Protocol Title: A Phase 1 Randomized, Single-Blind, Placebo-Controlled, Ascending Dose Study to Evaluate the Safety and Immunogenicity of rVSVΔG-MARV-GP [Angola] (PHV01, MARV GP Vaccine) in Healthy Adults

Protocol Number	PHV01-C-101
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Short Title	PHV01 (MARV GP Vaccine) Safety and Immunogenicity in Healthy Adults
IND Number	028937
Product	PHV01 (rVSVΔG-MARV-GP [Angola])
Sponsor	Public Health Vaccines, LLC One Broadway, 14th Floor Cambridge, MA 02142
Funders	Biomedical Advanced Research and Development Authority Department of Health and Human Services, Washington, DC

APPROVAL SIGNATURE PAGE

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REVIEWED AND APPROVED BY:

Richard T. Kenney, MD		
Chief Medical Officer for Marburg Public Health Vaccines, LLC	Signature	Date

This study will be conducted according to the Protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

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INVESTIGATOR STATEMENT

I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol.

I understand that I may terminate or suspend enrollment of the study at any time if it becomes necessary to protect the best interests of the study subjects. This study may be terminated at any time by Public Health Vaccines LLC (Sponsor) with or without cause.

I agree to personally conduct and supervise this investigation at my institution and to ensure that all associates, colleagues, and employees assisting in the conduct of this study are informed about their obligations in meeting these commitments.

I will conduct the study in accordance with Good Clinical Practice, the Declaration of Helsinki, and the moral, ethical, and scientific principles that justify medical research. The study will be conducted in accordance with all relevant laws and regulations relating to clinical studies and the protection of subjects.

I will ensure that the requirements relating to Institutional Review Boards/Independent Ethics Committees (IRB/IEC) review and approval are met. I will provide Public Health Vaccines LLC with any material that is provided to the IRB for ethical approval.

I agree to maintain adequate and accurate records and to make those records available for audit and inspection in accordance with relevant regulatory requirements.

I agree to promptly report to the IRB any changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB and Sponsor approval, except where necessary to ensure the safety of study subjects.

Investigator Name	Investigator Signature	Date

Investigational site or name of institution

This study will be conducted according to the Protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

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LIST OF ABBREVIATIONS

The following abbreviations are used in this study protocol.

Abbreviation	Definition
ADCC	antibody-dependent cell-mediated cytotoxicity
ADEM	acute disseminated encephalomyelitis
ADNP	antibody-dependent neutrophil phagocytosis
ADMP	antibody-dependent monocyte phagocytosis
AE	adverse event
AESI	adverse event of special interest
ALT (SGPT)	alanine aminotransferase
ANA	anti-nuclear antibody
AsaT	all subjects as treated, also known as the “Safety Cohort”
AST	aspartate aminotransferase
AUC	area under the curve
BMI	body mass index = subject weight [kilograms]/(height [meters])^2
BUN	blood urea nitrogen
CBC	complete blood count
CCP	cyclic citrullinated peptide
CFR	Code of Federal Regulation
CI	confidence interval
CEPI	Coalition for Epidemic Preparedness and Innovation
cGCP	Current Good Clinical Practices
cGLP	Current Good Laboratory Practices
CRP	C-reactive protein
CSR	Clinical Study Report (including Day 0-180)
DS	Drug Substance
DP	Drug Product
EBOV	Ebola virus
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
EoS	End of Study
ER	Emergency Room
FDA	United States Food and Drug Administration
FIH	first-in-human
FSH	Follicle-Stimulating Hormone
GBS	Guillain Barré Syndrome
GMT	geometric mean titer
GP	glycoprotein
GSD	geometric standard deviation
HbsAg	hepatitis B surface antigen
HgB	hemoglobin
HCG	human chorionic gonadotropin
HCV	hepatitis C
Hct	hematocrit

Abbreviation	Definition
HHC	household contact
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IA	Interim Analysis
IB	Investigator's Brochure
ICAM-1	intercellular adhesion molecule-1
ICF	informed consent form
ICH	International Conference on Harmonisation
ID	identification number unique to each subject
IEC	Independent Ethics Committee
IFN	interferon
Ig	immunoglobulin
IM	intramuscular/ intramuscularly
IND	Investigational New Drug application
INR	international normalized ratio
IP	Investigational product
IQR	interquartile range
IRB	Institutional Review Board
IWRS	Interactive Web Response System
kg	kilogram
LLOQ	lower limit of quantitation
MAAE	medically attended adverse event
MARV	Marburg virus, the etiologic agent of Marburg disease
MedDRA	Medical Dictionary of Regulatory Activities
mITT	modified intention-to-treat
MMRM	mixed models for repeated measures
NHP	non-human primate
NIAID	National Institutes of Allergy and Infectious Diseases
PCR	polymerase chain reaction
pfu	plaque-forming unit
PHV	Public Health Vaccines, LLC
PHV01	investigational agent; rVSVΔG-MARV-GP (Angola) [Live, replication competent attenuated recombinant vesicular stomatitis virus expressing the envelope glycoprotein gene of Marburg virus, code named PHV01]
PM	Pharmacy Manual
PMBC	Peripheral blood mononuclear cells
PI	Principal Investigator
PP	per protocol
PRBC	packed red blood cells
PsVNA	Pseudovirion neutralization assay
$PsVNT_{50}$	Pseudovirion neutralization titer of 50%
$PsVNT_{80}$	Pseudovirion neutralization titer of 80%
PT	prothrombin time

Abbreviation	Definition
PTT	partial thromboplastin time
RT-qPCR	quantitative reverse transcription polymerase chain reaction
RF	rheumatoid factor
rHA	recombinant human albumin
RNA	ribonucleic acid
ROG	remainder of Group
rVSV	recombinant vesicular stomatitis virus
rVSV-Marburg	rVSVΔG-MARV-GP (Angola), also known as “PHV01”
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SoE	Schedule of Events
SOP	standard operating procedure
SPEAC	Safety Platform for Emergency Vaccines (a joint Brighton Collaboration and CEPI project); https://brightoncollaboration.us/speac/
SRC	Safety Review Committee
SSP	study-specific procedures
SUDV	Sudan ebolavirus
SUSAR	suspected, unexpected serious adverse reaction
TBD	to be determined
TEAE	treatment-emergent adverse event
ULN	upper limit of the normal range
US	United States
USP	United States Pharmacopeia
VCAM-1	vascular cell adhesion molecule-1
VSV	vesicular stomatitis virus
WBC	white blood cell
WHO	World Health Organization
WT	wild type

1. INTRODUCTION

1.1. BACKGROUND ON MARV GP VACCINE DEVELOPMENT

1.1.1. Marburg Virus (MARV)

MARV disease is a severe hemorrhagic fever, occurring as sporadic cases and in small epidemics, and has a case-fatality rate of up to 90% (1). The natural reservoir host is the Egyptian fruit bat (*Rousettus aegyptiacus*), a medium-sized species of megabat that is found in Africa, the Middle East, the Mediterranean, and the Indian subcontinent (2); human infections can occur by exposure to bats or their excreta and outbreaks may be propagated by inter-human transmission of the virus, illustrating the potential for pandemic spread. Health workers and household contacts are at particular risk. Except for imported cases and laboratory infections, outbreaks have occurred only in sub-Saharan Africa with the most recent in March 2023 in Tanzania. There have been 17 recorded outbreaks since 1967 with the largest in 2004-2005 in Angola (374 cases with 329 deaths) (3).

There is no licensed vaccine or treatment for MARV in humans. Due to the widespread geographic distribution of the fruit bat *Rousettus aegyptiacus*, Marburg is an emerging zoonotic infectious disease with the potential to cause large outbreaks, resulting in its designation by the US Biomedical Advanced Research and Development Authority (BARDA/HHS/ASPR), the World Health Organization (WHO), and the Coalition for Epidemic Preparedness and Innovation (CEPI) as a priority pathogen for the development of medical countermeasures (4-6).

In response to this call, Public Health Vaccines, LLC (PHV) has developed PHV01, a live, attenuated, recombinant vesicular stomatitis virus (Indiana) (rVSV) in which the glycoprotein gene of VSV has been deleted and replaced with the corresponding envelope glycoprotein gene of Marburg virus (Angola) (MARV GP). This protocol is designed to test the safety, tolerability, immunogenicity, and dosage requirements for PHV01 as a single-dose vaccine against MARV.

This first-in-human (FIH) Phase 1 trial will enroll 36 healthy male and female adults (18 - 60 years of age), who will be randomized in cohorts to receive one of 3 graded doses ($1x10^5$, $1x10^6$, or $1x10^7$ pfu in 1.0 mL) or placebo (Lactated Ringer's 1.0 mL) in an ascending dose design. Participants will be followed for solicited and unsolicited adverse events, vaccinemia, shedding of vaccine virus in urine and saliva, and immune responses. The last visit will be 6 months after the dose.

1.1.2. Vesicular Stomatitis Virus as a Vaccine Vector

Rose and colleagues have pioneered the use of rVSV as an expression vector for generating vaccine candidates (7, 8). VSV viruses are in the family *Rhabdoviridae*, genus Vesiculovirus. They are bullet-shaped, single-stranded, negative-sense ribonucleic acid (RNA) viruses containing 5 genes, 1 of which is the viral GP. VSV viruses are limited in their distribution to the Americas. In the US, the 2 most common strains are the New Jersey and Indiana strains. VSV causes disease in hoofed livestock (cattle, horses, llamas, and swine), primarily manifesting as crusting and vesiculation of the mucous membranes and skin. The virus is transmitted between animals by direct contact or by insects.

Recombinant vesicular stomatitis viruses expressing foreign viral genes are desirable as vaccine candidates for multiple reasons. The rVSV virus used in the construction of PHV01, which has the VSV G protein gene deleted, is highly attenuated compared to wild-type (WT) VSV and, importantly, has lost its neurovirulence for mice and monkeys. rVSV vaccines induce strong cellular and humoral host immune responses as a result of the intracellular synthesis of specific antigens at high levels. rVSV elicits both mucosal and systemic immunity. The rVSV viruses grow to high titers in acceptable cell lines for manufacturing. Importantly, the single-stranded RNA genome of VSV does not undergo genetic reassortment or recombination. Since the VSV G protein containing neutralizing epitopes is deleted, the rVSV vectors are not susceptible to neutralization by pre-existing VSV antibodies. Additionally, the lack of serious pathogenicity in humans (9) is an advantage of using rVSV vaccines in humans.

For those reasons, numerous VSV-based prophylactic vaccines and therapeutics are being developed for use against both infectious and malignant disease targets. In addition to the PHV01 vaccine, several other VSV-based vaccines and therapeutics are also being studied in humans:

- VSV expressing the EBOV GP protein, a vaccine licensed by the FDA (ERVEBO®, Merck) for the prevention of Ebola (Zaire)
- VSV expressing the EBOV GP protein and the Nipah GP protein, now being studied by PHV in a Phase 1 study (NCT05178901)
- VSV expressing the SUDV GP protein vaccine in a Phase 1 study (NCT05724472)
- VSV expressing the SARS-CoV-2 virus spike protein, now being trialed in Phase 2/3 study (NCT04990466)
- VSV expressing Lassa virus glycoprotein (NCT04794218)
- VSV expressing interferon (IFN)-β TYRP1, in treating patients with stage III-IV melanoma (NCT03865212)
- VSV expressing IFN-β-sodium iodide symporter (NIS), in treating patients with refractory advanced/metastatic solid tumors (NCT02923466); endometrial and uterine cancers (NCT03212624); lymphoma, leukemia, and myelodysplastic syndrome (NCT03017820); solid tumors, refractory non-small cell lung cancer, or head and neck squamous cell carcinoma (NCT03647163); melanoma, hepatocellular carcinoma, non-small cell lung cancer, and endometrial cancer (NCT04291105); VSV expressing GP128, for Stage IV colorectal cancer (NCT04046445)
- VSV expressing human immunodeficiency virus (HIV) gag protein, as a HIV vaccine (NCT01438606 and NCT01578889)
- VSV expressing IFN-β, as treatment for hepatocellular carcinoma (NCT01628640)

In addition, there are clinical development efforts underway for other VSV-based vaccines to protect humans against Middle East respiratory syndrome (10), chikungunya (11), and Venezuelan equine encephalitis (12).

1.1.3. Non-Clinical Evaluation of rVSV Vectored Marburg Vaccines

A number of safety and proof-of-concept studies have been published demonstrating safety, immunogenicity, and protective activity of rVSV-MARV-GP (Musoke, 1980) in the non-human primate (NHP) model for pre- and post-exposure protection against intramuscular (IM) and aerosol challenge (13-17). Studies of a rVSV-MARV (Angola) vaccine candidate confirmed immunogenicity and protection against virus challenge (18). The vaccine virus was similar to PHV01 but it was derived from a different transfection and was not plaque-purified. NHPs were challenged with MARV (Angola) 14, 7, or 3 days after a single IM vaccination; 100% of the animals survived when vaccinated 7 or 14 days before challenge, and 75% survived when vaccinated 3 days before challenge. Both innate and adaptive immune responses were involved in early protection, as shown by induction of antiviral and interferon gene pathways and T-cell activation (19, 20).

1.1.3.1. Safety

A toxicity and local tolerance study following repeat IM dose administration of the PHV01 vaccine with a 14-day recovery was conducted in guinea pigs according to cGLP. There was no mortality observed following IM administration of rVSV-MARV-GP. There were no injection site observations, changes in group mean body weights, food consumption, and body temperatures attributed to IM administration of rVSV-MARV-GP. On necropsy (acute and recovery phase), there were no changes in organ weights and no gross abnormalities attributed to rVSV-MARV-GP administration. Test-article related microscopic lesions were limited to mild skeletal muscle degeneration and necrosis accompanied by inflammation at the site of injection during the acute phase and this had resolved during the recovery phase where muscle regeneration was observed.

A cGLP biodistribution study was conducted in NHPs inoculated IM with 1.6×10^7 pfu. The pattern of tissues with detectable viral RNA was similar to that reported for rVSVΔG-ZEBOV-GP (ERVEBO) (21). Multiple tissues were positive at 24 hours, fewer by 72 hours, and even fewer by 8 days after inoculation. By day 15 and later, the tissues with consistent persisting RNA were principally lymphoid tissues (lymph nodes, spleen, ileum with GALT). Infectious virus measured by plaque assay was not detected after day 15. Tissues representing potential sites of shedding (nasal turbinates, salivary gland, kidney ileum, lung) were positive for viral RNA, principally during the first week after inoculation. RNA was found in synovial tissue from one animal at 24 hours and in metacarpophalangeal joint tissue from 2 animals on day 8. Low levels of viral RNA (below assigned lower limit of quantitation [LLOQ]) were found in the spinal cord of 1 of 3 monkeys tested at each of the following timepoints after IM inoculation: 24 hours, at 8 days and at 15 days, but at no subsequent timepoint to day 113. The animals with positive spinal cord results at 24 hours and 8 days also had viral RNA detected in plasma. There was no detectable viral RNA in brain tissues from any animal. Given the absence of the VSV G gene (the principal neurovirulence factor) in PHV01, no evidence for infection of brain tissue in the biodistribution study and the lack of neurovirulence in a published study of a similar vector, rVSV-MARV (Musoke) (22), the risk of a neurotropic adverse events (AEs) resulting from inoculation of PHV01 is judged to be low. Collectively, the data suggest the safety profile is acceptable for human clinical trials. Please see the Investigator's Brochure (IB) for details of these studies.

1.1.3.2. Immunogenicity and Efficacy

Two studies in guinea pigs given graded doses of an uncloned, development lot, the cloned master viral seed, or final drug substance showed development of vaccinemia, vaccine-specific antibodies and protection against lethal challenge using a guinea pig adapted MARV. In animals inoculated with $2x10^4$ or $2x10^6$ pfu of the final drug substance, 83% and 100% survived challenge, respectively. See the Investigator's Brochure for details.

Two studies in cynomolgus macaques were conducted with a development lot and with the toxicology lot 155941of PHV01 at $6.7x10^7$ and $1.6x10^7$ pfu, respectively. All animals developed vaccinemia peaking on day 1 and clearing by day 4-7 indicating active replication of the vaccine virus. IgG GP-binding and neutralizing antibodies were elicited by day 14, and all animals survived lethal challenge on day 42 with MARV (Angola), whereas control animals inoculated with 0.9% saline succumbed within 8 days. Please see the IB for details of these studies.

1.2. CLINICAL EXPERIENCE

This is a FIH clinical trial. There has been no administration of this vaccine to humans prior to this study.

1.2.1. Post-Exposure Treatment

There has been no use of this vaccine as a post-exposure treatment. Post-exposure treatment is not a planned use or intended labelling indication for the PHV01 vaccine.

1.3. RATIONALE FOR A DOSE-FINDING STUDY

There is a high unmet medical need for a vaccine for the control of outbreaks and for persons with occupational exposure to MARV (23). MARV is widely distributed across Sub-Saharan Africa with a history of repeated outbreaks characterized by spill-over from the bat reservoir, followed by interhuman transmission. The events in Europe in 1967 illustrate the potential for virus exportation and spread outside the endemic region as well. Ebola, also a bat-associated filovirus, had a similar pattern of small outbreaks until it erupted in pandemic form in West Africa in 2014-2016, providing fair warning of a future episode of this kind in the case of MARV.

The severity of disease and potential for interhuman spread underlie the need for a safe and effective vaccine. MARV disease is a devastating and often lethal infection, with prostration, bleeding manifestations, and multiorgan failure. The overall case-fatality rate can be as high as 88-90% (24). There are few, if any, asymptomatic infections. Patients develop multiple signs of hemorrhagic diathesis, renal impairment, obtundation, hypotension, shock, and multiorgan failure. Most fatal cases succumb during the second week of illness. Autopsies have demonstrated focal necrosis without inflammation in the liver, spleen, testes, ovaries, and the pancreas, and signs of hemorrhagic diatheses in all organs, and glial nodule encephalitis (25).

The dose requirements for efficacy of the PHV01 vaccine are not determinable by review of the available pre-clinical data. Knowledge of the lower dose limit for successful vaccination with PHV01 is critical to defining the vaccine formulation for pre-exposure immunization. Importantly, such data will establish the scale of manufacturing required to meet current and future demands. This information is especially critical during a period of vaccine shortage. Moreover, the dose could modulate reactogenicity of the vaccine, as was found for the very similar Ebola vaccine (26).

As live vaccines replicate in vivo and expand their antigenic mass, only a single injection of a low dose of infectious units is generally required for primary immunization for general prophylactic use. However, time to onset of acquired immunity (seroconversion) is a function of inoculum level and could be a critical determinant in dose selection for outbreak use.

