer.

We have chosen to use $1x10^8$ $TCID_{50}$ (dose level 1 from the phase 1 dose-escalation study in patients with recurrent WHO Grade 4 malignant glioma) for the following reasons.

1. In the dose escalation study conducted in patients with malignant glioma, 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 I brain tumor study and use of dose level -1 (5×10^7 $TCID_{50}$) in the Phase 2 glioma trial. There were no signs of encephalitis, systemic toxicity or systemic inflammatory processes. Given that the breast is not in a physiologically confined space (like brain in the cranium) the effects of inflammation due to PVSRIPO are not of significant clinical impact. Additionally, the breast tumor will be resected 10-20 days following PVSRIPO infusion therefore limiting the time in which the patient may experience discomfort from tumor site hyper-inflammation.
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 phase 1 recurrent WHO Grade 4 malignant glioma study, to ensure that we elicit a response in tumor (both tumor killing and immune consequences) post-PVSRIPO in this pilot study.

7.3 Study Purpose and Rationale

PVSRIPO, a recombinant oncolytic poliovirus, has shown promising results in recurrent malignant glioma when administered intracerebrally. Once administered, PVSRIPO enters tumor cells through the poliovirus receptor CD155, which is overexpressed in solid malignancies. As such, it both specifically targets and destroys tumor cells that it enters and initiates innate and adaptive immune events in the host.

A phase I clinical trial of PVSRIPO in recurrent malignant glioma patients has yielded durable complete radiographic and clinical responses with a proportion of patients achieving prolonged disease survival up to 61+ months. The first subject treated with PVSRIPO is alive and well 5 years after PVSRIPO administration. PVSRIPO was granted *Breakthrough Therapy Designation* by FDA/CBER on May 16th, 2016.

As CD155 is also overexpressed in all subtypes of invasive breast cancer, a pilot study to examine the effects of intratumoral injection of PVSRIPO into invasive breast cancer tumors in 6 women is of interest. Importantly, if effective, this will lead to a phase I/II trial of PVSRIPO and combinations of PVSRIPO with immune checkpoint blockade (for example PD-1/PD-L1) in women with triple negative breast cancer. Of note, PD-1/PD-L1 blockade has already demonstrated some clinical efficacy in women with TNBC.

Rationale: The rationale for doing this pilot clinical study in six women with invasive breast cancer is as follows:

1. To characterize intratumoral PVSRIPO injection in a non-CNS cancer
2. To investigate inflammation and immunity in tumor tissue post-PVSRIPO treatment. PVSRIPO treatment is showing promise in malignant glioma patients but the tumor tissue is not accessible to examine PVSRIPO bioactivity. This pilot study in women with accessible invasive breast cancer would enable investigation of PVSRIPO bioactivity in tumor tissue.
3. To demonstrate that post-transcriptional gene regulatory principles that mediate PVSRIPO tumor cytotoxicity apply to breast cancer.

8 OBJECTIVES AND ENDPOINTS

To examine oncolytic poliovirus (OncPV) bioactivity in tumor tissue after intratumoral administration of PVSRIPO in women with invasive breast cancer.

Objectives (Exploratory):
To investigate PVSRIPO-mediated inflammation and immunity in women with stage II-IV invasive breast cancer.

1. **Primary Exploratory Objective**: To describe the change in the amount of tumor infiltrating immune cells in tumor tissue pre- and post-injection of PVSRIPO by H&E (Haemotoxylin and Eosin).
2. **Other Exploratory Objectives**:
    a. To examine tumor tissue pre- and post-injection of PVSRIPO for inflammatory and immune signature using arrays, CD155 expression by immunohistochemistry (IHC), immune cell infiltrate by IHC and tumor infiltrating immune cells using flow cytometry (post-injection only).
    b. To examine blood for inflammatory and immune signature using arrays, 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. Blood will be collected on day -7 (range day -7 to -10, before polio vaccine booster), day 0 (before PVSRIPO injection), day 2 (range day 1-2 after PVSRIPO), day 14 (range day 10 to 20, after PVSRIPO before surgery) and in follow-up at months 1 (± 10 days, after PVSRIPO) and 6 (± 1 month, after PVSRIPO).

