STATISTICAL ANALYSIS PLAN

STUDY SPONSOR:	Public Health Vaccines, LLC One Broadway, 14th Floor Cambridge, MA 02142 Tel: (617)-539-8224
SPONSOR REPRESENTATIVE:	Richard Kenney Chief Medical Officer for Marburg
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
PROTOCOL VERSION AND DATE:	Version 3.0: 23 January 2024
NAME OF TEST DRUG:	PHV01 (rVSVΔG-MARV-GP [Angola])
PHASE:	Phase 1
METHODOLOGY:	Randomized, Single-Blind, Placebo-Control
ANALYSIS PLAN DATE:	14 June 2024
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AUTHOR:	Christopher Kenwood

APPROVAL SIGNATURE PAGE

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Veristat, Inc. Author:	Veristat, LLC. 134 Turnpike Road, Suite 200 Southborough, MA 01772

Approval Signature	Job Title

Sponsor Signatory: Richard Kenney, MD
Chief Medical Officer for Marburg

Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol, clinical development plan, and all applicable regulatory guidances and guidelines.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report.

Approval Signature	Job Title

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE	Adverse event
AESI	Adverse event of special interest
ASaT	All subjects as treated, also known as the "Safety Cohort"
ATC	Anatomic therapeutic class
AUC	Area under the concentration versus time curve
BMI	Body mass index = subject weight [kilograms]/(height [meters])2
CI	Confidence interval
CRF	Case report form
CSR	Clinical study report
EDC	Electronic data capture
ELISA	Enzyme-linked immunosorbent assay
EU	ELISA Units
FDA	Food and Drug Administration
FIH	First-in-human
GEE	Generalized estimating equations
GMFI	Geometric mean fold increase
GMT	Geometric mean titer
GP	Glycoprotein
HCV	Hepatitis C
HepB	Hepatitis B
HIV	Human immunodeficiency virus
IA	Interim Analysis
ICAM-1	Intercellular adhesion molecule-1
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IM	Intramuscular/ intramuscularly
IRB	Institutional Review Board
ITT	Intent-to-treat
IWRS	Interactive Web Response System
LLOQ	Lower Limit of Quantitation
LOD	Limit of Detection
LS	Least squares
MAAE	Medically attended adverse event
MARV	Marburg virus, the etiologic agent of Marburg disease
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intention-to-treat

Abbreviation	Definition
mL	Milliliter
MMRM	Mixed models for repeated measures
%	Percent
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]
PI	Principal Investigator
Placebo	Lactated Ringer's solution
PP	Per-Protocol
PsVNA	Pseudovirion neutralization assay
$PsVNT_{50}$	Pseudovirion neutralization titer of 50%
$PsVNT_{80}$	Pseudovirion neutralization titer of 80%
PT	Preferred term
Rel Day	Relative study day
RNA	Ribonucleic acid
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
RTF	Rich text format
rVSV	Recombinant vesicular stomatitis virus
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SD	Standard deviation
SE	Standard error
SI	International System of Units
SOC	System organ class
SOE	Schedule of Events
SRC	Safety Review Committee
TEAE	Treatment-emergent adverse event
ULN	Upper Limit of Normal
VNA	Virus neutralization assay
VCAM-1	Vascular cell adhesion molecule-1
VSV	Vesicular stomatitis virus
WHO	World Health Organization

1. INFORMATION FROM THE STUDY PROTOCOL

1.1. Introduction and Objectives

1.1.1. Introduction

Marburg virus (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% (CDC, 2023). 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 (Miragalia, 2019). 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.

There is no licensed vaccine or treatment for MARV in humans. 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). Protocol PHV01-C-101 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 to 60 years of age), who will be randomized in cohorts to receive one of 3 graded doses (1×10^5, 1×10^6, or 1×10^7 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 (AEs), vaccinemia, shedding of vaccine virus in urine and saliva, and immune responses. The last visit will be 6 months after the dose.

This statistical analysis plan (SAP) is designed to outline the methods to be used in the analysis of study data in order to answer the study objective(s). Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this trial.

This SAP will also outline any differences in the currently planned analytical objectives relative to those planned in the study protocol.

1.1.2. Study Objectives

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 intramuscular (IM) injection in 1 mL to healthy adults.

Primary Immunologic Objective:

To evaluate Marburg-specific IgG ELISA antibody and neutralizing antibody responses to graded doses of PHV01 given by IM injection to healthy adults on Days 1 and 29.

Secondary Objective:

To evaluate vaccine viremia and shedding in saliva and urine after administration of PHV01 on days 1 (at 0 and 6 hours), 2, 4, 8, 15, and 29.