Outcome measures in this dose-ranging study will principally include the safety profile, as determined by incidence of AEs, evidence of replication in vivo (vaccinemia), and the occurrence of vaccine shedding and excretion. Immunogenicity measures will include the development of immunoglobulin (Ig)-G binding and neutralizing antibodies. Blood will also be collected for other immunogenicity and cell-based assays (Table 3), which may be performed in the future.

1.4. NAME AND DESCRIPTION OF THE INVESTIGATIONAL PRODUCT

rVSVΔG-MARV-GP (Angola) (referred to as PHV01), is a live attenuated viral vaccine produced in Vero cells. The virus consists of a VSV (Indiana) backbone that was deleted for the VSV-G envelope glycoprotein with substitution of the GP of the Marburg virus (Angola strain).

The drug product is a sterile solution for injection containing $\geq 1 \times 10^7$ pfu/mL PHV01 virus in 10 mM Tris HCl, 0.25% rHA, pH 6.8-7.8. It is a clear to opalescent, colorless solution, free from visible particulates. Residual host cell DNA is $\leq$ 10 ng/mL and endotoxin < 20 EU/mL. The vaccine is supplied in a 2 mL glass vial containing nominal 1 mL. Refer to the Pharmacy Manual for information about the specific lot to be used in the Phase 1 clinical trial.

The vaccine must be stored in the freezer at -80ºC ± 10 °C. The vaccine is delivered in a volume of 1.0 mL by IM injection with a standard needle/syringe according to the instructions provided. For use in clinical trials, dilution to specified dose (potency, pfu/mL) may be required. See the Pharmacy Manual for details for preparation of individual doses.

1.5. KNOWN AND POTENTIAL RISKS AND BENEFITS TO HUMAN SUBJECTS

1.5.1. Risks/Discomfort to Subjects and Precautions to Minimize Risk

This will be the first clinical trial involving PHV01 vaccine candidate administration to humans in the US under an Investigational New Drug (IND) application with FDA.

Potential risks associated with this vaccine may include those common to other vaccine products, including local injection site reactions and systemic reactions. The following sections discuss these potential risks in more detail and include brief descriptions for possible procedures to ameliorate risks and symptoms. All relevant risks and precautions are described and explained in detail in the Informed Consent Form (ICF) provided to potential subjects.

It is possible that the side effects of PHV01 will be improved by lowering the dose administered.

Local Reactions

In a study of the only licensed rVSV-based vaccine, rVSVΔG-ZEBOV-GP (ERVEBO) (N=1051) versus placebo (N=133) in healthy North American adult subjects, the most common AEs were injection site pain (69.5% vs 12.8%), swelling (16.5% vs 3.0%), and redness (11.9% vs 1.5%) (17). Similar local injection site reactions, with either PHV01 or placebo, may include pain, swelling, and/or redness at the injection site. Although highly unlikely, IM injections, even

independent of investigational product (IP), can result in acute muscle damage, bruising, and/or injection site infection.

Early Systemic Reactions

Transient mild to moderate lymphopenia and neutropenia have been described after receipt of rVSVΔG-ZEBOV-GP and are similar to findings associated with acute viral infections. No associated infections have been reported. Similar hematologic effects may be seen with PHV01.

Systemic reactions have been reported in individuals who received the rVSVΔG-ZEBOV-GP vaccine (17, 26). These events generally occur within the first 12 to 24 hours post-vaccination, are mild to moderate in severity, and resolve within 1 or 2 days. They include headache, fever, feeling "feverish", chills, arthralgia, myalgia, and less commonly, gastrointestinal symptoms. These early symptoms are associated with activation and release of cytokines and chemokines (27). For these and other systemic symptoms commonly seen in investigational vaccine studies, monitoring will be performed throughout the study and symptoms will be addressed as required to maximize subject safety. On occasion, individuals may feel lightheaded or faint after the act of injection (often an emotionally-related vasovagal reaction).

Late Systemic Reaction

Transient post-vaccination AEs seen with rVSVΔG-ZEBOV-GP primarily include arthritis, and macular, papular, or vesicular rash (17). In a Phase 1b study of 513 healthy volunteers (26), self-limited, post-vaccination arthritis occurred in 4.5% (19 of 418) of vaccinees (median onset 12.0 days [interquartile range (IQR) 10–14]; median duration 8.0 days [IQR 6–15]) versus 3.2% (three of 94) of controls (median onset 15.0 days [IQR 6–20]; median duration 47.0 days [IQR 37–339]), with no apparent dose relationship. Post-vaccination dermatitis occurred in 5.7% (24 of 418) of vaccinees (median onset 9.0 days [IQR 2–12]; median duration 7.0 days [IQR 4–9]) versus 3.2% (three of 94) of controls (median onset 5.0 days [IQR 3–53]; median duration 33.0 days [IQR 5–370]). These could also be observed with PHV01.

Other Potential Risks

Since WT VSV causes encephalitis after intracerebral inoculation of mice and NHP, there is a theoretical risk of aseptic meningitis or encephalitis following vaccination with rVSV. However, the vaccine vector has been specifically attenuated by removal of the VSV G to eliminate neurotropism/neurovirulence. The rVSVΔG-ZEBOV-GP vaccine has lost the ability to cause encephalitis after intracerebral inoculation of adult mice. Moreover, no neurological symptoms or histopathological lesions in brain or spinal cord were observed after intrathalamic inoculation of cynomolgus macaques with either rVSVΔG-ZEBOV-GP or rVSV-MARV (22). The rVSV-MARV virus used in the referenced study had the GP of a different MARV strain (Musoke) (23). No instances of post-vaccination meningitis or encephalitis have been associated with the administration of rVSVΔG-ZEBOV-GP in humans. As noted above in describing the biodistribution study of PHV01 in NHP, low levels of viral RNA were found in spinal cord of 1 of 3 monkeys tested at each of the following timepoints after IM inoculation: 24 hours, at 8 days, and at 15 days, but at no subsequent timepoint to day 113. There was no detectable viral RNA in brain tissues from any animal. Given the absence of the VSV G gene (the principal neurovirulence factor) in PHV01, no evidence for infection of brain tissue in the biodistribution study, and the lack of neurovirulence of the rVSV-MARV (Musoke) construct, the risk of a neurotropic AE

resulting from inoculation of PHV01 is judged to be low. However, this protocol includes careful monitoring for neurological AEs.

Adverse Events

AEs will be recorded starting with Screening. Once dosed, subjects will also be monitored for the occurrence and severity of indicators of reactogenicity. Treatment-emergent AEs (TEAEs) include all AEs that occur with and after dosing. It is expected that PHV01 may cause cytokine-mediated acute phase reactions similar to those associated with the similar Ebola vaccine (ERVEBO) during the first 1-3 days after inoculation (17). Thus, TEAEs will be solicited daily for 14 days after vaccination using a scripted questionnaire or Memory Aid (Section 5.4.1), including expected local and systemic AEs, as well as specific neurologic, joint, or dermatologic AEs, and unsolicited AEs; solicited AEs for Days 16-29 are limited to instances of neurological signs and symptoms, arthritis, and rash. Throughout the remainder of the study (e.g., until study day 181), subjects will be asked to report important changes in health status and concomitant medications, as well as any medically attended adverse events (MAAEs) (Section 5.4.2). AEs will be recorded throughout the rest of the study if noted on subsequent Memory Aids or if evaluated as MAAEs, serious AEs (SAEs), or AEs of special interest (AESIs), along with any concomitant medications (Section 6.4.3). The severity of all AEs will be graded in accordance with the FDA document "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (APPENDIX 1).

Medically-Attended Adverse Events

Any AEs that cause a subject to seek medical care (including extra visits to the clinical site) are considered to be MAAEs. These will be recorded for the entire study. Subjects will be asked to record MAAEs on Memory Aids that will be reviewed during the visits on days 29, 85, and 181.

Adverse Events of Special Interest

In order to maximize the value of vaccine safety data in clinical trials, the CEPI and the Brighton Collaboration have formed an international organization, "Safety Platform for Emergency Vaccines" (SPEAC), to promote standardization of data collection, presentation, and analysis of AESIs relevant to vaccines in general, to specific vaccine platforms (live virus vaccines, rVSV vaccines), and in specific cases to the target disease itself (28).

While Marburg disease cannot be acquired with PHV01 injection, as the only MARV component present is the GP gene in a background of rVSV, vaccines, in general, have been associated with neurologic disease (generalized convulsions, aseptic meningitis, optic neuritis, transverse myelitis, Guillain-Barré Syndrome (GBS), and acute disseminated encephalomyelitis (ADEM), hematologic disease (thrombocytopenia) and immunologic disease (anaphylaxis, vasculitides), and these conditions are considered AESIs in this study.

In order to capture vaccine-related AEs resembling those associated with WT MARV infection, AEs such as multiorgan failure, hemorrhagic diathesis, and neurologic complications (encephalitis, myelitis, aseptic meningitis, generalized convulsions) are considered AESIs.

Subjects' Memory Aids through day 181 will be reviewed for evidence of potential neurologic disease, i.e., blurry vision, changes in balance or coordination, confusion, disorientation, dizziness, severe headache, shaking or body tremors, slurred speech, tingling or numbness, weakness or

feeling weak. If present, a neurologic examination will be performed and documented. In addition, a neurologic examination will be performed and documented at baseline and day 29.

Neurologic examination will include assessment of affect, posture, involuntary movement, cognition, speech, cranial nerves, strength, gait, sensation, deep tendon reflexes, and coordination.

1.5.2. Pregnancy

Risks from the study vaccines to human fetuses are unknown at this time. As such, pregnant females will be excluded from participation, and female subjects of childbearing age will be required to use contraception during this study and for a period after vaccination.

Given the theoretical risk of shedding and transmission to a female partner of childbearing potential, male subjects will also be required to utilize effective contraception precautions for the same period of time.

Each pregnancy should be reported *immediately* (within 48 hours of identification) by email or fax to the Sponsor's safety office and the IRB. Subjects, or their partners, who become pregnant after day 1 will be followed to term. Any SAE experienced during pregnancy must be reported on the SAE report form.

In the absence of any pregnancy complications that meet the criteria of an SAE, a pregnancy should not be reported as an AE.

1.5.3. Lactation

Risks from the study vaccine to nursing infants are unknown at this time. As such, lactating females will be excluded from participation.

1.5.4. Venipuncture

Some discomfort from the needle stick for the blood draw is possible, including swelling or bruising, and there is a very small risk of infection at the site of the needle stick. A few subjects may feel lightheaded and may develop a rapid heartbeat during blood collection. These symptoms can be stopped by having the subject lie down and/or by stopping the procedure.

1.5.5. Allergic Reaction

As with any IP, there is the potential risk of a serious or even life-threatening allergic reaction to one or more components of the IP to be used in this study. The vaccine contains the virus and recombinant (yeast derived) human albumin in a buffer and is considered to have a low risk of eliciting allergic reactions. To further mitigate this risk, subjects with a history of severe allergic reaction of any kind, or significant allergic reaction to a known component of the experimental product, will be excluded from participation. Subjects will be observed for at least 60 minutes ($\geq$ 60 minutes and $\leq$ 90 minutes) after vaccine administration for any signs or symptoms of local and/or systemic intolerance to the study product. Vital signs will be checked within the same observational timeframe ($\geq$ 60 minutes and $\leq$ 90 minutes) after vaccination.

In the event of a severe allergic reaction, the study site is staffed with trained medical personnel and stocked with appropriate medical emergency equipment to provide acute care for conditions such as anaphylaxis. Further, if required, a formal emergency medical response service (rescue

squad, fire department, etc.), capable of treating and transferring any life-threatening injuries to a higher level of medical care, is available in close proximity to the trial site.

1.5.6. Skin Biopsy

In the event that a punch biopsy is indicated, there is a risk of local bleeding and bruising, pain, infection, allergic reaction to the anesthetic, or damage to the structures beneath the skin site such as an artery or a nerve. A separate consent would be used for this procedure.

1.5.7. Arthrocentesis

In the event that an arthrocentesis is indicated, in addition to the risk of pain and hemorrhage, there is a rare (1:2000) risk of iatrogenic septic arthritis (29). A separate consent would be used for this procedure.

1.5.8. Guillain-Barré Syndrome (GBS)

GBS, an auto-immune disorder of peripheral nerves, has been observed to be temporally related to administration of certain vaccines. There is a background incidence of GBS in North America and Europe of 0.62 to 2.66 per 100,000, with rates higher with advancing age and in males vs. females (30). Additionally, recent epidemiologic data from the Centers for Disease Control and Prevention indicate that there is no statistically significant increased risk of GBS in the 6 weeks following vaccination with licensed vaccines (31). Overall, the chance of developing GBS is very unlikely but it is possible. In rare situations when GBS does occur, approximately 85% of GBS patients make a complete or nearly complete recovery. Management includes supportive care, plasmapheresis, and administration of high dose IgGs. The death rate from GBS may be 3% to 7% (32).

To mitigate this risk, individuals with a history of GBS, other neurologic diseases per the Principal Investigator's (PI) discretion, or other significant reaction to vaccination will be excluded from participation.

1.5.9. Unknown Risks

As with all research of new vaccines, there is a remote possibility of risks that are unknown or that cannot be foreseen based on available information. This would include late effects that have been seen with some vaccines.

1.5.9.1. Shedding of Virus

There is a theoretical risk of shedding of vaccine virus and transmission to contacts. The finding of low levels of viral RNA in salivary gland, kidney, and nasal turbinates until day 15 in the NHP biodistribution study (Section 1.1.3) indicates the possibility of shedding of the vaccine virus in a clinical setting, and the potential for transmission, as reported for the preceding rVSV-ZEBOV-GP vaccine (33). Participants in this study are advised to take measures to limit contact spread of the vaccine virus to other persons or susceptible animals.

1.5.10. Alternatives to this IND Product or Study

At this time, there is no FDA-approved or European Medicines Agency-approved product that affords protection against infection with Marburg. PHV01 is one of five vaccines in development intended to protect against Marburg (34). Subjects may choose not to participate in this study.

1.5.11. Intended Benefit for Subject

There is no intended direct benefit for study subjects. It is possible that vaccination with PHV01 may result in an immune response to Marburg. The protective efficacy of such responses is unknown. The results of this study may be of benefit to humanity in the future.

1.5.12. Risks to the Study Personnel and the Environment

The principal risk for study personnel is exposure during dose preparation. In the clinical setting there is a risk of exposure to infectious pathogens from study subjects through an accidental needlestick during dosing with the live attenuated PHV01 vaccine, or to blood-borne pathogens. There is also the theoretical risk of transmission through contact exposure to blood or body fluids after dosing. Adherence to good hygiene practices and standard operating procedures (SOPs) for working with infectious agents and universal precautions will reduce the risk of exposure.

There are no known risks to the environment other than those associated with the generation of biohazardous waste attendant to vaccination of humans. All biohazardous waste will be disposed of as stipulated by local, state, and federal regulations and in accordance with study site SOPs. The environmental risk of PHV01 to livestock is thought to be very low due to the attenuation of the WT VSV by deletion of the G protein, which is a virulence factor.

1.6. ROUTE OF ADMINISTRATION, DOSAGE REGIMEN, TREATMENT PERIOD

PHV01 or placebo will be administered once to each subject by IM injection. Subjects will be enrolled into treatment groups and randomized to either study product or placebo.

Detailed instructions for preparation of IP are provided in a separate Pharmacy Manual. The diluted PHV01 vaccine and placebo will have similar appearance, ensuring blinding of study medication. It is anticipated that 1 mL of vaccine or placebo will be administered IM in the deltoid muscle using a 3-mL syringe with a 1.5-inch #23-gauge sterile needle. Prior to injection, the area to be injected will be prepped with an alcohol swab and allowed to dry before injection. While holding the needle at an approximate 90-degree angle to the skin, the needle will be directed through the skin, into the muscle of the deltoid. After injection, the needle will be disposed of into a rigid sharps container.

The study will enroll 36 subjects in a stepwise, dose ascension manner in 4 distinct Groups (Table 1) organized as 4 dosing Cohorts (Figure 1). Randomization will be stratified to ensure balance across the treatment groups with regards to gender. Different block sizes may be used.

Table 1: Dosage Regimen by Treatment Group

Treatment Group	Subjects	Test Article	PHV01 Dose (pfu & volume)	Route	Schedule
Group A	10	PHV01	1×10^5 pfu in 1 mL	IM	Day 1
Group B	10	PHV01	1×10^6 pfu in 1 mL	IM	Day 1

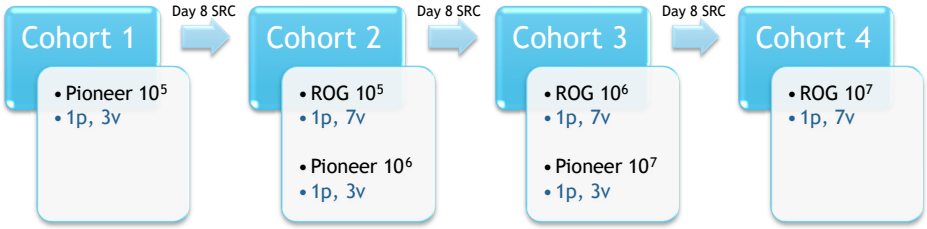
Group C	10	PHV01	1×10^7 pfu in 1 mL	IM	Day 1
Group D	6	Lactated Ringer's	1 mL	IM	Day 1

Abbreviations: IM = intramuscular; pfu = plaque forming units.
*Titer determined on release of drug product and will be ≥ $1x10^7$ pfu/mL

Subjects will be enrolled stepwise with safety reviews conducted between Dosing Cohorts to approve dosing the remaining subjects within that cohort and dose-escalation into the next Dosing Cohort (Figure 1).

Dosing Cohort #1 consists of Pioneer Group A (3 vaccinees at $1x10^5$ pfu and 1 placebo). *Note: As this is a FIH study, a maximum of 2 randomized subjects will be dosed per day in Cohort #1.* The Study Review Committee (SRC) (PI, Medical Monitor, and Sponsor's Chief Medical Officer) will review safety data through day 8, and if no pausing or stopping rules are met, will enroll Dosing Cohort #2, consisting of Remainder of Group (ROG) A (7 vaccinees at $1x10^5$ pfu and 1 placebo), and Pioneer Group B (3 vaccinees at $1x10^6$ pfu and 1 placebo). The SRC will review safety data through day 8 and, if no pausing or halting rules are met, will enroll subsequent Dosing Cohorts #3 and #4 in the same stepwise review and authorization process, to proceed to complete planned enrollment of all 36 subjects.