Hypothesis
Administration of oncolytic poliovirus, PVSRIPO, in the tumor causes inflammation, which stimulates innate and adaptive immune activation, in invasive breast cancer.

9 INVESTIGATIONAL PLAN

9.1 Study Design

This is a single institution pilot study designed to assess PVSRIPO-mediated intratumoral bioactivity, inflammation and immune cell infiltration in 6 patients with invasive breast cancer. To maximize immunological stimulation, we will wait at least 2 weeks (ideal 3-4 weeks) after the last dose of chemotherapy or steroids to administer the polio vaccine booster. PVSRIPO will be injected into the tumor mass at $1x10^8$ $TCID_{50}$ 7-10 days after polio vaccine booster is administered.

Biopsy material will be obtained from tumor tissue immediately prior to virus administration and resected tumor tissue will be obtained at 10-20 days (ideally 12-16) after PVSRIPO at the time of surgical resection. The PVSRIPO-treated tumor tissue will be transported to Surgical Pathology by the Brain Tumor BioRepository (BTBR) Team, as directed by Dr. Roger McLendon, MD or per clinical protocol, for gross examination and research tissue procurement, thereby safe-guarding the diagnostic integrity of the specimen. Additional expertise in breast pathology will be provided by Dr. Edgardo Parilla Castella, MD, PhD.

Tissue deemed appropriate for collection (i.e. wasteful excess and of no diagnostic value) will be collected by the Brain Tumor BioRepository (BTBR) Team, as directed by Dr. Smita Nair, PhD, and transported to her lab for correlative molecular and immune analyses. Our primary objective is to determine change in the amount of tumor infiltrating immune cells in tumor tissue pre- and post-injection of PVSRIPO by H&E. We will also evaluate inflammatory and immune signatures using inflammatory and innate/adaptive arrays, CD155 expression and immune cell infiltrate by IHC and tumor infiltrating immune cells by flow cytometry (post-injection only).

Additionally, inflammatory and immune analysis will be conducted using blood obtained on day -7 (range day -7 to -10, before polio vaccine booster), day 0 (before PVSRIPO injection), day 1-2 (after PVSRIPO), day 14 (range day 10 to 20 after PVSRIPO, which is before surgery), and in follow-up at months 1 (± 10 days) and 6 (± 1 month) after PVSRIPO. Blood will be analyzed for inflammatory and immune signature using arrays, 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. Immune cell composition and T cell activation in blood will be done using DuraClone IM panels from Beckman Coulter.

9.2 Safety Considerations

9.2.1 Surgical Complications

Patients undergoing biopsy and excision of breast tumors may develop infection at site of injection or breast tissue removal, cellulitis, or abscess formation. Seromas or hematomas may also be a complication in this setting. The risk of perioperative infection will be the same as encountered routinely for breast surgery. Patients will be monitored throughout the course of the study for any signs and symptoms of infection. If an active infection is suspected, patients will be cultured and treated with appropriate antibiotics and/or undergo incision and drainage procedure.

9.2.2 Anesthesia

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

9.2.3 Poliomyelitis

PVSRIPO has been tested in non-human primates (NHPs) according to the WHO standardized monkey neurovirulence tests. These tests revealed the absence of neuropathogenic properties, evident as failure to induce symptoms of poliomyelitis in NHPs after intracerebral inoculation of virus. In addition, the use of intracerebral PVSRIPO in human subjects with recurrent malignant glioma in phase I trials have not induced symptoms of poliomyelitis. However, PVSRIPO are replication-competent viral agents that, in principle, retain the potential to cause motor neuron damage. Possible complications include transient or permanent mono- or paraparesis, paralysis, and life-threatening respiratory insufficiency.

9.2.4 Virus Recombination and Mutation

PVSRIPO retains the ability to alter its genome during replication upon administration to

patients. Various mechanisms are known to lead to genetic adaptation, including spontaneous mutagenesis and recombination that may give rise to viral progeny with changed properties. Empirical studies in tissue culture and laboratory animals demonstrated that prolonged passaging in malignant glioma cells does not select for genetic changes in viral progeny. However, the occurrence of such events cannot be categorically excluded in patients receiving PVSRIPO. So far, the use of intracerebral PVSRIPO in human subjects with malignant glioma in phase I trials have not demonstrated these phenomena.