Exploratory Objective:

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

1.2. Study Design

1.2.1. Synopsis of 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 MARV disease.

The purpose of the PHV01-C-101 study is to test the safety and immunogenicity of the plaque-purified, vaccine injected IM undiluted (Group C: 1×10^7 pfu in 1 mL) and at ten-fold dilutions (Group B: 1×10^6 in 1 mL and Group A: 1×10^5 pfu in 1 mL). 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. Note, as this is a FIH study, a maximum of 2 randomized subjects will be dosed per day in Cohort #1. The remainder of group and a randomized placebo control will then be dosed at the lower dose, randomized with 3 Pioneer vaccinees at the next higher dose and a placebo control.

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 Interim Analysis will be performed on the safety and serological data from all subjects collected up to Day 29 using a cleaned and frozen dataset. The final CSR will be based on the safety and immunogenicity data collected on Days 1 to 181 using the cleaned and locked dataset.

1.2.2. Randomization Methodology

Subjects will be evaluated for eligibility during a screening visit prior to starting treatment. The randomization procedure will be performed centrally in an Interactive Web Response System (IWRS) within the electronic data capture (EDC) system.

Up to 36 subjects will be randomized to receive the 3 vaccines dose groups (1×10^5, 1×10^6, and 1×10^7 pfu of PHV01) and placebo. For each dosing level, a pioneer group of the first 4 subjects will be randomized 3:1 to receive the PHV01 or placebo, and the remainder of the group will be randomized (along with the pioneer group of next dose level) at 7:1. Randomization will be performed in a stepwise, dose ascension manner in 4 distinct cohorts; the proposed cohorts are presented in Table 1-1.

Table 1-1 Cohort Randomization

Cohort		PHV01: Placebo
1	Pioneer 10^5	3:1
2	Remainder of Group 10^5	7:1
	Pioneer 10^6	3:1
3	Remainder of Group 10^6	7:1
	Pioneer 10^7	3:1
4	Remainder of Group 10^7	7:1

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.

1.2.3. Stopping Rules and Unblinding

Criteria for Study Pause or Halt

The Safety Review Committee (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 AE 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 serious adverse event (SAE) or an adverse event of special interest (AESI) is considered to be related to PHV01 vaccine. In the event dosing is halted, the SRC and Institutional Review Board (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.

Individual Subject Unblinding

The group assignment for an individual subject may be unblinded during the course of the study if the Principal Investigator (PI) deems that, a safety event requires knowledge of that group assignment, 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 investigational product (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 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.

1.2.4. Study Procedures

The schedule of assessments, as outlined in the study protocol, is provided in Table 1-2.

Table 1-2 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.

1.2.5. Safety and Immunogenicity Parameters

1.2.5.1. Safety Parameters

Safety will be assessed by analysis of the occurrence of treatment emergent adverse event (TEAE) and vaccine viremia, as well as by adverse changes in laboratory evaluations (chemistry, hematology, urinalysis, etc.), vital signs, and physical findings, including neurologic examinations. Solicited AEs will be collected systematically through Day 29; unsolicited TEAEs, medically attended adverse events (MAAEs), SAEs, and AESIs will be collected for the entire duration of a subject's participation in the study with the help of Memory Aids reviewed at every visit The primary safety endpoints are:

- 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, Days 9-15, and up to Day 29 after vaccination, per treatment group (active vaccination versus placebo).

- 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, i.e., not related vs related.

- 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 AESIs up to Day 181, including 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.

Secondary safety endpoints include the following:

- 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 of PHV01, and AUC

1.2.5.2. Immunogenicity Parameters

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
- GMT of $PsVNT_{50}$ and $PsVNT_{80}$ MARV GP-specific neutralizing antibody titers (the reciprocals of the serum dilutions at which 50% and 80% of the input pseudovirion was neutralized, respectively) as measured by Pseudovirion neutralization assay (PsVNA) on Days 1 and 29

If the corresponding titer value is below the lower limit of quantitation (LLOQ), the titer value will be considered as LLOQ/2 at such visit for GMT computation. For ELISA titers below limit of detection (LOD), the titer will be assigned a value of 1 ELISA unit/mL for GMT computations.