Figure 1. Flow Diagram for Dosing Cohorts and SRC Decision Points



v-vaccine; p-placebo

1.7. COMPLIANCE STATEMENT

The study will be conducted according to the protocol and in compliance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP). All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guidelines.

1.8. STUDY POPULATION

The study will enroll and dose a total cohort of 36 healthy male and non-pregnant, non-lactating, adult female subjects, 18 to 60 years of age at the time of screening (inclusive). To avoid difficulties in interpretation, subjects are excluded if there is a history of a confirmed or suspected immunosuppressive or immunodeficient condition, including HIV-1 or HIV-2 infection, history of long COVID (35), cytotoxic therapy in the previous 5 years, and/or diabetes.

See Section 4.4 & 4.5 for inclusion and exclusion criteria. Refer to Section 7.1 for a statistical justification of the sample size.

1.9. SUBJECT IDENTIFIER CODE

In agreement with cGCP, each subject will be unambiguously identified by a code that allows the identification of all data reported for that subject.

Once the subject has provided informed consent at the screening visit, a unique subject screening number will be allocated.

Upon meeting all inclusion and no exclusion criteria, subjects will be deemed eligible for randomization into the study. Immediately prior to randomization, subjects will be assigned a unique treatment identification (randomization) number.

If for any reason after signing the ICF the subject fails to be randomized and dosed, the reason for not being dosed must be documented.

Once assigned, the subject, screening, and randomization number, cannot be reused. The Investigator must keep track of the names of the subjects enrolled and their identifying numbers in a Subject Identification Code List.

2. STUDY OBJECTIVES

2.1. PRIMARY SAFETY OBJECTIVE

To evaluate the safety and tolerability of the Marburg vaccine candidate, PHV01, when administered at graded doses (1×10^5, 1×10^6, 1×10^7 pfu/mL) given by the IM route in 1 mL to healthy adults.

2.2. PRIMARY IMMUNOLOGIC OBJECTIVES

To evaluate Marburg-specific IgG ELISA antibody and neutralizing antibody responses to graded doses of PHV01 given by the IM route to healthy adults.

2.3. SECONDARY OBJECTIVES

To evaluate vaccine viremia and shedding in saliva and urine after administration of PHV01.

2.4. EXPLORATORY OBJECTIVES

To obtain and preserve serum, blood, and peripheral blood mononuclear cell specimens for assays designed to dissect immunological mechanisms of protection and gene activation.

3. STUDY DESIGN

3.1. STUDY ENDPOINTS

3.1.1. Primary Safety Endpoints

- Incidence and severity of solicited injection site AEs (arm pain, local tenderness, erythema (redness), induration (swelling/firmness) and systemic AEs (objective fever, subjective fever, chills, sweats, headache, myalgia, arthralgia, fatigue, nausea, vomiting) and neurological AEs (blurry vision, changes in balance or coordination, confusion, disorientation, dizziness, severe headache, shaking or body tremors, slurred speech, tingling or numbness, weakness or feeling weak) between days 1 and 8, and days 9-15 after vaccination, per treatment group (active vaccination versus placebo) and stratified by relatedness.
- Incidence and severity of unsolicited AEs between days 1-8, days 9-15, and up to day 29 after vaccination, per treatment group (active vaccination versus placebo) and stratified by relatedness.
- Incidence and severity of arthritis and skin/mucosal lesions up to day 29 after vaccination using standard case definitions and stratified by relatedness. This includes where possible quantitative reverse transcription polymerase chain reaction (RT-qPCR) for detection of PHV01 and lesion biopsy for histopathology.
- Incidence of SAEs observed up to day 181 and stratified by relatedness.
- Incidence and severity of MAAEs observed up to day 181 and stratified by relatedness.
- Physical and neurological examination results, vital signs, normal and abnormal clinical laboratory values (hematology and clinical chemistry, urinalysis).
- Incidence of adverse events of special interest (AESIs), to include pneumonitis, acute respiratory distress syndrome, multiorgan failure, a hemorrhagic diathesis, certain neurologic events (encephalitis, myelitis, aseptic meningitis, optic neuritis, transverse myelitis, generalized convulsions, Guillain-Barré Syndrome, acute disseminated encephalomyelitis), thrombocytopenia, anaphylaxis, and vasculitides.

3.1.2. Secondary Safety Endpoints

- PHV01 in blood on days 1 (at 0 and 6 hours), 2, 4, 8, 15, and 29, as detected by quantitative reverse transcription polymerase chain reaction (RT-qPCR)
    - Proportion of subjects with viremia detected by RT-qPCR
    - Median duration of viremia, determined by RT-qPCR
    - Geometric mean level of viremia, geometric mean peak level of viremia, and area under the curve (AUC)
- PHV01 in urine, saliva, biopsy, joint fluid, vesicle fluid, or swab of other suspect lesions on days 1 (at 0 and 6 hours), 2, 4, 8, 15, and 29, as detected by RT-qPCR
    - Proportion of subjects with PHV01 detected by RT-qPCR
    - Median duration of PHV01, determined by RT-qPCR

- Geometric mean copy number of PHV01, geometric mean peak copy number of PHV01, and area under the curve (AUC)

3.1.3. Safety Endpoint Collection

Safety will be assessed by analysis of the occurrence of TEAE and vaccinemia, as well as by adverse changes in laboratory evaluations (chemistry, hematology, urinalysis), vital signs, and physical including neurologic examinations. All AEs will be collected systematically through day 29 (Section 1.5.1). SAEs, MAAEs, and AESIs will be collected for the entire period each participant is on study with the help of Memory Aides, reviewed at the day 29, 85, and 181 visits.

AEs will be coded according to Medical Dictionary of Regulatory Activities (MedDRA) Version 26.1 (or latest) (36). A TEAE is defined as an AE that starts or worsens on or after the date and time of the study vaccination. AE endpoints include:

- Solicited TEAEs.
  - Solicited local AEs (at the injection site) are reported daily through day 14 and systemic reactogenicity symptoms, facilitated through the use of a memory aid, occurring from vaccination through 15 days following vaccination. *Note: solicited AEs for days 16-29 are limited to instances of neurological signs and symptoms, arthritis, and rash.*
  - Local reactogenicity signs and symptoms, including arm pain, local tenderness, erythema (redness), and induration (swelling/firmness).
  - Systemic reactogenicity signs and symptoms, including subjective and objective fever, shivering/chills, sweats, myalgia (generalized muscle aches), arthralgia (joint aches/pain, general or while moving joints; affected joints will be captured), joint swelling, joint tenderness, fatigue, gastrointestinal symptoms (nausea, vomiting, abdominal pain, and diarrhea), mucosal lesion (any sores or lesions, including mouth ulcers), skin lesion (including any rashes or blisters), and neurological symptoms (blurry vision, changes in balance or coordination, confusion, disorientation, dizziness, severe headache, shaking or body tremors, slurred speech, tingling or numbness, weakness or feeling weak). As noted in this paragraph, a severe headache is an adverse event that may represent a potential neurologic AESI.
- Solicited joint-related (joint aches/pain, joint swelling, and joint tenderness), mucosal lesion, and skin lesion TEAEs will be collected from vaccination through day 29, facilitated through the use of a Memory Aid. TEAEs will be recorded on the Memory Aids throughout the remainder of the study. Enhanced evaluation of arthritis and rash TEAEs will be done if the subject triggers protocol-specified algorithm and/or is referred to rheumatologist or dermatologist.
- Unsolicited TEAEs will be similarly collected from vaccination through day 29 with the use of a Memory Aid, and throughout the remainder of the study.

All TEAEs and clinical laboratory evaluations, including a standard metabolic chemistry panel, CBC, prothrombin time, activated partial thromboplastin time, and fever will be graded according to the toxicity grading scales (APPENDIX 1), which are based on the FDA document "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (Sept 2007) (37). Vaccinemia is defined as a blood specimen

polymerase chain reaction (PCR) positive for rVSV (i.e., a detectable result ≥ LLOQ genome copies(gc)/mL).

3.1.4. Joint and Skin Related Adverse Events

Based on comprehensive review of the related rVSVΔG-ZEBOV-GP vaccine (17, 26, 38-43), a small percentage of subjects (approximately 5%) may experience post-vaccination arthritis with onset typically within one week of injection and resolving within days to weeks. The reported incidence by studies ranges from 0% to 23.5%, depending on the study methodology, definition of arthritis and method and timing of diagnosis. Arthritis may affect small or large joints, or the axial skeleton. Skin rash has been reported in 4% of subjects, commonly described as maculopapular or vesicular (33, 44). Subjects with suspected or confirmed diagnosis of post-vaccination arthritis (day 5 onwards) or petechial, purpuric, or vesicular rash or mucosal ulcers will be promptly evaluated according to standardized algorithms by Investigators trained on these case definitions. The algorithm for assessment of joint-related AEs is found in Figure 2 and the one for rash is found in Figure 3. A baseline serum sample will be stored at the immunology laboratory for use with the evaluation if needed.

Assessments may include some or all of the following:

- Targeted assessment of affected musculoskeletal areas.
- Detailed assessment of the involved joints to determine if joint aspiration is likely to yield joint fluid for further analysis.
- Full body evaluation for skin lesions and mucosal lesions (ulcers).
- At the discretion of the Investigator, and when feasible and acceptable to the subject, synovial fluid aspiration of at least one involved joint by a healthcare provider who is proficient in performing arthrocentesis in the affected joints. Arthrocentesis has only been required in rare cases in prior trials using this rVSV backbone (26).
- At the discretion of the physician, and when feasible and acceptable to the subject, 1 or 2 punch biopsies will be obtained of any petechial or purpuric rash. Biopsy has only been required in rare cases in prior trials using this rVSV backbone.
- Further laboratory evaluations from samples collected on days 1 and 29, and unscheduled samples to coincide with onset and resolution of symptoms:
  - Blood samples
  - Chemistries (creatinine, blood urea nitrogen [BUN], uric acid)
  - C-reactive protein (CRP)
  - Cytokines
  - Rheumatoid factor (RF), anti-cyclic citrullinated peptide (CCP), anti-nuclear antibody (ANA)
  - C3, C4
  - Urinalysis
- Skin biopsy of affected skin lesions (histopathology, RT-qPCR).
- Needle aspiration of vesicular skin lesions for RT-qPCR and virus isolation.
- Swab samples of mucosal lesions (e.g., mouth ulcers) for RT-qPCR.

- Synovial fluid assessment:
  - Cell count with differential
  - Crystal examination
  - Culture for bacteria, and virus isolation
  - RT-qPCR for vaccine viral RNA
- Exploratory analyses may be performed on other bodily fluids. These data (as available) will be provided in listings.

3.1.5. Primary Immunogenicity Endpoints

The primary immunogenicity endpoints are as follows:

- Geometric mean titers (GMT) of Marburg GP protein-specific IgG antibody as measured by (ELISA on days 1 and 29.
- $PsVNT_{50}$ and $PsVNT_{80}$ MARV GP-specific neutralizing antibodies titers (with 50% and 80% neutralization, respectively) as measured by PsVNA on days 1 and 29.

3.1.6. Secondary Immunogenicity Endpoints

- GMT Marburg GP protein-specific IgG ELISA antibody on all other study days through Day 181.
- Average $PsVNT_{50}$ and $PsVNT_{80}$ on all other study days to Day 181.
- Seroconversion rate (at least 4-fold increase in Marburg GP-specific IgG (ELISA)
- Seroconversion rate (at least 4-fold increase in Marburg GP-specific neutralizing antibody titer PsVNA)
- Geometric mean fold increase in Marburg GP-specific IgG (ELISA)
- Geometric mean fold increase in Marburg GP-specific neutralizing antibody titer (PsVNA)
- Reverse cumulative distribution of titers (IgG ELISA and PsVNA)

3.1.7. Exploratory endpoints

- Wild-type Marburg neutralization against one or more Marburg lineages
- Marburg ELISA against one or more Marburg lineages
- Determination of Fc-mediated cell-targeting vaccine-specific antibodies, e.g., ADCC, ADNP, ADMP, etc.
- Vaccine-specific T-cell responses to Marburg GP protein by intracellular cytokine staining (ICS) and/or enzyme-linked immune absorbent spot (ELISPOT)
- Determination of Marburg IgM ELISA antibody responses
- Determination of vaccine-specific pro-inflammatory markers, such as CRP, ICAM-1, VCAM-1 levels, and cytokine levels
- Determination of vaccine-specific gene activation by RNA transcript sequencing (transcriptomics)

- Determination of B or T cell responses to VSV proteins

3.1.8. Immunogenicity Endpoint Collection

Samples for immunogenicity testing will be collected per the Schedule of Events (Table 2). All subjects will have samples tested for serum Marburg GP-specific IgG ELISA and PsVNA.

3.2. OVERALL STUDY DESIGN

This Phase 1, FIH study of the Marburg vaccine candidate PHV01 is on the critical path for development of a VSV-vectored, single-dose vaccine for the prevention of Marburg virus disease. The purpose of the PHV01-C-101 study is to test the safety and immunogenicity of the plaque-purified, vaccine injected by the IM route undiluted (Group C: $1x10^7$ pfu in 1 mL) and at tenfold dilutions (Group B: $1x10^6$ in 1 mL and Group A: $1x10^5$ pfu in 1 mL) (Table 1). A blinded ascending dose design will be used in cohorts, such that lower doses are given first to 3 Pioneer vaccinees and a randomized placebo control (Group D: 1 mL Lactated Ringer's solution), with day 8 safety assessments reviewed before proceeding to higher doses in the next cohorts (Figure 1). *Note: As this is a FIH study, a maximum of 2 randomized subjects will be dosed per day in Cohort #1.* The ROG and a randomized placebo control will then be dosed at the lower dose, randomized with 3 Pioneer vaccinees and a placebo control at the next higher dose.

This trial of PHV01 will provide timely safety, immunogenicity, and dose-response data to guide further manufacturing, control, and clinical development of the vaccine. This trial will accelerate the development and availability of validated immunologic assays required to reliably define and qualify immunologic test methods and will guide dose selection for a subsequent Phase 2 trial in one or more endemic countries in Africa. An IA will be performed on the safety and serological data from all subjects collected up to day 29. The final Clinical Study Report (CSR) will be based on the safety and immunogenicity data collected on days 1-181. The schedule of assessments is provided in the Schedule of Events (SoE) (Table 2).

Table 2: Schedule of Events

Visit # and Timing	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9
	-28to -1	Day 1	Day 2	Day 4 ± 1 day	Day 8 ± 1 days	Day 15 ± 2 days	Day 29 ± 2 days	Day 85 ± 7 days	Day 181 ± 7 days
Informed consent[1]	X								
Inclusion/exclusion	X	Review							
Demographics[2]	X								
Medical history	X								
Vital signs[3]	X	X × 2[3]	X	X	X	X	X	X	X
Physical exam	X	X[4]	X[4]	X[4]	X[4]	X[4]	X	X[4]	X[4]
TEAEs, SAEs, MAAEs, AESIs[5]	X	X × 2	X	X	X	X	X		
Concomitant medications[5]	X	X × 2	X	X	X	X	X		
Pregnancy test[6]	X	X					X		
Screening for HIV, HCV, HepB (5 mL)[7]	X								
Rapid antigen test for SARS-CoV-2		X							
Hematology, chemistry, and coags (10.7 mL)[8]	X	X		X	X		X		
HLA-B27 Testing		X							
Urinalysis[9]	X			X	X		X		
MARV antibody tests (2 x 10 mL)[10]		X			X	X	X	X	X
Blood for research (100 mL)[11]								X	
PBMC/T-cell Assay[12]		X			X		X		
Transcriptomics (2.5mL PaxGene RNA tube)[13]		X × 2	X	X	X	X			
MARV viremia (7 mL) and shedding[14]		X × 2	X	X	X	X	X		
Cytokine levels (10mL)[15]		X		X	X		X		
Vaccination		X							
Post-vaccination observation[16]		X							
Provision of emergency contact card		X							
Training and review of Memory Aid		X	X	X	X	X	X	X	X

Abbreviations: AESI = Adverse Event of Special Interest; eCRF = electronic case report form; HBsAg = hepatitis B surface antigen; HCG = human chorionic gonadotropin; Hct = hematocrit; HCV = hepatitis C; HepB = hepatitis B; HgB = hemoglobin; HIV = human immunodeficiency virus; INR = international normalized ratio; MAAE = medically attended adverse event; RBC = red blood cell; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction; PT = prothrombin time; PTT = partial thromboplastin time; SAE = serious adverse event; TEAE = treatment emergent adverse event; WBC = white blood cell.

Footnotes:

[1] Obtain written informed consent for participation in the clinical study, clinical samples, and HIV testing.
[2] Demographic data include age, height and weight (for BMI), race, and ethnicity.
[3] Vital signs will include blood pressure and pulse while seated and at rest, and body temperature measured orally. On Visit 2, vital signs will be assessed up to 60 minutes before vaccination, then at 60-90 minutes after vaccination and at 6h ± 1h. (Shown as X × 2)
[4] Directed physical examination at discretion of Investigator. See the Protocol for the Arthritis Pathway algorithm for subjects with joint complaints. If rash is present on any visit, the PI should reference the Dermatology Pathway.
[5] SAEs, MAAEs, and AESIs will be recorded from day of consent until EOS. AEs will be recorded beginning with Screening. All TEAEs will be recorded from dosing on day 1 through day 29, and during the rest of the study.. Concomitant medications will be recorded up to 6 months prior to study entry and throughout the duration of the study. This will include dietary supplements and other prophylactic substances.
[6] Pregnancy tests (serum HCG) will be done in all female subjects at Screening. Repeat pregnancy tests (urine) will be done in all female subjects prior to vaccination and at day 29.
[7] Screening serology will include HIV-1, HIV-2, HCV, and HBsAg.
[8] Clinical laboratory assessments will include CBC (HgB, Hct, RBC, platelets), WBC with differential, metabolic chemistry panel, PT, PTT, and INR.
[9] Urinalysis assessments by dipstick. Reflex testing by microscopy for abnormal blood or leukocyte esterase.
[10] Two 10 mL tubes for serology: minimum of 6 (1.5 mL) aliquots of serum frozen. Four tubes will be used for viremia and neutralization studies and two tubes will be preserved at baseline and held for possible AE assessment. Collect one 10 mL tube for Rheumatologic or Dermatologic baseline
[11] Blood collection for serology and purification of IgG (100 mL). 10 × 10 mL SST tubes to be centrifuged, then the serum should be split as 10 mL aliquots in each of 5 x 15 mL conical sterile tubes and frozen at -60ºC.
[12] Collect 6 × 10 mL EDTA tubes for PBMC separation and cryopreservation.
[13] Collect in 2.5 mL PaxGene RNA tube for transcriptomics before dosing, then at 6h (± 1h) and on each subsequent visit as indicated.
[14] Blood (7 mL) for plasma, saliva (swab) and urine sample for virus detection before dosing, then at 6h (± 1h) and on each subsequent visit as indicated.
[15] Blood (10 mL) for plasma cytokines, aliquoted into 4 × 1 mL cryotubes and frozen at -60ºC.
[16] Includes TEAEs and examination of injection site 60-90 minutes after vaccination.