9.2.5 Long-Term Sequelae of Virus Injection

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

Prolonged gastrointestinal propagation of polioviruses, associated with prolonged virus shedding with stool, has been reported in select individuals with acquired or rare inherited immunodeficiency disorders. Primate toxicology results indicate that extraneural dissemination and shedding of PVSRIPO with stool do not occur after intracerebral inoculation. In addition, the use of PVSRIPO in human subjects with recurrent malignant glioma in phase I have not demonstrated this phenomena.

9.2.6 Risks of Phlebotomy

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

9.2.7 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. In addition, this also has not been observed with the use of PVSRIPO in human subjects with recurrent malignant glioma in phase I trials. Therefore, and because poliovirus transmission occurs via the fecal-oral route, the likelihood of unintended exposure of patient household contacts is exceedingly low. If accidental exposure occurred, it would equal the risk of exposure to any type 1 Sabin vaccine virus or vaccine virus derivatives. Thus, in essence, exposure with PVSRIPO is equal to oral immunization with a safe version of type 1 Sabin. Since type 1 Sabin vaccine virus or vaccine virus derivatives have to be considered part of the human environment, exposure to PVSRIPO would not represent an added risk beyond the possibility for exposure that already exists. Study subjects will be advised of the risks of exposure to unvaccinated household contacts.

9.2.8 Unacceptable Toxicities

Clinical trials using PVSRIPO in breast tumor patients have not been performed. Since the overall risk classification of this research is unknown we plan to take the following approaches to ensure patient safety:

I. We will stagger enrollment of each patient by at least 4 weeks, which will be sufficient time to implement dose reduction or other safety measures based on adverse events observed.

II. The study will only treat 6 patients total. However, the trial will be paused for enrollment and undergo reevaluation if two patients have any Grade 3 or any Grade 4 toxicity that is not reversible within 2 weeks after surgery (~24-34 days after PVSRIPO, depending on when the surgery is performed), or any life-threatening event, or treatment-related death will be considered an unacceptable toxicity. Any grade 2 or higher serious autoimmune toxicities particularly those affecting vital organs (e.g. cardiac, renal, CNS) will be considered an unacceptable toxicity. Also, the study will be paused to enrollment if a patient death occurs within a month after PVSRIPO injection possibly or likely related to PVSRIPO.

9.3 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 I study in adults with recurrent WHO grade IV malignant glioma. For more information on the results from the dose-finding study, please refer to the Investigator's Brochure for PVSRIPO.

<u>We have chosen to use $1x10^8$ $TCID_{50}$ (dose level 1 from phase 1 dose-finding malignant glioma trial) for the following reasons.</u>

1. In the dose escalation study conducted in patients with recurrent malignant glioma, 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 I brain tumor study. Because of this, dose level -1 (5×10^7 $TCID_{50}$) is being investigated in the phase 2 recurrent malignant glioma study in adults and phase 1b study in pediatric patients. There were no signs of encephalitis, systemic toxicity or systemic inflammatory processes. Given that the breast is not in a physiologically confined space (like brain in the cranium) the effects of inflammation due to PVSRIPO are not of significant clinical impact. Additionally, the breast tumor will be resected 10-20 days following PVSRIPO infusion therefore limiting the time in which the patient may experience discomfort from tumor site hyper-inflammation.
3. A higher dose is warranted in the breast cancer 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 phase 1 malignant glioma study, to ensure that we elicit a response in tumor (both tumor killing and immune consequences) post-PVSRIPO in this pilot study.

4. If a study pause occurs due to adverse events observed according to Section 9.2.8 and these adverse events are concluded to be possibly or likely related to PVSRIPO injection and it is deemed safe to continue the study, then we will continue to enroll patients at a lower dose of $5x10^7$ $TCID_{50}$, the dose currently being investigated in the phase 2 recurrent malignant glioma study in adults and a phase 1b study in recurrent malignant glioma in pediatric patients.