Secondary immunogenicity endpoints include the following:

- GMT Marburg GP protein-specific IgG ELISA antibody on all other study days through Day 181
- GMT $PsVNT_{50}$ and $PsVNT_{80}$ on all other study days to Day 181
- Seroconversion rate defined as at least 4-fold increase in Marburg GP-specific IgG (ELISA) titer compared to Day 1 for subjects with baseline titers > LLOQ. Otherwise, if baseline titer < LLOQ or <LOD, seroconversion is defined as at least 4-fold increase over LLOQ/2.
- Seroconversion rate defined as at least 4-fold increase in Marburg GP-specific neutralizing antibody titer PsVNA compared to Day 1 for subjects with baseline titers > LLOQ.

Otherwise, if baseline titer < LLOQ, seroconversion is defined as at least 4-fold increase over LLOQ/2.

- Geometric mean fold increase in Marburg GP-specific IgG (ELISA)
- Geometric mean fold increase in Marburg GP-specific neutralizing antibody titer (PsVNA)
- Seroresponse rate defined as at least 2-fold increase in Marburg GP-specific IgG (ELISA) compared to Day 1 for subjects with baseline titers > LLOQ. Otherwise, if baseline titer < LLOQ or <LOD, seroresponse is defined as at least 2-fold increase over LLOQ/2.
- Seroresponse rate defined as at least 2-fold increase in Marburg GP-specific neutralizing antibody titer PsVNA compared to Day 1 for subjects with baseline titers > LLOQ. Otherwise, if baseline titer < LLOQ, seroresponse is defined as at least 2-fold increase over LLOQ/2.
- Reverse cumulative distribution of titers (IgG ELISA and PsVNA)
- The correlation between the IgG ELISA and neutralizing antibody titers on all study days tested
- The correlation between blood viremia AUC and IgG ELISA and neutralizing antibody

Exploratory immunogenicity endpoints include the following:

- 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

2. SUBJECT POPULATION

2.1. Population Definitions

The following subject populations will be evaluated and used for presentation and analysis of the data:

- All Subjects as Treated (ASaT) Population: 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.

- Per-Protocol (PP) Population: All randomized subjects who received the vaccine dose to which they were randomized, have PHV01 IgG ELISA titer or PsVNA results on Days 1 (baseline) and 29 (± 5 days), and do not have any protocol violations that influence interpretation of immunogenicity endpoints.

- Modified Intent-to-Treat (mITT) Population: All randomized subjects who received a single study injection and have at least 1 post-injection immunogenicity evaluation. The subjects will be analyzed according to treatment received in case of a treatment error.

The ASaT population is the primary population for the analysis of safety endpoints. The PP population is the primary population for the analysis of immunogenicity parameters. The mITT population may be used for additional analyses of immunogenicity endpoints, should there be a 10% difference or more in the sizes of the mITT and PP analysis sets (see Section 4.3). Intervals for analysis, i.e., visit windows, are further defined in the Schedule of Events.

2.2. Protocol Violations

At the discretion of the Sponsor, major protocol violations, as determined by a review of the data prior to unblinding of the study results and the conduct of statistical analyses, may result in the exclusion of a subject's data from the PP population. Veristat will be responsible for producing the final protocol violation file (formatted as a Microsoft Excel file), in collaboration with the Sponsor and the data monitoring group as applicable; this file will include a description of the protocol violation and clearly identify whether this violation warrants exclusion from the PP population. The decisions on PP exclusions will be finalized prior to interim analysis and the complete file of protocol violations will be finalized prior to final database lock for CSR.

All protocol violations will be presented in a data listing.

3. GENERAL STATISTICAL METHODS

3.1. Sample Size Justification

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 and 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, the sample size was established to provide reasonable information for descriptive purposes and not specifically designed for formal hypothesis testing.

Safety: With a sample size of 10 subjects, an upper bound of 30.9% is established for the 2-sided 95% confidence interval (CI) for the incidence of an AE in the case that the event is not observed according to the two-sided 95% (Clopper-Pearson) exact CI. Alternatively, when pooled together all 30 vaccinees, an upper bound of 11.6% is provided. This established the sample sizes needed to ensure minimal AE rates could be detected with confidence.

Immunogenicity: Clinical data for the rVSV-ZEBOV-GP Ebola vaccine showed that a dose of 3×10^6 pfu/mL yielded a Day 29 GMT IgG ELISA titer of 1400 EU/mL and geometric standard deviation (SD) 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.

3.2. General Methods

All data listings that contain or imply an evaluation date will contain a relative study day (Rel Day). Pre-treatment and on-treatment study days are numbered relative to the day of the first dose of study medication which is designated as Day 1. The preceding day is Day -1, the day before that is Day -2, etc. Post-treatment study days are numbered relative to the first dose and are designated as Day 1, Day 2, etc. for table and listing display. Data for time interval analysis and graphing will be displayed as days since dosing where Hour 6 timepoint on Day 1 is converted to 0.25 days.