Table 3: Blood Collection Volume Estimates

Blood Volumes	Volumes	D -28 to -1	Day 1	Day 2	Day 4	Day 8	Day 15	Day 29	Day 85	Day 181	Total
Blood for HepB, HepC, HIV (5 mL)	**5.0**	5.0									**5.0**
HLA-B27 testing	**5.0**		5.0								**5.0**
Safety Labs (10.7 mL)	**10.7**	10.7	10.7		10.7	10.7		10.7			**53.5**
Blood for MARV antibody tests (2 x 10 mL)	**20.0**		20.0			20.0	20.0	20.0	20.0	20.0	**120.0**
Blood for assay development (100 mL)	**100.0**								100.0		**100.0**
Blood for PBMC/T-cell Assay (6 x 10 mL)	**60.0**		60.0			60.0		60.0			**180.0**
Blood for RNA (2.5mL PaxGen tube)	**2.5**		2.5	2.5	2.5	2.5	2.5				**12.5**
Blood for viremia (7 mL)	**7.0**		14.0	7.0	7.0	7.0	7.0	7.0			**49.0**
Blood for Cytokine levels (10 mL)	**10.0**		10.0		10.0	10.0		10.0			**40.0**
	Totals	**15.7**	**122.2**	**9.5**	**30.2**	**110.2**	**29.5**	**107.7**	**120.0**	**20.0**	**565.0**

3.3. MEASURES TAKEN TO MINIMIZE/AVOID BIAS

3.3.1. Randomization

This is a single-blind study, with group assignment hidden from site staff (apart from the compounding pharmacist) and subjects, along with a blinded CRA who will monitor the site records. To further avoid the potential for bias, the Medical Monitors (Sponsor and CRO) will remain blinded as well, until the database is frozen for the interim analysis (IA). Randomization will be stratified to achieve balance of sex across PHV01 dose levels and placebo recipients. For each randomization number, 2 replacement subject randomization numbers will be generated. The treatment assignment for replacement subjects will be the same as the subject who is being replaced.

Study personnel will obtain treatment identification (ID) numbers from the Interactive Web Response System (IWRS) as eligible subjects, who have successfully completed the screening process and are deemed eligible to participate by the PI (or another designated Investigator), are enrolled and proceed to vaccination.

Care will be taken to ensure that recruiting and screening processes are unbiased with regard to race and gender. To maintain randomization, alternates will be used, if necessary, in the order in which they were deemed eligible. Alternates are allowed to join the next cohort if they still meet entry criteria.

3.3.2. Blinding and Unblinding

Dosing materials will be prepared by an unblinded study pharmacist, labeled with the subject identifier, and administered by a member of the study team. All dosing materials will have identical appearance and all members of the site study team (apart from the unblinded pharmacist) will be blinded to the treatment assignment, along with the study participants. Apart from the individuals noted above, the Sponsor and CRO personnel will be unblinded, although the blind will be maintained during discussions with the site PI and staff. Subjects and Investigators will remain blinded to the subject's group assignment (PHV01 dose or placebo) until the study database is locked.

Interim Analysis of Safety and of Immunogenicity

One IA will be performed when all 36 subjects have completed the day 29 follow-up. For this IA, the records to be included from each subject will be 'frozen' after being monitored and queries will be resolved without unblinding the site staff. Pre-specified tables, listings, and figures will describe solicited local and systemic AEs, unsolicited AEs, and any MAAEs or SAEs, along with a simple listing of labs by subject without analysis of the latter.

For immunogenicity analyses, tables, listings, and figures will describe the serum IgG ELISA and PsVNA for each Group. In addition, RT-qPCR results from plasma, urine, and saliva samples will be used to assess viremia and shedding. Details regarding the IA for safety, immunogenicity, and sample algorithms will be provided in the Statistical Analysis Plan (SAP).

Individual Subject Unblinding

The group assignment for an individual subject may be unblinded during the course of the study if the PI deems that, for health purposes, the blind must be broken emergently to evaluate an AE;

the other SRC members should be included in the decision if time allows. Only in the case of an emergency, when knowledge of whether the subject has received the IP is essential for the clinical management or welfare of the subject, may the unblinded study pharmacist unblind that subject's treatment assignment. Unblinding at the study site for any other reason other than to confirm rVSV shedding positivity will be considered a protocol violation and reported as a major deviation.

Otherwise, the group assignments will be shared with the site, and subjects will be given the opportunity to request the identity of the IP he or she received, after the conclusion of all study visits (day 1 to 181) and locking of the database.

3.4. INVESTIGATIONAL PRODUCT

The IP, PHV01, was manufactured under GMP conditions by National Resilience (formerly Ology Bioservices) in Alachua, FL for PHV. Subjects will receive a 1 mL IM injection of PHV01 or placebo, according to the randomization plan. Lactated Ringer's solution USP will be used as the diluent and placebo. Sites will source the Lactated Ringer's solution USP from their pharmacy and will only utilize solution that is approved for parenteral use in humans, noting lot number and certificate of testing. See the Pharmacy Manual for details on the specific lot of IP to be used, the vial label, and methods for preparing dosing materials for administration to study participants.

3.4.1. Investigational Product Storage and Preparation

PHV01 Drug Product (DP) is provided as a sterile, frozen, preservative-free liquid for IM injection containing ≥ 1×10^7 pfu of rVSVΔG-MARV-GP, pH 6.8-7.8. No novel excipients or components of animal or human origin are used in the manufacture of the Drug Substance (DS) or DP.

Each single-dose vial contains 0.9 mL of IP. In addition to the virus, PHV01 contains 2.5 mg/mL recombinant human albumin (rHA) and 10 mM Tris at pH 7.2. No preservative is included in these single-use vials. The composition of PHV01 DP is shown below.

Table 4. PHV01 Drug Product Composition

Component	Purpose	Grade	Concentration
rVSVΔG-MARV-GP virus (PHV01)	Active ingredient	GMP	≥ 1×10^7 pfu/mL
Recombinant human albumin	Stabilizer of virus	USP-NF	2.5 mg/mL (0.25%)
Tris pH 7.2	Buffer (pH control)	GMP, USP/EP	10 mM

The DP is a clear to opalescent, colorless solution, free from visible particulates. Residual host cell DNA is ≤ 10 ng/mL and endotoxin < 20 EU/mL. The vaccine is supplied in a 2-mL glass vial containing an extractable volume of 0.9 mL. For use in clinical trials, dilution to specified dose (potency, pfu/mL) may be required. NOTE: the DP concentration (titer) of virus varies from lot to lot. Refer to the Pharmacy Manual for the composition of the lot to be used in the clinical trial.

The vaccine must be stored in the original container/vial in the freezer at -80º C ± 10ºC. The vaccine is delivered in a volume of 1 mL by IM injection according to the instructions in the Pharmacy Manual as briefly described below. Temperature during IP shipment will be monitored and recorded. Acknowledgement of receipt of the study vaccine should be completed by a designated person at the study site. Drug must be handled and stored, safely and properly, and kept in a secured location to which only the Investigator or designee have access.

To make each dose, the IP vial will be removed from the ultralow temperature freezer and thawed at room temperature for approximately 10 minutes. The thawed PHV01 should appear as a colorless liquid with no particulates visible and should be stored on wet ice until dilution.

Each thawed vial of PHV01 will be swirled gently before dilution. The vaccine may be diluted into glass vials containing Lactated Ringer's Injection, USP in each, using serial dilution as described in the Pharmacy Manual. The diluted vaccine will be stored, protected from light, on wet ice or in a refrigerator (2-8°C) until administration within 4 hours.

3.4.2. Investigational Product Administration

The vaccine and placebo dosing materials are given IM into the deltoid muscle in a volume of 1 mL using a 3-mL syringe fitted with a 1.5-inch #23-gauge needle. The 1.5-inch gauge needle is preferred to ensure IM inoculation in all subjects, including obese subjects. The arm used should be recorded for reference when inspecting the injection site.

Precaution: Although anaphylaxis is rare, facilities for its management must always be available during vaccination. Epinephrine (1:1000) for injection, resuscitation equipment, and personnel competent in their use should be immediately available. Subjects should be warned about the possibility of delayed allergic reactions and should be advised to contact a study Investigator in case of the appearance of signs of allergy (urticaria, angioedema).

3.4.3. Investigational Product Accountability

The Sponsor's representative/designee is responsible for distributing the IP to the study site. After the IP is released to the study site, the unblinded site pharmacist is responsible for and will maintain logs of IP receipt, storage, dilution, accountability by subject, and IP remaining before final disposition. At the study site, the logs will be maintained in an unblinded pharmacy binder, separate from other study files to avoid risk of unblinding. The PI may delegate, in writing, this responsibility to the unblinded institutional pharmacist, but the PI is ultimately responsible for the IP and its proper storage upon receipt at the study site until it is transferred back to the Sponsor's representative/designee or is destroyed as directed by the Sponsor's representative/designee.

All vials (unused, partially used, and spent) will be retained by the unblinded site pharmacist for accountability. No vials should be destroyed or disposed of without specific instructions from the Sponsor's representative/designee and as stipulated by local, state, and federal regulations. This occurs after the study monitor has completed the final accountability inspection. The disposition records will account for all remaining IPs.

3.5. CONCOMITANT MEDICATIONS

Concomitant medications will be recorded up to 6 months prior to study entry and throughout the duration of the study. This includes dietary supplements and other prophylactic substances.

3.6. DURATION OF SUBJECT PARTICIPATION

The expected duration of individual enrolled subject participation will be approximately 7 months, depending on the length of the screening period.

3.7. DISCONTINUATION CRITERIA

3.7.1. Discontinuation of an Individual Subject

As there is only 1 dose of study vaccine administered, there are no discontinuation rules for an individual subject.

3.7.2. Stopping and Pausing Rules

Criteria for Study Pause or Halt

Study pause and halting criteria related to AEs are as follows:

The SRC will review the day 8 safety data in each Dosing Cohort to determine if enrollment may continue.

Dosing will be paused if 2 or more subjects experience the same or similar Grade 3 adverse event considered to be at least possibly related to vaccination. In the event of a study pause, the SRC will meet before resumption of dosing.

Dosing will be halted if a SAE or an AESI is considered anything other than not related to PHV01 vaccine. In the event dosing is halted, the SRC and IRB will review the event and other relevant safety data from the study and the IRB must agree before any continuation of dosing.

Enrollment

If no pause or halting rules are triggered in each Dosing Cohort, enrollment and dosing may proceed after review of day 8 safety data by the SRC. In the event dosing is paused or halted, scheduled follow up visits and procedures will continue for subjects already dosed.

3.7.3. Study Termination

The PI, Sponsor's representative, the SRC, IRB, or the FDA may stop or suspend the use of this product at any time.

3.8. TRIAL TREATMENT RANDOMIZATION CODES

A single document delineating the randomized assignment of treatment ID numbers (according to the methodology outlined in Section 3.3.1) to subjects will be generated and maintained in a secure location as part of the regulatory file. During the study, the unblinded site pharmacist, representatives of the Sponsor, and applicable regulatory authorities will have access to this list if required by their duties.

3.9. IDENTIFICATION OF DATA TO BE RECORDED ON THE CASE REPORT FORMS

The eCRF data will be transcribed from source documentation to ensure data completeness and accuracy as required by study protocol. No source data will be recorded directly in the eCRF (i.e., without prior written or electronic record of data). The transcribed data will be consistent with the source documents; any discrepancies will be queried and resolved. The Investigator and/or site staff must make the eCRFs and source documents of subjects enrolled in this study available for inspection by PHV or its representative at the time of each monitoring visit.

At a minimum, source documentation must be available to substantiate subject identification, eligibility and participation, proper informed consent procedures, dates of visits, adherence to the protocol procedures, adequate reporting and follow-up of AEs, administration of concomitant medication, study vaccine receipt/dispensing/return records, study vaccine administration information, and date of termination or completion and reason.

The subject must allow Medical Monitor, Sponsor, SRC, IRB, and FDA access to the subject's medical records. Each subject should be informed of this prior to the start of the study. For more information on data handling, refer to Section 8.

4. SELECTION AND WITHDRAWAL OF SUBJECTS FROM THERAPY

4.1. RECRUITMENT OF SUBJECTS

Recruitment of subjects will conform to accepted human subject protections. Details of recruitment may be included in a separate study recruitment plan.

4.2. INFORMED CONSENT PROCESS

Eligible subjects may only be included in the study after providing written (witnessed where required by law or regulation) informed consent using an IRB-approved ICF. Informed consent must be obtained before conducting any study-specific procedures (SSPs) (i.e., all of the procedures described in the protocol). The record of obtaining informed consent should be documented in the subject source documents.

PHV or its representative will provide Investigators with an ICF that complies with the ICH GCP guideline and regulatory requirements, is considered appropriate for this study, and has been approved by a relevant IRB/Independent Ethics Committee (IEC).

All men and women of child-bearing potential should be informed that taking the study vaccination may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study, they must adhere to the contraception requirements for the first eight weeks after vaccination (Section 5.3). In case of doubts on the ability of a subject to adhere to these requirements, that subject should not be allowed in the study. The original signed ICFs for each subject will be kept on file in a secure location by the Investigator. Each potential subject will receive a copy of their signed and dated written ICF and any other written information provided to the subjects.

4.3. ELIGIBILITY SCREENING

Each subject must meet all inclusion and no exclusion criteria. The PI or designee will make the final decision of the eligibility. Only eligible subjects will be given the IP or placebo.

Refer to Section 1.8 for a detailed description of the subject population.

4.4. SUBJECT INCLUSION CRITERIA

1. Healthy adult male or non-pregnant, non-lactating adult female, ages 18 to 60 years old (inclusive) at the time of screening
2. Have provided written informed consent prior to screening procedures
3. Free of clinically significant health problems
4. Available, able, and willing to participate in all study visits and procedures
5. Primary COVID vaccination, defined as two doses of licensed SARS-CoV-2 vaccine, administered > 1 month prior to day 1
6. Negative rapid antigen test for SARS-CoV-2 on the day of and prior to dosing
7. Be willing to practice abstinence from sexual intercourse, or willing to use effective methods of contraception from the time of screening until eight weeks after dosing

8. Be willing to minimize blood and body fluid exposure of others or handling of livestock after dosing through six weeks after vaccination
9. Not anticipate changes in living conditions through six weeks following vaccination that will put them in contact or association with children less than 1 year of age or household contacts (HHC) who are immunodeficient, on immunosuppressive medications, are human immunodeficiency virus (HIV)-positive, are pregnant or breastfeeding, or have an unstable medical condition
10. Must plan to reside in the geographic area of the clinical study site for at least 6 months after vaccination

4.5. SUBJECT EXCLUSION CRITERIA

1. Presence of objective, measured fever >100.4°F/38°C or acute illness within 1 week before study product injection
2. Inability to observe possible local reactions at the eligible injection site (either deltoid region), which is unacceptably obscured due to a physical condition or permanent body art, in the opinion of the Investigator
3. Has BMI of 35 or greater
4. Have an active malignancy or history of metastatic or hematologic malignancy
5. Subjects with atopic dermatitis (eczema), recurrent oral/mucosal vesicles, or ulcerative lesions
6. Subjects with a history of chronic inflammatory disease (e.g., rheumatoid arthritis, psoriatic arthritis, reactive arthritis, ankylosing spondylitis, systemic lupus erythematosus, psoriasis, Crohn's disease, ulcerative colitis, and gout), symptomatic osteoarthritis, or any other autoimmune or autoinflammatory disorder
7. History of severe local or systemic reactions to any vaccination or a history of severe allergic reactions
8. Known allergy to the excipients of the PHV01 vaccine product (Tris buffer, recombinant human albumin)
9. Any chronic or active neurologic disorder, including seizures and epilepsy (excluding a single febrile seizure as a child), or history of Guillain-Barré Syndrome
10. Receipt of investigational product up to 30 days prior to randomization
11. Receipt of licensed or authorized non-live vaccines within 14 days of planned study immunization (30 days for live vaccines)
12. Administration of IgGs and/or any blood products within the 120 days preceding study entry or planned administration during the 6-month study period
13. Administration of systemic chronic immunosuppressants (defined as more than 14 days) or other immune modifying drugs within 6 months of study entry
14. History of prior infection with VSV, receipt of a VSV vectored or filovirus vaccine, or employment or activity that involves potential contact with Marburgvirus or other Filoviruses

15. History of blood donation within 60 days of enrollment or plans to donate within the study period
16. Any baseline laboratory screening tests that, in the opinion of the Investigator, are considered clinically significant
17. Any serologic evidence of acute or chronic hepatitis B or C infection (unless HCV RNA RT-qPCR is negative)
18. Any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV-1 or HIV-2 infection, history of long COVID, cytotoxic therapy in the previous 5 years, and/or diabetes
19. Ongoing participation in another clinical trial, without approval by PHV
20. Clinically significant psychiatric, neurologic, hematologic, pulmonary, cardiovascular history, as determined by the Investigator
21. Suspected or known alcohol and/or illicit drug abuse within the past 5 years
22. Pregnant or lactating female, or female who intends to become pregnant during the 6-month study period
23. Unwilling to allow storage and use of samples for future vaccine research
24. Unwilling to consider diagnostic evaluation of joint signs and symptoms (which may include arthrocentesis for an effusion) or skin rash (to include punch biopsy if clinically indicated), and if the procedure is acceptable to the subject at the time
25. Research staff or the immediate family of research staff directly involved in the clinical study
26. Elective surgery or hospitalization planned during the period of study participation
27. Any other significant finding that, in the opinion of the Investigator, would increase the risk of the individual having an adverse outcome from participating in this study

4.6. SUBJECT WITHDRAWAL CRITERIA

Each subject may withdraw consent at any time during the study without penalty or loss of benefit to which the subject is otherwise entitled. Counseling about the subject's health will be provided by the PI or Co-Investigator if he/she decides to discontinue participation in the study. Medical advice regarding what is in the best interest of the subject will be provided.