9.4 Rationale for Correlative Studies

As this is a pilot study with exploratory endpoints only, correlative studies will be used to assess PVSRIPO tumor bioactivity. Specifically, the study will investigate PVSRIPO-mediated inflammation and immunity in women with stage II-IV invasive breast cancer. Greater detail is provided in Section 8.

9.5 Early Study Termination

This study can be terminated at any time for any reason by the PI or Sponsor. If this occurs, all subjects on study will be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 12.6, which describes criteria for Early Withdrawal of Subject(s).

10 STUDY DRUG

10.1 Names, Classification, and Mechanism of Action

PVSRIPO is the live attenuated poliovirus (PV) serotype 1 (SABIN) vaccine with its internal ribosomal entry site (IRES) element replaced with that of human rhinovirus type 2. IRES exchange causes an inability of PVSRIPO to drive translation of its genome in normal cells (*35, 36*). Thus, PVSRIPO is devoid of neuropathogenicity after high-dose intracerebral inoculation in non-human primates (*13*) and in humans. Because PVSRIPO is a version of the serotype 1 live-attenuated (Sabin) poliovirus vaccine (PV1), its immunogenic properties and potential for long-term sequelae are expected to be similar. PV1S has been safely administered to >10 billion individuals worldwide without untoward long-term immunogenic sequelae. The main immunogenic effect of administration of PV1S to human subjects is neutralizing immunity to poliovirus.

10.2 Supply, Receipt, and Storage

PVSRIPO is formulated in 50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% human serum albumin. It is provided in sterile, single use containers. PVSRIPO must be stored at less than or equal to -70°C. Once thawed, it is a clear colorless liquid with no evidence of particulates or foreign matter. The PVSRIPO being utilized in this study was manufactured at the Biopharmaceutical Development Program/National Cancer Institute (NCI)-Frederick.

10.3 Dispensing and Preparation

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 (e.g., less than or equal to -80°C freezer).

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. The supplier 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 Investigational Pharmacy. All handling of the study agent will occur in a biosafety cabinet or a similarly contained environment.

The study agent will be in a disposable, hypodermic needle with a safety sheath to cover the needle using a one-handed technique after the agent is drawn into the syringe. The sheathed needle is then removed from the syringe containing the study agent, aliquoted at the desired dose, and the syringe will be capped. The capped syringe will be transported to the site of patient injection in a 'ziplock' bag placed in a portable cooler while maintaining a temperature of 4°C.

Each patient will be injected with a total intratumoral dose of $1x10^8$ $TCID_{50}$ PVSRIPO in physiologic saline stabilized with 0.2% human serum albumin. The injection surface will be cleaned with Chloraprep or betadine and allowed to dry. A local anesthetic agent will be applied to the injection area. There will be a single entry point into the breast. The study agent in 0.6 mL will be loaded into a 1 cc syringe connected to the injection needle. Under direct ultrasound visualization, a 20 or 21-gauge needle will be inserted into the tumor. Following visualization of the needle tip within the tumor, study agent will be injected over 10-15 seconds by hand injection. The needle tip will then be repositioned to another site within the tumor and the injection repeated. A total of 3-6 sites will be injected with the total study dose evenly divided among sites. The location of sites will be selected by the performing radiologist with the aim of evenly distributing the study agent over the volume of a typically irregularly shaped tumor. All injections will be performed under direct real-time ultrasound visualization. The needle will then be removed very slowly.

Post-Injection pressure will be applied to the injection site(s) with sterile gauze for at least 30 seconds. The injection site and surrounding area will be swabbed with alcohol. After a change of gloves, the injected lesion(s) will be covered with an absorbent pad and dry occlusive dressing. Exterior of the occlusive dressing will be wiped with alcohol. Following injection and bandaging, the patients will be transferred to clinic 2.2. Vital signs will be monitored every 10 minutes for an hour prior to patient discharge. Patients will be advised to keep the injection site(s) covered. Bandage will be replaced when the patient returns to the clinic 1-2 days after PVSRIPO injection. Patients will be advised to leave the dressing on for 2-3 more days. If the bandage falls off at home, the patients will be asked to replace the bandage. They will receive instructions on how to replace bandage and will be provided with gloves and plastic Ziploc disposal bags. The old bandage and gloves will be placed in the plastic Ziploc bag, the bag closed and then put in regular plastic trash bag for disposal.