All outputs will be incorporated into Microsoft Word or Excel files, or Adobe Acrobat PDF files, sorted and labeled according to the International Conference on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, immunogenicity, and safety parameters. For categorical variables, summary tabulations of the number and percentage of subjects within each category (with a category for missing data) of the parameter will be presented. For continuous immunogenicity variables, the number of subjects, geometric mean, median, geometric standard deviation (SD), minimum, and maximum values will be presented.

Other continuous variables (e.g., BMI) will be summarized by the number of subjects, mean, median, SD, minimum, and maximum values. Summarizations will be tabulated by treatment group.

Formal statistical hypothesis testing will be performed on the primary and secondary immunogenicity endpoints with all tests conducted at the 2-sided, 0.05 level of significance. Summary statistics will be presented, as well as CIs on selected parameters, as described in the sections below.

3.3. Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software Version 9.4, unless otherwise noted. Medical history and adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 26.1. Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary Version Global B3 Sep 2023.

3.4. Baseline Definitions

For all analyses, baseline will be defined as the most recent measurement prior to administration of study vaccine.

3.5. Methods of Pooling Data

In addition to presenting results by PHV01 vaccine dose levels, data will be pooled across PHV01 vaccine dose levels for analyses as an PHV01 overall group and all placebo subjects will be pooled as another group.

3.6. Adjustments for Covariates

Additional exploratory analyses adjusted for covariates may be performed on an ad-hoc basis.

3.7. Multiple Comparisons/Multiplicity

No adjustments will be made for multiplicity. Study objectives will be evaluated through a combination of statistical and clinical assessments, which is not consistent with an approach to adjust for multiple comparisons.

3.8. Subpopulations

The immunologic endpoints will be analyzed separately for males and females, and by age group (< 40 and ≥ 40 years old).

3.9. Withdrawals, Dropouts, Loss to Follow-up

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.

3.10. Missing, Unused, and Spurious Data

In general, there will be no substitutions made to accommodate missing data points. All data recorded on the case report form will be included in data listings that will accompany the CSR.

Subjects with negative post-baseline antibody response (defined as the corresponding titers < LLOQ) will have a titer value assigned as LLOQ/2.

When tabulating AE data, partial dates will be handled as follows. If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as study treatment. In this case, in order to conservatively report the event as treatment-emergent, the onset date will be assumed to be the date of treatment. If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study treatment. In this case, the event onset will be coded to the day of treatment in order to conservatively report the event as treatment-emergent. A missing onset date will be coded as the day of treatment.

3.11. Visit Windows

It is expected that all visits should occur according to the protocol schedule. All data will be tabulated per the evaluation visit as recorded on the CRF even if the assessment is outside of the visit window. In data listings, the relative day of all dates will be presented.

For analyses of IgG ELISA antibody titers and neutralizing antibody titers, analysis visits will be derived based on the relative study day (i.e., Rel Day, defined in Section 3.2) as outlined in the schedule of assessments with an expanded interval of +/-5 days. If there is more than one immunogenicity assessment during a given interval for analysis, the immunogenicity assessment within the protocol-specified interval (+/- 2 days) will be used. If there is still more than one immunogenicity assessment with the protocol-specified interval, the assessment closest to the target protocol-specified day will be used, i.e., if there is more than one immunogenicity assessment at the protocol-specified Day 29±2 days, the assessment closest to Day 29 will be used.

3.12. Interim Analyses

One administrative Interim Analysis (IA) will be performed when all 36 subjects have completed the Day 29 follow-up, to guide dose selection for a subsequent clinical trial in one or more endemic countries in Africa. 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. Tables, listings, and figures will be provided by the treatment group to describe solicited local and systemic AEs, unsolicited AEs, and any MAAEs or SAEs, along with simple descriptive

statistics of clinical laboratory data. Neurologic findings through Day 29 will also be summarized.

For immunogenicity analyses, tables, listings, and figures will describe the serum IgG ELISA and PsVNA for each treatment group. In addition, RT-qPCR results from plasma, urine, and saliva samples will be used to assess viremia and shedding.

4. STUDY ANALYSES

4.1. Subject Disposition

Subject disposition will be tabulated and include the number screened, the number randomized, the number treated in total and by dose level, the number in each subject population for analysis (ASaT mITT, PP), the number who withdrew prior to completing the study (lost to follow up) and reason(s) for withdrawal, including withdrawal due to intolerance to the study injection.