The Investigator can withdraw a subject if, in his or her clinical judgment, it is in the best interest of the subject or if the subject cannot comply with the protocol. The PI may discontinue the subject’s activity without the subject’s consent if any of these criteria are met:

- The discovery or development of any health condition with a subject that would make his/her continued participation in the protocol dangerous to himself/herself.
- The failure of the subject to comply with the requirements of the protocol.
- Any significant finding that, in the opinion of the Investigator, would increase the risk of the subject having an adverse outcome from further participation in the study.

4.6.1. When and How to Withdraw Subjects

A subject may end his or her participation in the study at any time. If a subject withdraws, the Investigator will make a reasonable effort to determine the reason for the withdrawal from the study and to complete a final follow-up visit following the outline for the next scheduled visit as described in Section 5.1.3. Telephone calls, registered letters, and email correspondence are considered reasonable effort. For subjects leaving the study, a targeted examination may be performed, if medically indicated and if permitted by the subject.

Subjects who are withdrawn for SAE/AEs will be clearly distinguished from participants who are withdrawn for other reasons. Information relevant to the withdrawal will be documented in the appropriate section of the eCRFs. The Investigator will document whether the decision to withdraw from the study was made by the subject or the Investigator and which of the following possible reasons was responsible for withdrawal: SAE, Non-serious AE, Protocol violation (e.g., poor compliance with study visits), Consent withdrawal, not due to an AE, Moved from the study area, Lost to follow-up, Other (specify). If a subject meets withdrawal conditions for a concomitant medication violation or noncompliance, this should clearly be stated in the source document and the study termination eCRF.

When a subject withdraws due to an AE or is withdrawn by the PI due to an AE, the SRC and the Sponsor must be notified within 48 hours (Table 5). Investigators must follow the specific policy at each institution regarding the timely reporting of AEs and SAEs to the IRB. In all cases, the PI will make a reasonable effort to complete study termination procedures.

4.6.2. Data Collected for Withdrawn Subjects

All data collected up to the time of withdrawal, including any final evaluation and lab results that may be pending at the time of withdrawal, will be reported. Likewise, any specimens collected up to the time of withdrawal, including any sample collected for storage and use in future research, will be kept and utilized as outlined in the protocol and ICF. The study termination eCRF will be completed, with the reason for withdrawal specified.

If a subject withdraws consent but does not revoke the Health Insurance Portability and Accountability Act (HIPAA) authorization, PHV will have full access to the subject's medical records, including termination visit information. If a subject revokes only the HIPAA authorization, PHV will have full access to all the subjects' medical records prior to the date and time of written revocation.

4.6.3. Replacement of Subjects

To ensure that a required minimum number of subjects (36) are available for vaccination, any subject scheduled for vaccination who is not available at time of vaccination will be replaced with a screened and eligible alternate subject. If the replacement occurs after randomization, the replacement subject will be randomized to the same treatment group. Once vaccinated, up to 3 subjects may be replaced should they be withdrawn from the study or lost to follow-up before day 29.

4.6.4. Follow-up for Withdrawn Subjects

Investigators will follow subjects who are withdrawn as a result of an SAE/AE until the event has returned to normal or stabilized, the event is otherwise explained, or the subject is lost to follow-up.

5. TREATMENT OF SUBJECTS

5.1. DETAILED DESCRIPTION OF STUDY VISITS

Note: If a subject is not able to visit the study site for scheduled visits or due to transient circumstances not precluding continuation in the study, they are asked to contact the study site by telephone immediately. AE and concomitant medication details may be collected during a telephone call. It may not be possible to collect all clinical laboratory samples or conduct clinical assessments during this time, but the study site is asked to make every effort to collect and record all possible available information designated by the protocol. If alternative arrangements can be agreed for the sample collection or clinical assessments, the details should be documented.

5.1.1. Screening Visit

Visit 1/Screening (days -28 to -1)

Reconfirm interest in this study before starting; significant time and travel is required in the first month and multiple resources are committed by the site and Sponsor to each subject.

Screening and enrollment procedures will be the same for all potential subjects. Any potential subject falling outside the 28-day window will require rescreening. The following procedures will be performed during the screening/briefing visit:

- Explain the study and review the informed consent with subject. Obtain written informed consent for participation in the clinical study and for HIV testing. Provide a copy of the signed ICF to the subject.
- Collect demographic information and medical history (including concomitant medications).
- Check each inclusion and exclusion criterion.
- Weight, vital signs, and physical examination, including baseline neurologic examination
- Sample collection including:
  - Complete blood count (CBC) with WBC differential
  - Coagulopathy tests, including prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio [INR]
  - Serum chemistry metabolic panel, including at a minimum creatinine, glucose, AST, ALT, and total bilirubin (plus direct bilirubin if elevated)
  - Pregnancy test (serum) (all female subjects who may become pregnant – see below)
  - Urinalysis
  - Viral serologies (HIV-1, HIV-2, hepatitis C [HCV], hepatitis B surface antigen [HBsAg])

Potential subjects will be notified of their eligibility as soon as laboratory results are available and an assessment can be made by the Investigators. If determined to be eligible, subjects will be scheduled for their initial vaccination (day 1, Visit 2).

Regardless of eligibility, individuals will be informed of any significantly abnormal test results in an expeditious manner via telephone, in person, or by written communication. Appropriate counseling will be given regarding necessary medical follow-up. Participants may rescreen once.

5.1.2. Treatment Visit

Visit 2/Enrollment and Injection (day 1)

Subjects will have only 1 dosing visit during the study. Each subject will receive a 1 mL IM injection of PHV01 or placebo into the outer aspect of the subject's upper arm (deltoid area).

The following procedures will be performed during the injection visit, prior to the injection:

- Vital signs check
- Review of inclusion/exclusion criteria to confirm eligibility, then randomize
- Review of any AEs (including concomitant medications) since previous visit
- Directed physical examination (including proposed injection site)
- Rapid antigen test for SARS-CoV-2
- Urine pregnancy test (females only)
- Human leukocyte antigen (HLA) B27 typing (once only)
- Hematology, chemistries, and coagulation tests as above
- Collection of baseline samples for:
  - PHV01 antibody, including sera for rheumatology and dermatology assessments
  - PBMCs
  - Transcriptomics
  - Vaccinemia
  - Cytokines levels

Injection Procedures

- Administration of PHV01 or placebo
- Provision of emergency contact card
- Post-injection observation period: examination of injection site and vital signs check (≥60 minutes and ≤90 minutes post-vaccination)
- Explanation of the Memory Aid (see Section 5.4), distribution of thermometer and ruler, and review of use. Subjects are to record the severity of symptoms, including injection site reactions, joint-related AEs (joint pain, pain on motion of joints, joint swelling, joint tenderness), neurological issues, and rash, with the concomitant medications taken, and body temperature.

Subjects will remain in the clinic on day 1 for lunch and a blood draw at 6h ± 1h, including;

- Vital signs check
- Review of any immediate AEs or change in health status
- Collection of samples for vaccinemia, shedding, and transcriptomics

5.1.3. Follow-Up Visits

Subjects will be followed closely in the first month in the clinic, then at 3 and 6 months as outlined below. They will use a Memory Aid with a list of graded solicited symptoms, including injection site reactions, self-recorded body temperature, and concomitant medications to help record any issues. There is space for unsolicited symptoms as well. This should be filled out daily until day 14, then 3 times a week until day 29, then once a week until day 181. Subjects should pay special attention to neurological symptoms, joint-related symptoms (joint pain, pain on motion of joints, joint swelling, joint tenderness) and rash.

If an AE of post-vaccination arthritis or rash is reported, the appropriate evaluations will be triggered as outlined in Section 6.2 and Section 6.3, respectively. Always swab an open skin lesion or aspirate vesicular fluid if present, along with oral mucosa. Swabs should be placed in Viral Transport Medium and frozen at < -60ºC. The following procedures will be performed during each follow-up visit:

Visits 3-6/Interim Evaluation (days 2, 4, 8, and 15)

- Vital signs check
- Review of subject's AEs, concomitant medications, and any change in health status since previous visit including joint or neurological symptoms and rash
- Perform directed history and physical, including neurologic examination at discretion of the Investigator to assess possible AEs. Document abnormalities, including neurological, joint, or skin exam, signs if symptoms are present. Photograph rash if present.
- Hematology, chemistries, coagulation tests, and urinalysis as above (days 4 and 8 only)
- Collection of samples for:
  - PHV01 antibody (day 8 and 15 only)
  - PBMCs (day 8 only)
  - Transcriptomics at each visit
  - Vaccinemia and shedding at each visit
  - Cytokine levels (days 4 and 8 only)
- At the day 4 visit, please remind subjects to call the site if they have any joint-related AEs, rash or neurologic complaints.

Visit 7/Final Acute Evaluation (day 29)

- Vital signs check
- Review Memory Aid for days 1-29
- Review of subject's AEs, concomitant medications, and any change in health status since previous visit including joint or neurological symptoms and rash
- Perform directed history and physical, as well as a neurologic examination. Document joint and skin exam if symptoms present. Photograph rash if present.
- Hematology, chemistries, coagulation tests, and urinalysis as above (days 4 and 8 only)
- Urine pregnancy test (females only)

- Collection of samples for:
  - PHV01 antibody
  - PBMCs
  - Vaccinemia and shedding
  - Cytokine levels

Visit 8/3-month Follow up (day 85)

- Vital signs check
- Review Memory Aid for days 29-85, including any unsolicited symptoms
- Review of subject's AEs, concomitant medications, MAAEs, SAEs, AESIs, and change in health status since previous visit
- Perform directed history and physical
- Collection of samples for:
  - PHV01 antibody
  - Blood for research (100 mL)

Visit 9/6-month Follow up (day 181)

- Vital signs check
- Review Memory Aid for days 85-181, including any unsolicited symptoms
- Review of subject's AEs, concomitant medications, MAAEs, SAEs, AESIs, and change in health status since previous visit
- Perform directed history and physical
- Collection of samples for:
  - PHV01 antibody

Visit 9 (day 181) will be the End of Study (EoS) visit for all subjects. Subjects completing this visit will be considered to have completed the study. Subjects with an ongoing AE at this visit will be followed until the event has returned to normal or stabilized, the event is otherwise explained, or the subject is lost to follow-up.

5.1.4. Early Termination Visits

Subjects who withdraw from the study for any reason between scheduled study visits will be asked to complete assessments for the next scheduled visit (at least 28 days after dosing) as their EoS Visit.

5.1.5. Biological Samples

Samples collected under this protocol will be used to conduct protocol-related safety and immunogenicity evaluations related to the vaccine. Immunological testing will be performed at a specialty laboratory that does not have access to the randomization code group assignment.

All specimens for clinical laboratory testing will be collected at the site and processed by the site. The amount of blood to be drawn, by visit, is provided in the study-specific laboratory manual and in Table 3.

All samples will be collected using standard techniques and safety precautions. To maintain clarity and confidentiality, specimens will be labeled with at least the collection date and the applicable study ID code for the subject. Labels may be in barcode format or handwritten.

If necessary, biological samples will be stored temporarily at the site to await transport to appropriate laboratories for processing and analysis. Transport and storage of these biological samples will be handled according to applicable SOPs/SSPs and instructions in the Sponsor-approved laboratory manual.

Any study for the future use of these biological samples that are not identified in this protocol shall have IRB approval. In addition, a subject may decide at any point to withdraw consent for the future use of his/her samples. Should a subject withdraw consent for the use of his/her samples, any unused samples will be destroyed. All other study subject samples will be transported to PHV or designee and stored indefinitely. Samples will be stored in a locked, controlled-access freezer.

5.2. CONCOMITANT MEDICATIONS

The restrictions on the use of concomitant medications by subjects apply through day 29 only and include immunosuppressive medications, including oral or injected corticosteroids.

Drugs that are used to reduce fever and pain are generally not allowed 24 hours before injection or 24 hours after injection. This protocol places no restrictions on rescue medications, and the Investigator will recommend medication for symptomatic relief if necessary.

5.3. CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

All subjects must be willing to minimize blood and body fluid exposure of others until six or eight weeks after dosing by:

- Using effective barrier prophylaxis, such as latex condoms during penetrative sexual intercourse or oral sex with opposite-sex or same-sex partners (until eight weeks after dosing)
- Abstaining from sexual activity, including oral sex, if effective barrier prophylaxis cannot be used (until eight weeks after dosing)
- Avoiding the sharing of needles, razors, or toothbrushes (until six weeks after dosing)
- Avoiding open-mouth kissing (until six weeks after dosing)
- Covering of any open-skin lesions (until six weeks after dosing)

5.3.1. Woman of Childbearing Potential

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below). If fertility is unclear (e.g., amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Premenarchal or premenopausal women with one of the following surgical histories are not considered to be women of childbearing potential:

- Documented hysterectomy
- Documented bilateral salpingectomy
- Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (e.g., mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry. *Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.*

5.3.2. Postmenopausal female

A postmenopausal state is defined as having no menses for 12 months without an alternative medical cause. This can be documented by:

- A high follicle stimulating hormone level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, confirmation with more than 1 FSH measurement (> 35 IU/L or mIU/mL) is required.
- Females on hormone replacement therapy and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue hormone replacement therapy during the study. Otherwise, they must discontinue hormone replacement therapy to allow confirmation of postmenopausal status before study enrollment.

5.3.3. Contraception Guidance

If a subject is sexually active or becomes active, then she and her male partner must use a male or female condom in addition to one of the following effective methods of adequate contraception until eight weeks after dosing. Highly effective birth control includes the following methods:

- Coil (intrauterine device)
- Oral contraceptive pill containing combined estrogen and progesterone
- Depot progesterone injections
- Progesterone implant
- Vaginal ring
- Transdermal patch containing combined estrogen and progesterone

5.3.4. Collection of Pregnancy Information

5.3.4.1. Male participants with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in the first 29 days of this study.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy.
- The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than two months following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

5.3.4.2. Female participants who become pregnant

- The Investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The initial information will be recorded on the Pregnancy Report Form and submitted to the Sponsor within 24 hours of learning of a participant's pregnancy.
- The participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than twelve months beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE (Section 6.7).
- A spontaneous abortion (occurring at < 22 weeks gestational age) or still birth (occurring at > 22 weeks gestational age) is always considered to be an SAE and will be reported as such.
- Any poststudy pregnancy related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.
- Details of all pregnancies in female participants and, if indicated, female partners of male participants will be collected after the start of study intervention and until EOS or ET.
- If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in this section.
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs. Therefore, they follow the same reporting requirements.

5.4. SUBJECT MEMORY AID COMPLETION

Memory Aids will be provided to subjects following the injection and subjects will be instructed in their use. On a daily basis during the 14-day period after the injection, then 3 times a week until day 29, then once a week until the end of the study, subjects will evaluate, record, and grade any local injection-site reactions (e.g., redness, swelling, tenderness, or pain), any general symptoms (e.g., fever, chills, sweat, myalgia, arthralgia, fatigue, skin or mucosal lesions, or gastrointestinal symptoms), and any neurological signs and symptoms (blurry vision, changes in balance or coordination, confusion, disorientation, dizziness, severe headache, shaking or body tremors, slurred speech, tingling or numbness, weakness or feeling weak). These types of neurological symptoms should be evaluated within 24 hours. To more accurately account for the size of any local reactions, a ruler will be given to the subjects, and they will be instructed on how to use it. To more accurately account for possible fevers, subjects will be given an oral thermometer, instructed on its use, and asked to take and record their temperature on the same schedule.

Memory Aids will be reviewed by site staff at each follow-up visit during the post-injection period. If Memory Aids are not completed or returned, the Investigator will review the symptoms that occurred during that timeframe to the best of the subject's recollection to determine AEs and the subject will be retrained.

Although subjects will be instructed on the use of the Memory Aid and they are encouraged to fill it out as directed, although if it is not completed it will not be considered a protocol deviation. The information, if available, will be reviewed with the subject, and any positive responses confirmed. There will be three distinct Memory Aid periods:

5.4.1. Memory Aid / Period 1: Days 1-29

The Memory Aid for days 1-29 will ask subjects to record and grade the severity of local and systemic symptoms; grade and measure local injection-site reactions (redness, swelling, tenderness, and pain); body temperature; and concomitant medications. The Memory Aid for Days 16-29 will ask subjects to record any symptoms of arthritis (joint aches/pain [general or while moving joints] or joint swelling), and location of affected joint(s), as well as mouth ulcers, mucosal lesions, or dermatologic reactions such as rash, and neurologic symptoms.

The information will be reviewed with the subject and any positive responses confirmed. The Memory Aid includes a scripted section for solicited symptoms and a general question to elicit any unforeseen symptoms. If a symptom is reported by telephone that makes a personal contact desirable, an unscheduled optional visit can be performed at any time. Subjects who develop arthritic events or rash will be followed up until resolution of the AE.

5.4.2. Memory Aid / Period 2 and 3: Days 29-85, 85-181

The Memory Aid for days 29-85 and 85-181 will ask subjects to record and grade symptoms, MAAEs, SAEs, any important changes in health status, along with concomitant medications. All subjects will be followed until at least day 181. Subjects who develop arthritic events or rash requiring evaluation will be followed up until resolution of the AE. The information, if available on the Memory Aid, will be reviewed with the subject, and any issues considered to be AEs will be transcribed into the source documents for inclusion in the eCRFs.

5.5. SAFETY LABORATORY AND IMMUNOGENICITY ASSESSMENTS

5.5.1. Safety Laboratory Assessments

Safety laboratory assessments will be performed by the site's local laboratory. If an AE on day 5 or later post-vaccination of arthritis or petechial/purpuric or vesicular rash is reported at any follow-up visit, additional blood (i.e., chemistries and rheumatology panel) and urine will be obtained from the subject for laboratory testing. Repeat laboratory testing will be conducted as outlined in Section 6.1 and graded according to APPENDIX 1. Details regarding collection and processing of subject samples will be provided in the Study Procedure Manual.

Subjects will have blood, saliva, and urine assessed for evidence of rVSV via RT-qPCR. Plasma and urine samples for rVSV PCR will be collected and stored frozen until they are transferred for analysis. Saliva samples will be collected with an oral swab, placed in storage reagent, and stored frozen pending transport.

5.5.2. Blood for Immunogenicity Assessment and Vaccinemia

A blood sample for antibody tests will be drawn to obtain the minimum required serum volume for immunological assays and vaccinemia determinations (Table 3). All sample analysis will be performed at a laboratory designated by PHV.

Detailed instructions for separating serum and plasma, labeling, storage, and shipping of serum samples are included in the Laboratory Manual, provided separately. This will include the collection of blood (100 mL) for large volume serum on day 85.