10.4 Compliance and Accountability

Compliance and accountability will be monitored by the Investigational Chemotherapy

Service (ICS) according to their standards of practice (SOPs).

10.5 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 trepanation 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. All surgical materials used in the procedure and potentially coming into contact with the study agent will be disposable. Used sharps with 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.

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.

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

11 SUBJECT ELIGIBILITY

11.1 Inclusion and Exclusion Criteria

Please refer to Section 5.4.

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Informed consent will occur after confirming tumor size by imaging per SOC between days -28 and -7 After obtaining informed consent and during screening (days -28 to -7), women with invasive breast cancer will undergo complete history and physical exam, blood work including CBC, CMP, and pregnancy test (if applicable). An ultrasound, mammogram, or breast MRI showing a residual breast tumor at least 1 cm in size before PVSRIPO injection is required.

After completing informed consent for the protocol, the patient will receive a polio vaccine booster (day -7). On day 0, an ultrasound guided core biopsy will be performed at radiology prior to injection of PVSRIPO into the tumor. Injection will be performed in Duke Breast Radiology and the patients will be transferred to Duke Cancer Center clinic 2-2. Vital signs will be monitored every 15 minutes for an hour prior to patient discharge. On day 10-20

(ideal 12-16 days), the patient will undergo SOC surgery. Part of the excised tumor will be transported to Surgical Pathology by the Brain Tumor BioRepository (BTBR) Team, as directed by Dr. Roger McLendon, MD or per clinical protocol, for gross examination and research tissue procurement. Additional expertise in breast pathology will be provided by Dr. Edgardo Parrilla Castellar, MD, PhD.

Tissue deemed appropriate for collection (i.e. wasteful excess and of no diagnostic value) will be collected by the Brain Tumor BioRepository (BTBR) Team, as directed by Dr. Smita Nair, PhD, and transported to her lab for correlative molecular and immune analyses. Surgically resected tissue will be used fresh and/or processed into formalin-fixed paraffin-embedded (FFPE) blocks, required for immunohistochemistry and immunofluorescence. Additionally, inflammatory and immune analysis will be conducted using blood obtained on day -7 (range day -7 to -10, before polio vaccine booster), day 0 (before PVSRIPO injection), day 1-2 (range day 1-2 after PVSRIPO), day 14 (range day 10 to 20 after PVSRIPO, which is before surgery) and in follow-up at months 1 (± 10 days) and 6 (± 1 month) after PVSRIPO. Blood will be analyzed for inflammatory and immune signature using arrays, 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. Whole blood will be collected for immunological correlates on day -7 (range -7 to -10 just before polio vaccine booster), day 0 (before PVSRIPO injection), day 1-2 and day 14 (range day 10 to 20 after PVSRIPO, just before surgery) and in follow-up at months 1 and 6 after PVSRIPO.

Table 1. Screening and On-Study Tests and Procedures.

Anticipated Time Frame	**Screening Day -28 to -7**	**Day -7**	**Day 0**	**Day 1-2**	**Day 10-20**	**Follow-up**
Scheduling Window (Days)	-28 to -7	-7 to -10	0	1-2	10 to 20	1, 6 months
Informed consent	X					
Medical history	X					
Physical Exam, vitals	X	X	X			X
Wound/injection site evaluation				X	X	
Concomitant medications	Continuous					
12-Lead ECG	X					
Pregnancy test (whole blood - only if premenopausal prior to chemotherapy)	X					
CBC w/diff	X	X	X	X	X	
CMP	X		X		X	
Urinalysis	X					

Anticipated Time Frame	Screening Day -28 to -7	Day -7	Day 0	Day 1-2	Day 10-20	Follow-up
SOC MRI, MMG, or ultrasound of tumor	X		X		X[1]	X[2]
Polio vaccine booster		X				
PVSRIPO injection			X			
Tumor biopsy			X			
Tumor resection					X	
Whole blood for immune analysis (35 mL)	X	X	X	X	X	X
Blood for polio titer (5 mL)	X	X	X		X	X[3]
SAEs / AEs		Continuous				
Stool sample*						X[4] Days 7,14,21,56