5.5.3. Blood for Investigation of Post-Vaccination Arthritis and Post-Vaccination Rash (Rheumatology Panel)

In the event of a case diagnosed as post-vaccination arthralgia/arthritis or petechial, purpuric, or vesicular rash occurring on or after day 5, specific samples of blood are drawn at time of diagnosis. In addition, these same samples may be drawn more frequently as needed, depending upon symptoms, and will be run only in the event of the occurrence of either event, according to specific guidelines in Figure 2 and Figure 3.

5.6. METHODS/TIMING FOR ASSESSING, RECORDING, AND ANALYZING IMMUNOGENICITY MEASUREMENTS

5.6.1. Determining Antibody Titers of Study Subjects

Sera for immunogenicity will be collected as outlined in the Schedule of Events (Table 2).

5.6.2. Immunogenicity Measurement

Anti-PHV01 IgG titers will be determined by conventional ELISA utilizing recombinant Marburg GP as the antigen. Anti-PHV01 neutralizing antibodies will be determined as measured by PsVNA.

5.6.3. Exploratory Measurements

There are multiple exploratory endpoints related to the immunological response to the vaccine. At the discretion of the Sponsor, additional immunology tests may be performed for exploratory purposes:

- Wild-type Marburg neutralization against one or more Marburg lineages
- Marburg ELISA against one or more Marburg lineages
- Determination of Fc-mediated cell-targeting antibodies, e.g., ADCC, ADNP, ADMP, etc.
- T-cell responses to Marburg GP protein by intracellular cytokine staining (ICS) and/or enzyme-linked immune absorbent spot (ELISPOT)
- Determination of Marburg IgM ELISA antibody responses
- Determination of pro-inflammatory markers, such as CRP, ICAM-1, VCAM-1 levels, and cytokine levels
- Determination of gene activation by RNA transcript sequencing (transcriptomics)
- Determination of B or T cell responses to VSV proteins

5.6.4. Vaccinemia and Shedding

Vaccinemia and shedding will be determined by PHV01 RT-qPCR assay using primer-probe set(s) specific for the vaccine vector VSV.

6. SAFETY ASSESSMENT

Safety monitoring will be conducted throughout the study; therefore, safety concerns will be identified by continuous review of the data by the PI, clinic staff, Medical Monitor, and Sponsor.

An SRC will be chartered by the Sponsor to monitor human subject safety during the study and will review any safety concern. Significant issues will be conveyed to the IRB.

6.1. ASSESSMENT OF SAFETY

All AEs will be assessed as described in Section 6.4 according to the FDA document "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" (37), using toxicity grading tables excerpted from that guidance shown in APPENDIX 1: Clinical and Laboratory Toxicity Grading , Tables A1-A3. Safety will be assessed by evaluating reactogenicity (local reactions and systemic reactions) and the occurrence of post-vaccination solicited local reactions and systemic AEs through day 15. Solicited AEs for days 16-29 are limited to instances of neurological signs and symptoms, arthritis, and rash. Arthritis, rash, and neurologic events should be evaluated throughout the study and may include expert consultation.

Grading scales for laboratory value abnormalities are shown in APPENDIX 1: Clinical and Laboratory Toxicity Grading, Tables A4 and A5. Local laboratory normal ranges will be used to classify laboratory results. Clinically significant abnormal laboratory values will be graded as AEs according to the ranges specified in the table.

All vaccine-related SAEs must be reported as soon as possible following confirmation. The toxicity grading tables should be followed when determining the severity of an SAE.

Signs and symptoms of an AESI, as listed in Section 6.4.3, will be assessed throughout the study. If neurologic symptoms are present, a neurologic examination should be performed and documented. Neurologic examinations will also be performed at baseline and day 29.

The nature, frequency, and severity of MAAEs and SAEs from the time of injection through the final study visit should be recorded for each subject. MAAEs are defined as AEs leading to medically-attended visits that were not a routine visit for physical examination or vaccination, such as an emergency room visit or an otherwise unscheduled visit to or from medical personnel (medical doctor) for any reason.

6.2. ASSESSMENT OF SUBJECTS WITH JOINT-RELATED ADVERSE EVENTS

In the event of cases of acute aseptic arthritis, data collection, analysis, and presentation should be performed in accordance with Brighton collaboration guidelines (45) and may require expert consultation.

Subjects who have joint pain on or after day 6 following administration of vaccine will undergo assessment by the PI within 72 hours of onset with a targeted exam, guided by the Arthritis Pathway titled "Evaluation of Subjects with Joint Signs and Symptoms" (Figure 2). In brief:

- The subject self-monitors for joint pain or rash. The subject should identify the primary source of pain and any other affected joints.

- Determine if findings of arthritis are present, defined as any 1 of the following:
  - Clinically significant reduction in range of motion
  - Synovitis (tenderness or swelling or redness or heat)
  - Effusion
- Document findings using the standardized form.
- If arthritis present, draw a CBC, serum chemistries (including BUN, creatinine, and uric acid), PT/PTT, fibrinogen, a rheumatology panel (CRP, RF, anti-CCP, C3, C4, ANA, anti-CCP, sample for cytokine levels), and obtain a urinalysis.

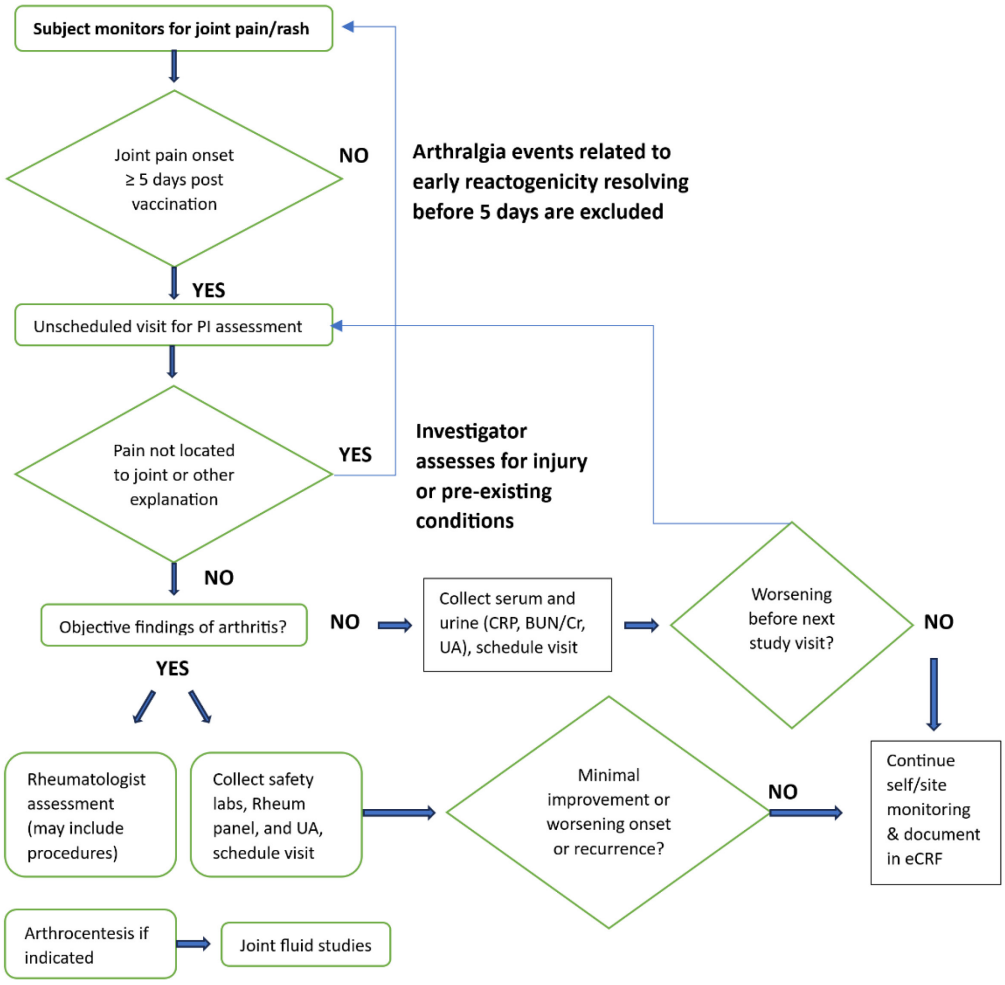
If arthritis is present, the subject should be assessed either by a Physician Investigator who is proficient in arthrocentesis or a consultant. If effusion is confirmed, consent should be obtained for the subject to undergo arthrocentesis for joint fluid assessment.

- The synovial fluid assessment should include:
  - Cell count with differential
  - Crystal examination
  - Culture for bacteria, and virus isolation
  - RT-qPCR for vaccine viral RNA

Follow-up is determined by clinical course. If symptoms are mild (Grade 1), follow up as per Schedule of Events: days 8, 15, and 29, then monthly until resolved. If at day 29 the ongoing symptoms are Grade 2 or worse, follow weekly until mild (Grade 1) and then schedule as above. Repeat BUN, creatinine, uric acid, and rheumatology panel, and obtain urinalysis at follow up visits until resolved. The Investigator may evaluate the subject more frequently if clinically indicated.

If rash is present on any visit, this triggers the Dermatology Pathway, as outlined in Section 6.3.

Figure 2: Evaluation of Subjects with Joint Signs and Symptoms



6.3. ASSESSMENT OF SUBJECTS WITH DERMATOLOGIC ADVERSE EVENTS

In the event of rash, including mucosal involvement, evaluation, data collection, and analysis will be performed in accordance with Brighton collaboration guidelines (46). Any new rash will be investigated and reported by the PI within 72 hours of onset.

Subjects who develop a rash following administration of vaccine will undergo assessment by a Physician Investigator at each site. The Investigator's assessment will be guided by the "Evaluation and Work-Up of Adverse Event of Petechial/Purpuric/Vesicular Type Rash" (Figure 3). In brief:

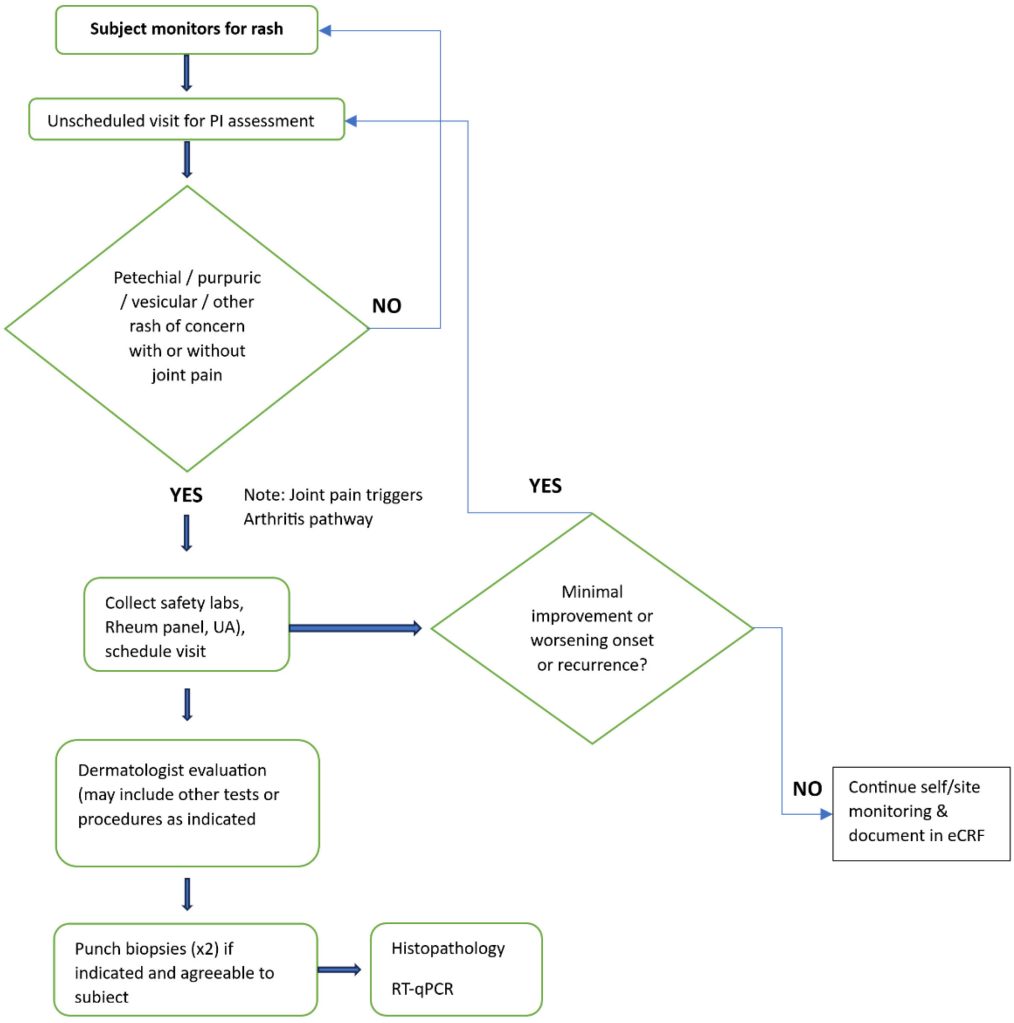
- Subject self-monitors for rash or mucosal lesions with onset on or after day 5.
- Physician Investigator assesses skin to determine if significant findings are present:
  - Petechiae, Purpura, and/or Vesicles
  - Mucosal lesions
  - Urticaria
  - Other

- Document the examination in the eCRF and photograph the lesions.
- If petechial, purpuric, or vesicular rash is present, draw CBC, serum chemistries (BUN, creatinine, uric acid), PT/PTT, fibrinogen, and rheumatology panel (CRP, RFF, anti-CCP, C3, C4, ANA, and a sample for cytokines), and obtain urinalysis.
    - If the subject is amenable, the Physician Investigator should obtain informed consent and perform 1-2 punch biopsies. Portions of the biopsy should be Formalin fixed and stained for H&E, immune complex deposition, and immune-stained for VSV and/or MARV GP.
    - Placed into RNA Later for processing to do RT-qPCR (see Laboratory Manual).
- Refer to Dermatologist for additional specialty consultation within 72 hours if possible.

Follow-up is determined by clinical course. If symptoms are mild (Grade 1) and participant stable or improving, follow up per SoE, i.e., days 8, 15, and 29, then monthly until resolved. If at day 29, ongoing symptoms are moderate (Grade 2) or worse, follow weekly until mild and then schedule as above. The Investigator will evaluate the subject more frequently if clinically indicated.

If arthralgia/arthritis is present on any visit on or after day 5 this triggers the Arthralgia Pathway as outlined in Section 6.2.

Figure 3: Evaluation and Work-Up of Adverse Events of All Rashes of Concern



6.4. IND SAFETY REPORTING

The terms described below, as defined by 21 Code of Federal Regulation (CFR) Part 312.32, apply to IND safety reporting.

6.4.1. Adverse Event or Suspected Adverse Reaction

An AE is defined as any untoward medical occurrence associated with the use of a pharmaceutical product in humans, whether considered product-related or not. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, regardless of whether it is related to the medicinal (investigational) product. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions.

A suspected adverse reaction is defined as any AE for which there is a reasonable possibility that the product caused the AE. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the product and the AE. "Suspected adverse reaction" implies less certainty about causality than "adverse reaction", which

means any AE caused by a product. An AE is any adverse change or exacerbation from a baseline condition which occurs following the initial administration of an IP, whether or not the event is considered to be related to the IP. Examples of this include but are not limited to the following:

- Adverse changes, including new signs and symptoms, intercurrent illness modifying the clinical course, or the worsening of a baseline condition, including the increased frequency of an event or increased intensity of a condition.
- Concomitant disease with onset or increased severity after the start of study product administration.
- A new pattern in a pre-existing condition, occurring after the receipt of IP, that may signal a clinically meaningful change.
- Any abnormal laboratory value, whether clinically significant or not.

All AEs will be monitored until resolution or, if the AE becomes chronic, a cause is identified. If an AE is unresolved at the conclusion of the study, a clinical assessment will be made by the Investigator or Medical Monitor whether continued follow-up of the AE is warranted. The severity of events will be determined by the Investigator as described in Section 6.5.

The Investigator must assign a relationship of each AE to the receipt of the IP. The Investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the IP, and identification of possible alternate etiologies including underlying disease, concurrent illness, or concomitant medications. The following guidelines should be used by Investigators to assess the relationship of an AE to study product administration. **ONLY A LICENSED PHYSICIAN CAN MAKE THIS DETERMINATION.**

Not related: No relationship to IP. Applies to those events for which evidence exists that there is an alternate etiology.

Possible: An association between the event and the administration of IP cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the subject's clinical status or underlying factors including other therapy.

Probable: There is a high degree of certainty that a relationship to the IP exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the subject's clinical state or factors including other therapy.

Definite: An association exists between the receipt of IP and the event. An association to other factors has been ruled out.

6.4.2. Solicited Adverse Events

A solicited AE is a predetermined event which may reflect safety concerns related to the IP. The solicited AEs for this study include:

Local Events:

- Redness, swelling, or pain at site of injection

Systemic Events:

- Subjective or objective fever
- Chills
- Sweats
- Myalgia (described to the subject as generalized muscle aches)
- Fatigue
- Gastrointestinal symptoms (nausea, vomiting, abdominal pain, and/or diarrhea)

Rheumatologic Events:

- Arthralgia (described to the subject as generalized joint aches/pain [general or while moving joints]); affected joints will be captured
- Joint swelling
- Joint tenderness

Dermatologic Events:

- Mouth ulcer
- Skin or mucosal lesion (rash, blister, ulcer)

Neurologic Events:

- Blurry vision, changes in balance or coordination, confusion, disorientation, dizziness, severe headache, shaking or body tremors, slurred speech, tingling or numbness, weakness or feeling weak

6.4.3. Adverse Events of Special Interest

The following are AESIs that will be monitored with increased frequency for safety purposes. AESIs that meet the serious criteria as defined in Section 6.4.4 will be reported as SAEs.

Respiratory Events:

- Acute respiratory distress syndrome/pneumonitis

Neurologic Events:

- ADEM
- Aseptic meningitis
- Encephalitis
- Generalized convulsions
- GBS
- Myelitis
  - Optic neuritis
  - Transverse myelitis

Hematologic Events:

- Thrombocytopenia ≥ Grade 2

Immunologic Events:

- Anaphylaxis
- Vasculitides

6.4.4. Serious Adverse Event or Serious Suspected Adverse Reaction

An AE or suspected adverse reaction is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE, defined as any adverse drug experience that, in the opinion of the Investigator, places the subject at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity defined as a "substantial disruption of the ability to conduct normal life functions"
- Congenital anomaly/birth defect (in the offspring of a subject)

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization; or the development of drug dependency or drug abuse.

6.4.5. Unexpected Adverse Events or Unexpected Suspected Adverse Reactions

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure (IB) or is not listed at the specificity or severity that has been observed. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the IB referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the IB listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of biologics or as anticipated from the pharmacological properties of the biologic but are not specifically mentioned as occurring with the particular biologic under investigation.

6.4.6. Unanticipated Problems Involving Risks to Subjects or Others

Unanticipated problems involving risks to subjects or others must be reported to the IRB within 24 hours. These events encompass a broader category of events than SAEs and may include issues

such as problems with loss of control of subject data or the IP; adverse psychological reactions; or breach of confidentiality. Risks to others (e.g., program personnel) must also be reported.

Unanticipated problems involving risks to subjects or others are any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the procedures that are described in the protocol, IB or ICF, and (b) the characteristics of the subject population.
- Related or possibly related to a subject's participation in the study.
- Suggests that the study places subjects or others at a greater risk of harm than was previously known or recognized.

The IRB will evaluate the PI's and Medical Monitor's reports to determine whether a given incident, experience, or outcome constitutes an unanticipated problem involving risk to subjects or others and, in coordination with the Sponsor, will ensure upward reporting of the unanticipated problems involving risk to subjects or others to the appropriate regulatory offices.

6.5. SEVERITY ASSESSMENT

All AEs will be assessed for severity by the Investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event, including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or life-threatening. Refer to the grading scale in APPENDIX 1 for further guidance in the assignment of severity. Any Grade 4 (life-threatening) AE must be reported as an SAE.

The eCRF for AEs will reflect only the highest severity for continuous days an event occurred. The following criteria below may be used for any symptom not included in the grading scale:

Mild (Grade 1): Does not interfere with routine activities; minimal level of discomfort.

Moderate (Grade 2): Interferes with routine activities; moderate level of discomfort.

Severe (Grade 3): Unable to perform routine activities; significant level of discomfort.

Potentially life-threatening (Grade 4): Hospitalization or ER visit for potentially life-threatening event.

The FDA guidelines for toxicity will be followed; however, if a subject is evaluated in an emergency room for nonlife threatening illness or symptoms (i.e., visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed and severity of the AE will be assessed according to the subject's clinical signs and symptoms.

As defined by the ICH guideline for GCP, the term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is **not** the same as "serious", which is based on subject/event **outcome** or **action** criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

6.6. RECORDING ADVERSE EVENTS

The PI will report all AEs occurring from time of ICF signature to the Sponsor's representative/ designee (as noted in) and the IRB in the appropriate safety, annual, and/or final reports. The study site will provide data files to the Sponsor's representative for preparation of annual and final reports to the FDA.

6.6.1. Methods/Timing for Assessing, Recording, and Analyzing Safety Endpoints

AEs, MAAEs, and SAEs will be assessed at all study visits, documented in the source records, and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event.

Solicited and unsolicited AEs will be reviewed within the 29 days after vaccination, utilizing the provided study Memory Aids. For unsolicited AEs, when a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The Investigator will assess all AEs for seriousness, relationship to IP, severity, and other possible etiologies. When an event has not resolved by study closure, it will be documented on the AE eCRF as "ongoing".

The Memory Aid from days 29-181 (Memory Aid 2 and 3) will ask subjects to record and grade changes in health status, along with all MAAEs, and to record concomitant medications.

In the follow-up period, subject-reported changes in health status, either by Memory Aid or at a visit, will be evaluated by the Investigator and reported as a change in health status if the following criteria are met:

- New medical condition (e.g., not present at baseline)
- Considered clinically significant in that the event requires an ongoing change in the subject's medical management and an important change in the subject's long-term health status.

When both criteria are met, the condition will be reported as an unsolicited AE.

6.6.2. Duration of Follow-Up of Subjects after Adverse Events

Investigators are required to follow SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will not become chronic. The SAE outcomes will be reported to the Sponsor's representative using the Serious Adverse Event Report Form.

Investigators are not obligated to actively seek SAEs in former subjects; however, if an SAE considered to be related to the IP is brought to the attention of the Investigator *at any time* following completion of the study, the event will be reported to the Sponsor (Section 6.7).

6.7. REPORTING ADVERSE EVENTS

The PI will report all AEs to the Sponsor and the IRB in the appropriate safety, annual, and/or final reports. SAEs, MAAEs, and AEs for inclusion in annual and final reports to the FDA will be provided from the clinical database by the Sponsor's clinical data manager. The study site will provide data files to the Sponsor's representative for preparation of annual and final reports to the FDA.

6.7.1. Reporting Serious and Serious Unexpected Suspected Adverse Reactions, and Unanticipated Problems Involving Risks to Subjects or Others

Contact information for reporting SAEs is provided in Table 5. All notification will be provided to the Sponsor's safety office.

All SAEs and serious unexpected suspected adverse reactions (SUSARS) must be reported promptly (within 24 hours) to the Sponsor's representative/designee as per 21 CFR Part 312.64, regardless of whether the event is considered related to study product. Further, the Investigator should comply with relevant study site SOPs on reporting SAEs, to include reporting to the IRB.

The minimum information that the Investigator will provide is specified in Table 6. The Sponsor's representative/designee may request additional information for purposes of the study.

Table 5: Study Contacts for Reporting SAEs and Unanticipated Problems Involving Risk to Subjects or Others

Priority List	
Veristat Safety Line	Safety Phone: 1-888-662-0657 (+1-416-620-2200) Safety Fax: 1-888-662-0647 (+1-416-620-2205) Safety Email: pharmacovigilance@veristat.com
CRO Medical Monitor (primary) and Sponsor's Safety Officer	Rachel Abu Taleb, MD 24-hr Emergency Phone: +1-613-203-6623 Email: rachel.abu.taleb@veristat.com
Sponsor CMO (backup)	Richard Kenney, MD Cell: +1-415-741-6990 Email: rkenney@phvaccines.com

Table 6: SAE Information to Be Reported to the Sponsor

Notification Method	Information to be Provided
Email or Telephone (within 24 hours)	IND number, Sponsor study number, name of the IP, and Investigator name and contact number Subject identification number SAE, onset date, date of IP administration, severity, relationship, and subject's current status
AND	
Email or Fax	Cover sheet or letter Adverse event case report form Serious AE report form Concomitant medication case report form or a list of concomitant medications Medical record progress notes including pertinent laboratory/diagnostic test results
NOTE: When submitting SAE reports via email, the subject line of each email should read as follows: SAFETY REPORT – IND # _____, Sponsor Study #_____, Subject# _____, Event term: _____	

Abbreviations: IND = investigational new drug; SAE = serious adverse event

In order to comply with regulations mandating Sponsor notification of specified SAEs to the FDA within 7 calendar days, Investigators must submit additional information as soon as it is available. The Sponsor's representative/designee will report unexpected SAEs associated with the use of the drug to the FDA as specified in 21 CFR Part 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy regarding the timely reporting of SAEs to the Medical Monitor.

Reporting to the Sponsor's safety office does not fulfill the Investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The PI will notify the IRB and the Medical Monitor.

Reporting to the IRB:

Unanticipated problems involving risk to subjects or others, SAEs related to participation in the study, and all subject deaths related to participation in the study should be promptly reported (within 24 hours) by telephone, email, or fax to the IRB. A complete written report with information regarding follow-up examinations required (if any) will follow the initial notification within 10 working days. All unanticipated problems and SAEs occurring within the reporting period should also be summarized in the continuing review reports submitted to the IRB.

Investigators are required to forward safety information provided by the Sponsor's representative to the IRB.

6.7.2. Reporting Additional Immediately Reportable Events

6.7.2.1. Pregnancy

Each pregnancy must be reported *immediately* **(within 48 hours of identification)** by email or telephone to the Sponsor and to the IRB.

Subjects, or their partners, who become pregnant after day 1 will be followed to term, and information will be gathered for outcome, date of delivery, and health status of the mother and child including the child's gender, height, and weight. Complications and/or abnormalities should be reported, including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the IP may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

6.7.2.2. AE-Related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported *immediately* **(within 72 hours of identification)** by email or telephone to the Sponsor's representative/designee. Report the withdrawal to the IRB in accordance with IRB policy.

6.7.2.3. Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, Form FDA 483, warning letters, or actions taken by any regulatory agency, including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements, will be reported immediately to the IRB and the Sponsor's representative/designee.

6.7.3. IND Annual Report to the FDA

The Sponsor is responsible for the preparation of a detailed annual synopsis of clinical activity, including AEs, for submission to regulatory agencies. Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date.

6.7.4. Final Report

A final CSR will be prepared in accordance with FDA's "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications" (Sept 1999) and ICH E3 Guideline "Structure and Content of Clinical Study Reports" (July 1996) and provided to the Sponsor's representative/designee for review and approval. The Sponsor's representative/designee will use this report to prepare the final CSR for submission to the FDA.

The PI will report all AEs to the Sponsor or delegated authority and the IRB in the appropriate safety, annual, and/or final reports. After appropriate data cleaning and query resolution between the clinical site, Sponsor's Clinical Monitor, and clinical data manager, SAEs from the clinical database will be reconciled with the Sponsor's SAE database. SAEs and AEs for inclusion in annual and final reports to the FDA will be provided from the clinical database by the Sponsor's clinical data manager or delegated authority.

7. STATISTICAL METHODS

An SAP, providing details about the specific planned analyses and statistical tests, will be prepared and approved by the Sponsor and its designees prior to study database lock and unblinding of the single-blind subject treatment assignments.

For safety and immunogenicity endpoints, tests will be performed comparing each dose level to the placebo group. Additional tests may be performed between dose levels to determine dose response. All statistical tests will be performed at the 2-sided 5% level of significance. No adjustments for multiple testing will be considered. No imputations will be made for missing immunogenicity data.

7.1. PLANNED ENROLLMENT AND REASON FOR SAMPLE SIZE

This is a Phase 1 study, where both descriptive and inferential statistical methods will be used to fully explore the preliminary data for planning of future studies and will define future study endpoints. This study is intended to provide initial safety, tolerability, and immunogenicity data for the vaccine candidate. In addition, immunogenicity endpoints will be evaluated for the potential of a dose-response relationship to increasing doses of the IP, including placebo as a 0-dose level.

The sample size for this Phase 1 study (N=36 subjects total: N=10 per vaccine dose level (30 vaccinees) and 6 placebo) was selected to provide initial data to obtain estimates of safety and immunogenicity parameters and their respective variabilities. Group size is based on prior experience with ERVEBO safety studies and assumptions from the responses seen in the preclinical studies. This Phase I study is therefore designed with the goal of providing further information for better planning of future in-human studies. As this is a FIH study of PHV01, no current human data exist to substantiate power or treatment effect estimates.

Safety: With a sample size of 10 subjects, an upper bound of 30.9% is established for the two-sided 95% confidence interval for the incidence of an AE in the case that the event is not observed according to the two-sided 95% (Clopper-Pearson) exact confidence interval.

Immunogenicity: Clinical data for the rVSV-ZEBOV-GP Ebola vaccine showed that a dose of $3x10^6$ pfu/mL yielded a day 29 GMT IgG ELISA titer of 1400 EU/mL and geometric standard deviation of 3.3. Assuming that the variability in responses to PHV01 is similar, the proposed group sizes afford 80% (alternate 90%) power to detect a 6.4-fold (alternate 8.6-fold) increase, comparing geometric mean ELISA titer using a two-sided test with alpha=0.05 for each vaccine group with the placebo group. Alternatively, performing the same comparison against placebo by pooling all three treatment groups with a total 30 vaccinated subjects, we would have 4.7-fold and an alternate 5.9-fold with 80% and 90% power, respectively.

7.2. ADMINISTRATIVE INTERIM ANALYSIS

The Sponsor will perform an administrative IA on cleaned and frozen data after all subjects have been vaccinated and observed through the day 29 visit to guide dose selection for a subsequent Phase 2 trial in one or more endemic countries in Africa. The IA will include laboratory measurements through day 29 of seroconversion by ELISA and PsVNA, GMT of the IgG ELISA and PsVNA, and reverse cumulative distribution curves by dose group, with descriptive statistics. Measures of safety through day 29 will include SAEs, MAAEs, AEs, and the frequency and severity of AEs including solicited and unsolicited AEs. Solicited local and

systemic AEs will be collected for days 1-15; solicited AEs for days 16-29 are limited to instances of neurological signs and symptoms, arthritis, and rash. As soon as vaccinemia, shedding, and excretion data are available, they may be added to the IA. Neurologic findings through day 29 will be summarized, including those identified during scheduled neurological examinations at Screening and day 29.

This is a single-blind trial. Summarized results, including treatment assignments of individual subjects, will be provided to the Sponsor and Regulatory Agencies as described in the Unblinding Plan. In addition, selected summarized results by treatment group may be presented at meetings with BARDA, the Phase 2 clinical study sites, and others who are engaged in making strategic decisions on further clinical development of the PHV01 vaccine. The clinical site and subjects will remain blinded until the full data set is cleaned and locked at the end of the trial.

7.3. STUDY ANALYSIS POPULATIONS: ASAT, MITT, AND PER PROTOCOL

ASaT Population: All safety analyses will be based on the population of all subjects as treated (ASaT). The ASaT population will comprise all subjects who received a partial or full dose of 1 single study injection, excluding subjects who have no on-study safety data. The subjects will be analyzed according to treatment received in case of a treatment error.

mITT Population: The mITT analysis population for the immunogenicity analysis will comprise all randomized subjects who received a single study injection and have at least 1 post-injection immunogenicity evaluation. Subjects who received the wrong study treatment(s) will be analyzed "as-treated" in the mITT analysis population.

PP Population: The per protocol (PP) analysis population consists of all randomized subjects who were dosed, have PHV01 IgG ELISA titer or PsVNA results on days 1 (baseline) and 29, and do not have any protocol violations that influence interpretation of immunogenicity endpoints.

The ASaT population is the primary population for analyses of safety endpoints. The PP population is the primary population for analyses of immunogenicity endpoints. The mITT population will be used for additional analyses of immunogenicity endpoints. Intervals for analysis, i.e., visit windows, are further defined in the Schedule of Events (Table 2).

7.4. DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographic and baseline characteristics will be listed by subject and summarized by treatment group. There will be 3 vaccine treatment groups and 1 placebo treatment group. Descriptive summary statistics (sample size, mean, median, standard deviation [SD], and range, when appropriate) will be provided for the continuous variables such as age, weight, and height. Number and percentage will be reported for categorical variables such as race and ethnicity.

7.5. SAFETY DATA

All safety data will be based on the ASaT populations. AEs will be coded using MedDRA Version 26.1 or subsequent. The vaccine treatment groups will be summarized separately.

7.5.1. Analysis Addressing the Primary Study Objective (Safety)

Solicited symptoms occurring during the 28 days following vaccination will be summarized by treatment group and by relatedness. The incidence and severity of solicited AEs to the vaccine over the 28-day follow-up period will be calculated. Presentations will include the number and percentage of subjects with at least 1 local AE, and at least 1 general (systemic) AE, as well as the incidence of each AE individually.

The number of subjects with at least 1 incidence of an unsolicited AE reported up to 28 days after vaccination will also be summarized overall and by treatment group. The severity and temporal relationship of the unsolicited AEs to vaccination will also be assessed. Presentations will also summarize unsolicited AEs by grade and vaccine-relatedness. Serious AEs occurring at any point during the trial will be summarized and relatedness to vaccine will be assessed. The incidence rate of solicited AEs may be compared between each treatment group and placebo using Fisher's exact test and logistic regression.

The level of vaccinemia (on days 1 [at 0 and 6 hours], 2, 4, 8, 15, and 29) and shedding will be assessed on all subjects. The proportion of subjects with detected vaccinemia, as well as the geometric mean and AUC of vaccinemia level, will be presented at each time point where data are collected. The relationship between vaccinemia level and dose will be explored in a mixed model for repeated measures (MMRM) analysis that incorporates dose level, time, and other potential predictive factors. A similar model will be used to explore the relationship between dose and the AUC of the vaccinemia level.

For hematology and serum chemistry tests, the mean, mean change, median, median change, and range of all values for each test, for each treatment group at baseline and for the final 'on therapy' values, will be printed in a summary table. A second table (a 'shift table') will be made, showing for each laboratory variable the percentage of subjects in each treatment group whose values decreased, stayed the same, or increased between the baseline or pre-treatment period and the end of the study. A third table will be prepared displaying the numbers of subjects in each treatment group who had values below, within, and above the normal range at baseline and at the final visit. Abnormal laboratory measurements that occur following vaccination will be summarized overall and by toxicity grade. A fourth table will be prepared displaying positivity or negativity for rVSV PCR from blood, urine, and saliva; and from other suspect lesions if applicable, on days it is assessed.

These tables will be reviewed by the PI, Medical Monitor, Sponsor, and SRC to evaluate whether any significant trends in laboratory values occurred. The urinalysis data will also be reviewed by inspecting the laboratory data tabulations, but no summary tables of these will be prepared. Vital signs and change from baseline in vital signs will also be summarized at each study visit.

7.5.2. Analysis Addressing the Immunologic Study Objective

Primary analysis of immunogenicity will be based on the PP analysis population; a secondary analysis of immunogenicity may be based on the mITT population. All immunogenicity analyses will be performed by vaccine treatment group. If a subject withdraws from the study, only the data collected to the point of withdrawal will be included. Immunogenicity will be described in terms of the number of responders and the magnitude of response over time.

For the continuous immunogenicity endpoints such as IgG ELISA antibody titers (on days 1, 15, 29, 85, and 181) and neutralizing antibody titers PsVNT (on days 1, 15, 29, 85, and 181). The 95% confidence limits will be obtained by first obtaining the 95% confidence limits for the mean of $\log_{10}$ translated values and then exponentiating the confidence limits.

Mean fold increase in PHV01 IgG ELISA titers and PsVNT on days 8, 15, 29, 85, and 181 compared to baseline (day 1) will be provided. For each respective study day, $\log_{10}$ translated values from baseline will be subtracted from the respective study day for each subject and averaged within vaccine treatment group and dose level. The result will be exponentiated to return to the original scale. The analysis of mean fold increase in PHV01 IgG ELISA titers and PsVNT over time will be performed using MMRM.

Distribution of PHV01 IgG ELISA titers and PsVNT on days 8, 15, 29, 85, and 181 will be displayed as reverse cumulative distribution curves. Median duration of vaccinemia determined by RT-qPCR will be provided. The duration will be presented graphically using the Kaplan-Meier method. Proportion of subjects with vaccinemia detected by RT-qPCR, by study day through days 1 (at 0 and 6 hours), 2, 4, 8, 15, and 29 and by vaccine treatment group, will be provided with 95% CIs.