Screening and On-Study Tests and Procedures

- History: Complete history, ECOG status, and physical exam including vital signs, and weight, must be obtained within 7-28 days prior to completion of informed consent.
- Blood work: CBC with differential, CMP, and pregnancy test. Pre-study laboratory tests must be obtained within 7-28 days prior to completion of informed consent.
- Imaging: All participants must have a MRI, MMG or ultrasound after completion of chemotherapy (days -28 to -7) showing a breast tumor at least 1 cm in size.
- Pathology: Pathological report confirming diagnosis of invasive breast cancer prior to start of chemotherapy will be required.
- Systemic imaging: CT chest/abdomen/pelvis, PET scans, ultrasounds, and bone scans during the trial are at the discretion of the PI and will be ordered as indicated to assess for extent of disease or disease relapse per SOC.
- *Stool samples: Stool samples will be collected on days 7, 14, 21, and 56 after PVSRIPO injection to comply with Duke Biosafety Protocol. This is not a clinic visit. Patients will be given a kit to collect stool that includes the following items: a toilet hat specimen collector, tongue depressor(s), a urine sample container, 2 plastic bags, and a brown paper bag. Samples will be stored frozen until the next clinic visit.

12.1 Pre-treatment Period

Women with stage II-IV invasive breast cancer with a residual tumor mass of at least 1 cm by imaging, after completion of neoadjuvant chemotherapy will be included in this study. A polio vaccine booster will be given 7 to 10 days before PVSRIPO injection. Thirty-five ml whole blood will be drawn for immunological profiling before polio vaccine booster.

[1] At the discretion of the physician, MMG or ultrasound may be done the day prior to surgery
[2] MMG or ultrasound only done at the 6 (± 1) month follow-up visit.
[3] At 1 month follow-up visit only.
[4] This is a take-home kit and does not involve a clinic/hospital visit. Days refer to days after PVSRIPO.

12.2 Treatment Period

Patients will undergo a core biopsy of the tumor after which PVSRIPO will be administered via intratumoral injection using ultrasound guidance in Radiology. Core biopsy will be tested for CD155 expression, immune infiltrate by H&E and IHC, and inflammatory and immune signature by arrays. After injection, the patient will be transferred to clinic 2.2. Vital signs will be monitored every 10 minutes for an hour prior to patient discharge. Thirty-five ml of whole blood will be drawn for immunological profiling prior to PVSRIPO injection. Thirty-five ml of whole blood will be drawn for immunological profiling 1-2 days after PVSRIPO injection.

12.3 End of Treatment

End of treatment, defined as definitive standard-of-care surgery, will be performed on patients 10-20 days after the PVSRIPO injection. Tumor samples obtained from the surgical resection will be tested for CD155 expression, immune infiltrate by H&E and IHC, tumor infiltrating immune cell profile using flow cytometry, and inflammatory and immune signatures using arrays. The excised tumor will be transported to Surgical Pathology by the Brain Tumor BioRepository (BTBR) Team, as directed by Dr. Roger McLendon, MD or per clinical protocol. Dr. Edgardo Parilla Castellar will provide additional expertise in breast pathology. Tissue deemed appropriate for collection will be transported to Dr. Smita Nair, PhD, by the BTBR Team for correlative molecular and immune analyses. Surgically resected tissue will be used fresh and/or processed into FFPE blocks, required for immunohistochemistry and immunofluorescence. Thirty-five ml of whole blood will be drawn for immunological profiling.

12.4 Follow-up Period

Follow up will be at 1-2 days, 1 month, and 6 months after PVSRIPO. Thirty-five ml of whole blood will be drawn at each time point for immunological profiling. At 6 month SOC visit, the patient will undergo an MMG or ultrasound to monitor disease progression.

12.5 End of Study

End of study is defined as after the 6 month follow-up visit. Patient will be removed from the trial if they choose to withdraw from the trial or if unable to comply with the study requirements, please refer to Section 12.6.1 for more details on early withdrawal. The decision to remove a patient from trial due to failure to comply with study requirements is at the discretion of the PI. Patients will continue to be monitored indefinitely for progression, further treatment regimens, and survival.

12.6 Early Withdrawal of Subject(s)

12.6.1 Criteria for Early Withdrawal

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

- Adverse events
- Abnormal laboratory values
- Abnormal test procedure results
- Protocol deviation

- Administrative issues
- Pregnancy

13 SAFETY MONITORING AND REPORTING

13.1 Adverse Events

An 'Adverse Event' will be defined as any adverse change from the subject's pre-treatment baseline condition, including any clinical or laboratory test abnormality that occurs during the course of research after the polio vaccine booster is given on day -7. Adverse events will be categorized and graded in accordance with the NCI CTC (Version 4).

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

A summary of all adverse events (not just those considered related to the study drug) will be maintained in 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.

13.2 Reporting of Serious Adverse Events

SAEs must be reported by the PI to the Sponsor or their designee 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 their designee must be transmitted via secure e-mail **within 24 hours of discovery to pv_istari@klserv.com**.

All suspected adverse events that are both serious and unexpected should be reported immediately to the PI, Dr. Shelley Hwang, or her designee. Unexpected fatal or life-threatening suspected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the Sponsor or their designee followed by a complete report within 8 additional calendar days. Unexpected adverse events that are both serious and suspected, but not fatal or life-threatening, will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the Sponsor or their designee.

All adverse events that are considered serious, unanticipated or unexpected, and related or possibly related to the research (as defined by 21CRF312.32[a]) will be reported to the Duke University Medical Center IRB using the appropriate Serious Adverse Event (SAE) report form within the following guidelines:

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

At the time of the annual report to the FDA, a summary of the overall toxicity experience will be provided.

13.3 Safety Oversight Committee

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

13.4 External Data and Safety Monitoring

Dr. Elizabeth A. Mittendorf will serve as the research monitor for this protocol. Per DoD 3216.02, at a minimum, the research monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the Human Research Protection Office (HRPO). IND safety reports and other notable safety information from all ongoing PVSRIPO clinical investigations will be provided to the PI by the Sponsor or their designee, who will then communicate relevant information with Dr. Mittendorf and the DoD, per their communication plans.

If the PI or DCI has a potential conflict of interest with conduct of this protocol, the protocol may require additional oversight beyond or in place of the SOC in the form of an external DSMB. If a potential conflict of interest exists, the PI should contact the Duke Research Integrity Office (RIO) to develop a conflict of interest management plan for the protocol. The plan should describe who will comprise the external DSMB, how frequently it will meet, what it will review, and the criteria it will use to determine whether it is safe to proceed with the protocol. Due to a potential conflict of interest, an external DSMB-Plus was created for the

original phase I clinical trial in adult patients with Grade IV malignant glioma performed by the Preston Robert Tisch Brain Tumor Center (PRTBTC) at Duke University Medical Center and was extended to all other studies with PVSRIPO conducted by the PRTBTC. As the current study is being supported by a grant from the DoD, which requires and provides independent medical monitoring, RIO has determined that no additional oversight is required by an external DSMB.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

This clinical research study will be monitored both internally by the PI and institutionally by the Duke Cancer Institute (DCI) and possibly by the Sponsor or their designee. In terms of internal review, the PI will continuously monitor and tabulate adverse events. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of Grade III or IV events 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:

- If stopping rules for toxicity are met, the study will be halted;
- 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 reasonable and timely manner.

The DCI Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. DCI review and monitoring of this protocol occurs in accordance with the NCI-approved Data and Safety Monitoring Plan. Briefly, protocol review begins with an initial review by the Cancer Protocol Committee (CPC), which assesses the ethics and safety of the protocol. Documentation of these assessments will be maintained. Formal, independent monitoring will be conducted by the DCI Monitoring Team after the first 3 subjects are enrolled, followed by annual monitoring of 1-3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

DCI Monitoring Team reports and additional data/safety/toxicity reports submitted by the PI will be reviewed by the Safety Oversight Committee on an annual basis. Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns. Monitoring visits may also be initiated upon request by DUHS and DCI Leadership, CPC, Safety Oversight Committee, a sponsor, an investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including, but not limited to the National Institute of Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

14.2 Audits

The Duke School of Medicine Office of Audit, Risk, and Compliance (OARC) office and the Sponsor or their designee may conduct confidential audits to evaluate compliance with the protocol and the principles of GCP. The 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 auditor(s) in order to discuss findings and any relevant issues.