Seroconversion rate determined on days 8, 15, 29, 85, and 181 and corresponding 95% CI will be provided. Logistic regression models may be performed for comparison between dose levels and placebo.

Additional immunogenicity dose response between dose groups and over time for continuous and categorical endpoints may be assessed using MMRM and logistic regression models, respectively. For continuous endpoints, $\log_{10}$ translated values will be entered in the model as the dependent variable(s). Regarding dose-response analyses more details will be provided in the SAP.

7.5.3. Analysis of Exploratory Endpoints

Exploratory endpoints to examine aspects of both cell mediated and humoral immunity will be defined in the SAP if testing is performed. The exploratory endpoints will be analyzed using the population of subjects enrolled in the trial who receive vaccination and have at least 1 value for the measurement underlying the endpoint. Additional details will be provided in the SAP.

Analysis will be done using the previously mentioned assays, and appropriate statistical tests will be performed according to the finalized SAP.

7.5.4. Subgroup Analysis

Multivariable logistic regression may be used to evaluate the association between age, sex, race, and seroconversion rate.

7.6. ACCOUNTING FOR MISSING, UNUSED, AND SPURIOUS DATA

Non-analyzable data will be documented in the deviations.

7.7. PROCEDURES FOR REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

Any deviation(s) from the original SAP as indicated in the protocol will be described in an amendment to the protocol and the SAP. Deviations from the SAP will be documented in accordance with site SOPs.

8. SUBJECT DATA HANDLING AND RECORDKEEPING

8.1. CONFIDENTIALITY

In this research, the subject's health information will be collected and used to conduct the study; to monitor the subject's health status; to measure effects of the IP; to determine research results; and possibly to develop new tests, procedures, and commercial products. Health information is used to report results of research to the Sponsor's representative/designee and federal regulators, and may be reviewed during study audits for compliance with study plans, regulations, and research policies. After the study ends, each subject has the right to see and receive a copy of his/her information.

The Sponsor, the IRB, the Office of Research Protection, and the FDA are eligible to photocopy and review records related to this protocol as a part of their responsibility to protect the participants of this protocol. In addition, these representatives are eligible to witness the applicable study procedures to assure the safety of subjects.

No personal identifier will be used in any publication or communication used to support this research study. The subject ID number will be used in the event it becomes necessary to identify data specific to a single subject.

8.2. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Subjects will be identified on eCRFs by a unique subject ID number and on source documents by name and unique subject ID number. No personal identifier will be used in any publication or communication used to support this research study. The subject ID number will be used if it becomes necessary to identify data specific to a single subject. Representatives of the Sponsor, the IRB, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research. Personal identifiers will be removed from photocopied medical and research records.

8.3. CASE REPORT FORMS

The primary source document for this study will be the subject's research file which will contain the study source documents. The source documents will be retained at the site.

For this study, an electronic data capture (EDC) system will be used for the collection of the study data in an electronic format. The EDC system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the EDC database system. The Investigator is ultimately responsible for the accuracy of the data transcribed on the eCRF.

Data monitoring and management will be performed in the EDC database system by the study Clinical Monitor and the designated Data Management group.

A detailed data management plan will be written and approved by the study team prior to study start, with approval by the Sponsor. All updates to the data management plan must be approved before study close-out and database lock.

8.4. RETENTION OF RECORDS

The PI must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or, if not approved, for 2 years following the discontinuance of the IP for investigation. The PI will notify the Sponsor before destroying any documents. If it becomes necessary for the Sponsor's representative or designee or the FDA to review any documentation relating to the study, the Investigator must permit access to such records. Completed, monitored eCRFs will be stored in a secure location by the Sponsor's representative or designee.

The PI will be responsible for retaining sufficient information about each subject, i.e., name, address, telephone number, Social Security number, and subject identifier in the study, so that the Sponsor's representative, the IRB, the FDA, or other regulatory authorities may have access to this information should the need arise.

9. STUDY MONTORING, AUDITING AND INSPECTION

9.1. MONITORING

The Sponsor is delegating most trial oversight and monitoring responsibilities to a Clinical Research Organization (CRO). Upon successful approval of the protocol and establishment of the regulatory file, the CRO will establish a clinical monitoring plan. To ensure that the Investigator and the study staff understand and accept their defined responsibilities, the CRO will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records. As needed, the CRO may witness the informed consent process or other applicable study procedures to assure the safety of subjects and the Investigators' compliance with the protocol and GCP guidelines.

Monitoring visits will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last subject has completed the study.

The Medical Monitor will be a qualified physician not associated with this protocol who is able to ensure the responsibilities of the study Sponsor, especially with regard to the ethics, clinical safety of a study, and the assessment of AEs. The monitor will review all unanticipated problems involving risk to subjects or others, SAEs, MAAEs, and all subject deaths associated with the protocol, and will provide an unbiased written report of the event to appropriate authorities, including at a minimum the outcomes of the event or problem, and commenting on the relationship of the event(s) to participation in the study.

Details of study monitoring, including potential action required due to COVID-19, will be included in a separate Study Monitoring Plan.

9.2. AUDITS AND INSPECTIONS

Authorized representatives of the Sponsor, the FDA, or the IRB may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported, according to the protocol, cGCP guidelines of the ICH, and any applicable regulatory requirements.

Auditing of the clinical trial may be conducted at any time during the study to ensure continued compliance with regulations, policies, and procedures. Sponsor-initiated auditing will be undertaken as needed by independent personnel designated by the Sponsor. Audit findings from audits initiated by the Sponsor will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

The Investigator should contact the Sponsor's representative/designee immediately if contacted by a regulatory agency about an inspection.

9.3. INSTITUTIONAL REVIEW BOARD

The IRB will review the protocol, ICF, and progress reports on a continuing basis in accordance with all applicable regulations, including Title 21 CFR, Parts 50 and 56.

The PI must obtain IRB approval for the study. Initial IRB approval and all materials approved by the IRB for this protocol, including the subject ICF and recruitment materials, must be maintained by the PI and made available for inspection.

10. ETHICAL CONSIDERATIONS

10.1. ETHICS REVIEW

The study is based on experience with a related licensed vaccine (rVSVΔG-ZEBOV-GP) (17, 26), as well as adequately performed laboratory and animal experimentation with rVSVΔG-MARV-GP and will be conducted under a protocol reviewed by the IRB. The study is to be conducted by scientifically and medically qualified persons. The IRB will determine whether the benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; subjects will give their informed consent and will be competent to do so and not under duress; and the study will be conducted in accordance with applicable regulations and policies including the Declaration of Helsinki, ICH Guidelines, US 32 CFR Part 219 (Protection of Human Subjects), US 21 CFR Part 50 (Protection of Human Subjects [Informed Consent]) and Part 56 (IRBs), and the principles described in the Belmont Report (https://www.hhs.gov/ohrp/regulations-and-policy/belmont-report/index.html).

10.2. REVIEW/APPROVAL OF STUDY PROTOCOL

Before a clinical study can be initiated, the study protocol and other required documents will be submitted to the following departments for review and/or approval, in the order listed, with the final review by the FDA. This order may be altered during the course of the review process if it is determined that additional IRBs or regulatory agencies are required to participate or if alterations

made during the review process require acknowledgement by groups that have previously given approval:

- Sponsor's Representative Team
- IRB
- Sponsor's Representative/designee

Enrollment in this protocol may not begin until all approvals have been obtained and the formal authorization letter is received by the PI from the Sponsor's representative/designee.

10.3. PROTOCOL AMENDMENTS

An amendment is a written description of change(s) to or formal clarification of a study protocol which may impact on the conduct or potential benefit of the clinical study, or may affect subject safety, including changes of study objectives, study design, subject population, sample sizes, study procedures, or significant administrative aspects. An administrative change of a study protocol is a minor correction or clarification that has no significant impact on the way the clinical study is to be conducted and no effect on subject safety (e.g., change of telephone number(s), logistical changes).

All amendments to the protocol and supporting documents (ICF, SSPs, SOPs, recruitment materials, etc.) must be reviewed and approved by PHV, Health Authorities where required, and the IRB/IEC prior to implementation. The ICF must be revised to concur with any amendment as appropriate and must be reviewed and approved with the amendment. Any subject already enrolled in the study will be informed about the revision and asked to sign the revised ICF if the modification directly affects the individual's participation in the study. A copy of the revised, signed, and dated ICF will be given to the subject. All original versions of the ICF will be retained in the protocol regulatory file.

10.4. PROTOCOL ADHERENCE

Investigators will apply due diligence to avoid protocol deviations. Under no circumstances should the Investigator contact PHV or its agents monitoring the trial to request approval of a protocol deviation, as no authorized deviations (i.e., exemptions) are permitted. If the Investigator feels a change to the protocol would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by PHV and approved by the IRB/IEC it cannot be implemented. All significant protocol deviations will be recorded and reported in the CSR.

All subject-specific deviations from the protocol (e.g., failure to return for follow-up visits or blood collection within the time indicated in the protocol) are to be documented. The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or SSPs. Action taken in response to the deviation, and the impact of the deviation, will be assessed by the PI or sub-investigator and recorded as significant or nonsignificant. Deviations will be reported in the continuing review report to the IRB/IEC, in the closeout report, and, if appropriate, in the final CSR submitted to the FDA.

For any protocol deviation that adversely affects the safety or rights of a subject or scientific integrity of the study, the deviation will be reported within 48 hours to the Sponsor's representative and the IRB/IEC.

10.5. ETHICAL CONDUCT OF THE STUDY

This study will be conducted in accordance with all applicable federal human research protection requirements and the Belmont Principles of respect for persons, beneficence, and justice.

The procedures set out in this study are designed to ensure that the Sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline and the CFR. The PI confirms this by signing this study protocol and Form FDA 1572.

10.6. WRITTEN INFORMED CONSENT

The informed consent process and document will be reviewed and approved by the IRB and Sponsor's representative with administrative review by the Office Research Protection prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR Part 50. The consent document indicates that by signature, the subject permits witnessing of applicable study procedures by the Sponsor's representative/designee, as well as access to relevant medical records by the Sponsor's representative/designee and by representatives of the FDA. The Sponsor's representative/designee will submit copies of IRB and Sponsor's representative approved ICFs to the FDA.

A written ICF, in compliance with 21 CFR Part 50, 32 CFR Part 219, and the Belmont Principles, will be signed by the subject before any study-related procedures are initiated for that subject. This consent document must be retained by the Investigator as part of the study records. Each subject will receive a copy of the signed ICF. The Investigators or their designees will present the protocol in lay terms to individual subjects. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the PI. No subject should grant consent until questions have been answered to his/her satisfaction. The subject should understand that the study product is an investigational drug and is not licensed by the FDA for commercial use but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the subject be informed about the principal potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary.
- Subjects may withdraw from participation at any time.
- Refusal to participate involves no penalty.
- The subject is free to ask any questions that will allow him/her to understand the nature of the protocol.

Should the protocol be modified, the subject consent document must be revised to reflect the changes to the protocol. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised ICF. The approved revision will be read, signed, and dated by the subject.

The subject will be informed that a description of this clinical trial will be available on https://clinicaltrials.gov as required by US law.

10.7. INSPECTION OF RECORDS

The Sponsor's representative or designee will be allowed to conduct site visits at the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, IP stocks, drug accountability records, subject charts, study source documents, and other records relevant to study conduct.

Subjects' health information is used to report results of research to the Sponsor's representative and federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. The consent document indicates that by signature, the subject permits access to relevant medical records by the Sponsor's representative and by representatives of the FDA and BARDA.

Upon a subject's termination from the trial, completed eCRFs will be ready and available for on-site review by the Sponsor's representative or the designated representative within 14 days after receipt of the subject's data.

11. PUBLICATION POLICY

All data collected during this study will be used to support this IND. All data may be published in the open medical literature with the identity of the subjects protected. Anyone desiring to publish or present data obtained during the conduct of the study will conform to the Sponsor's policies and then forward the publication for review to the Sponsor prior to submission.

12. REFERENCES

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APPENDIX 1: CLINICAL AND LABORATORY TOXICITY GRADING SCALES

Table A1: Toxicity Grading Scale for Local Reactions

Local Reaction	Normal (Grade 0)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Redness/ Erythema[a]	< 25 mm	25-50 mm	51-100 mm	>100 mm	Necrosis or exfoliative dermatitis
Induration/ Swelling [b]	< 25 mm	25-50 mm	51-100 mm	>100 mm	Necrosis
Pain	None	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	None	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization

[a] In addition to grading the local reaction, the measurement at the greatest single diameter should be recorded.
[b] Induration/swelling should be evaluated and graded using the functional scale.

Table A2: Toxicity Grading Scale for Systemic Adverse Events

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	Emergency Room (ER) visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Joint swelling	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Chills	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Sweats	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Subjective Fever	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Nausea /vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Abdominal Pain	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	Life-threatening consequences; urgent intervention indicated
Other Systemic Symptoms	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization

Table A3: Toxicity Grading Scale for Vital Signs

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever[b] (°C) (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute[c]	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

[a] Subject should be at rest for all vital sign measurements.
[b] Oral temperature, no recent hot or cold beverages or smoking.
[c] When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Table A4: Laboratory Toxicity Grading Scale for Hematology Adverse Events

Hematology Parameter[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
PT – increase by factor (prothrombin time)	1.0 – 1.10 × ULN	1.11 – 1.20 × ULN	1.21 – 1.25 × ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 × ULN	1.21 – 1.4 × ULN	1.41 – 1.5 × ULN	> 1.5 × ULN
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,000 – 1,499	500-999	<500	Hospitalization
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hospitalization
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000

Abbreviations: ULN = upper limit of normal; WBC = white blood cell.
[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. The clinical signs or symptoms associated with laboratory abnormalities may result in characterization of the laboratory abnormalities as AEs if they are considered to be clinically significant.

Table A5: Laboratory Toxicity Grading Scale for Blood Chemistry and Urinalysis Adverse Events

Serum[a,b]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Blood Urea Nitrogen BUN (mg/dL)	23-26	27 – 31	> 31	Requires dialysis
Creatinine (mg/dL)	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Liver Function Tests – ALT, AST	1.1 – 2.5 × ULN	2.6 – 5.0 × ULN	5.1 – 10 × ULN	> 10 x ULN
Bilirubin –accompanied by any increased LFT	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when LFT is normal	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Urine Protein	Trace	1+	2+	Hospitalization for dialysis
Urine Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) RBCs / high power field[c]	1-10	11-50	>50 and/or gross blood	Hospitalization for pRBC transfusion

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; PRBC = packed red blood cells; RBC = red blood cell; ULN = upper limit of normal

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[b] The clinical signs or symptoms associated with laboratory abnormalities may result in characterization of the laboratory abnormalities as AEs if they are considered to be clinically significant.

[c] Positive test for blood will not be considered clinically significant in a female subject who is menstruating, and microscopic analysis will not be performed unless clinically indicated. If the subject is not menstruating a repeat urinalysis will be performed.

APPENDIX 2: SKIN AND JOINT FLUID PROCESSING

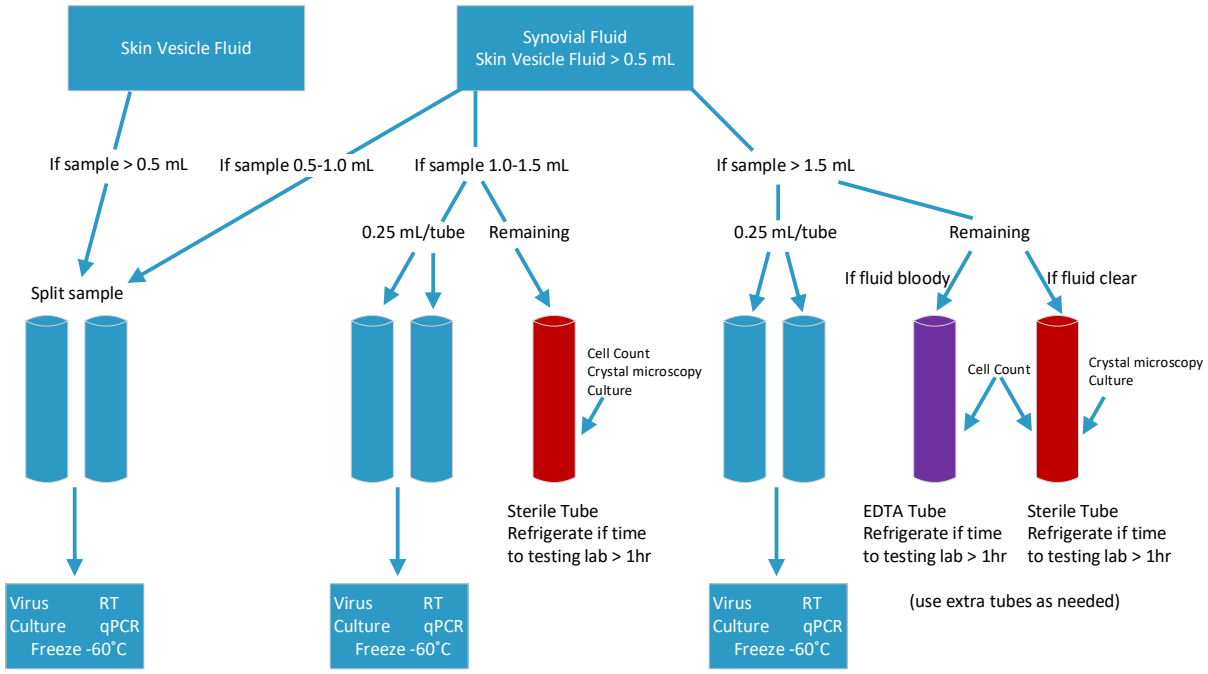
Skin Punch Biopsy

1. Anesthetize the site to be biopsied.
2. Perform Skin biopsy with ChloraPrep and 3mm punch biopsy, cut in half.
3. Retrieve formalin cup and label.
4. Place ½ biopsy in formalin and ½ in RNALater in labeled cryovials as soon as possible. Formalin sample is stored on site at room temperature. Sample in RNALater is stored O/N at 4°C, then RNALater is removed, and sample stored at ≤60°C.

All samples are to remain on-site until the Sponsor provides further instructions.

Synovial Fluid Collection

The following guide should be followed depending on the amount of fluid obtained from the skin vesicle aspiration or arthrocentesis. If a vesicle is smaller than 0.5 mL, it should be nicked and the fluid collected on a swab. Larger volumes should be collected with a syringe as follows:



Note: The following tests must be done by the local site laboratory:

- Cell count with differential
- Crystal microscopy
- Culture for bacteria

The samples for viral culture and RT-PCR are to remain frozen on-site until the Sponsor provides further instructions.