Audits are designed to protect the rights and well-being of human research subjects. OARC audits may be routine or directed. Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, investigator initiated investigational drugs or devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized institutional official.

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

14.3 Data Management and Processing

14.3.1 Case Report Forms (CRFs)

The electronic CRF (eCRF) will be the primary data collection document for the study and is developed in conjunction with statistical oversight. The CRFs will be updated in a timely manner following acquisition of new source data. Only the 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 DCI IT staff.

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 manager and the statistical team will cross-reference the data to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed

only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

14.3.3 Study Closure

Following completion of the studies, the 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

Six women with breast cancer will participate in this pilot clinical study. The study is designed as an exploratory pilot study only to assess PVSRIPO bioactivity. Any subjects who do not have evaluable tumor tissue from both the pre-treatment biopsy and post-treatment surgery will be replaced.

Our primary objective is to describe the change in the amount of tumor infiltrating immune cells in tumor pre- and post-injection of PVSRIPO by H&E. Given the small sample size of this study, descriptive statistics will be used to summarize inflammatory and immune signatures in tumor and blood, tumor infiltrating immune cell analysis, changes in immune cell composition in blood and changes in T cell activation in blood. Some statistical comparisons may be conducted in an exploratory manner.

Tumor specimens collected pre and post injection of PVSRIPO will be examined for inflammatory and immune signature using arrays, tumor infiltrating cells by H&E and IHC, and CD155 expression by IHC. The level of the immune checkpoint PD-L1 expression in tumor tissue before and after PVSRIPO injection will also be examined. Mean changes in these outcomes will be computed, and a paired t-test or Wilcoxon signed-rank test may be conducted with appropriate adjustment for multiple comparison.

Changes over time in inflammatory and immune signature using arrays, immune cell composition (antigen presenting cells, B cells and T cells) and T cell activation by flow cytometry will be described. Changes over time in B cell activation using ELISA and peptide arrays will be described. Measurements will be obtained on day -7 (range day -7 to -10, before polio vaccine booster), day 0 (before PVSRIPO injection), day 2 (range day 1-2 after PVSRIPO), day 14 (range day 10 to 20, after PVSRIPO before surgery) and in follow-up at months 1 and 6 (after PVSRIPO).

Additional descriptive analyses besides those described here may be conducted using the samples collected.

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 will be submitted to the DUHS Institutional Review Board (IRB) and DCI Cancer Protocol Committee (CPC) for review. The study will be initiated only after the Principal Investigator has received written and dated approval from the CPC, IRB, and Sponsor representative or their designee.

The Principal Investigator will submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The CPC will 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 will 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 will be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form will 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/their designees 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 protocols, approved informed consent forms, FDA Forms 1572, and laboratory certifications.

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

An electronic database, specifically RAVE, will be the primary documentation for case report forms (CRFs) in this study. The CRFs will be updated within two weeks of acquisition of new source data. Only approved staff as indicated in the in key personnel log, are permitted to make entries, changes, or corrections in the CRF. Changes or corrections will be dated, initialed, and explained (if necessary). The Principal Investigator or authorized key personnel will maintain a record of the changes and corrections.

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 limited to designated key personnel as indicated in the delegation log. Subject data may be stored temporarily on encrypted and password-protected portable memory devices such as flash drives and external hard drives, but only when absolutely necessary. Data stored on portable memory devices will be de-identified. Subject data will be deleted from the portable memory device at the earliest opportunity. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

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

16.6 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan. For a more detailed description, please refer to Section 13.3 and 14.1 in this protocol.

16.7 Protocol Amendments

All protocol amendments must be initiated by the Principal Investigator and approved by the IRB and Sponsor 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.

Though not yet required, 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, etc.).

16.8 Records Retention

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

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

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