A by-subject data listing of study completion information including the reason for premature study withdrawal, if applicable, will be presented.

4.2. Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized and presented by treatment group. Age, height, weight and BMI will be summarized using descriptive statistics (number of subjects, mean, standard deviation, median, minimum, and maximum). The number and percentage of subjects in each gender, ethnicity and race category will also be presented.

Medical history will be tabulated and provided in data listings.

Demographic and baseline data such as for pregnancy test and screening results for HIV, HCV and HepB for each subject will be provided in data listings.

4.3. Immunogenicity Evaluation

Immunogenicity analyses will be conducted using the PP population. Supportive analyses of immunogenicity will be based on the mITT population if the number of subjects excluded from PP population is more than 10%.

4.3.1. Geometric Mean Titers and Mean Fold Increase of IgG ELISA Antibody and Neutralizing Antibody PsVNT

For IgG ELISA antibody titers and neutralizing antibody titers PsVNT ($PsVNT_{50}$ and $PsVNT_{80}$), GMT, and 95% confidence limit will be provided on Days 1, 8 (if tested), 15, 29, 85, and 181, where the GMT is calculated as the anti-log of the base-2 log transformed mean and the same for the corresponding confidence limit. Results will be presented by treatment group along with a combined PHV01 group, and the descriptive statistics will include the GMT, the geometric standard deviation, 25th percentile, median, 75th percentile, minimum, and maximum values. If appropriate, the analyses will be repeated for the GMT using the anti-log of the base-10 log transformed mean.

Pairwise comparison of the above antibody titer levels for each PHV01 dose level to one another and to the placebo group, as well as the comparison of the combined PHV01 dose group relative to the placebo group will be performed with Satterthwaite t-tests. T-tests will be performed on log-2 transformed values and the resulting estimated mean difference and associated 95% CI will then be back transformed using base-2 antilogarithms to obtain estimates

on the ratio scale. If appropriate, the analyses will be repeated using base-10 log transformed values and back transformed using base-10 antilogarithms to obtain estimates. In the event of extreme outliers or non normally distributed data, Wilcoxon rank-sum test may be performed to support the comparison.

Additionally, Analysis of Variance (ANOVA) models will be used to compare each PHV01 dose level to one another and to placebo for Days 8 (if tested), 15, 29, 85 and 181. The response variable will be the log-2 transformed ELISA titer values for the time point under consideration. If there is a significant difference between the GMTs, i.e., where the p-value from the F-test is < 0.05, then post-hoc pairwise tests using Tukey's method will be used to compare each dose with one another and each dose with placebo. The estimated mean differences on the log-2 scale between the treatment groups will be expressed as the ratio of GMTs. The base-2 antilogarithm of each mean difference represents the GMT ratio and the base-2 antilogarithms of the 95% CI limits for the mean difference represent the confidence limits for the GMT ratio.

Similarly, the mean fold increase of ELISA titers and PsVNT will be calculated by subtracting the base-2 log transformed values at baseline (Day 1) from the respective study day (Day 8 [if tested], 15, 29, 85, 181) for each subject and averaged within treatment group. If baseline titer value is <LLOQ or <LOD (ELISA only), baseline will be assigned as LLOQ/2 for purposes of deriving fold increase. The results will be presented by transforming back the anti-log. The 95% CI will be calculated based on the inverse of the log2 transformed fold increase values from the t-distribution. Results will be presented for each study day (Day 8 [if tested], 15, 29, 85, 181) by treatment group along with a combined PHV01 group, and the descriptive statistics will include the geometric mean fold-increase from baseline, the geometric standard deviation, 25th percentile, median, 75th percentile, minimum, and maximum values.

Pairwise ratios of the geometric mean fold rise and the associated 95% confidence intervals will be estimated for each PHV01 dose group relative to one another and to the placebo group, as well as the ratio of the combined PHV01 dose group relative to the placebo group on Days 8 (if tested), 15, 29, 85, and 181. Pairwise comparisons will be performed using Satterthwaite's t-test. T-tests will be performed on log-2 transformed values of the individual fold-rise values. The resulting estimated mean difference and associated 95% CI will then be back transformed using base-2 antilogarithms to obtain estimates on the ratio scale.

4.3.2. Seroconversion Rate and Distribution of ELISA Titers and PsVNT

Seroconversion is defined as at least 4-fold increase in the titer when compared to baseline (Day 1). If the baseline titer is <LLOQ or <LOD (ELISA only), the baseline value used to determine seroconversion will be assigned as LLOQ/2. In these cases, seroconversion occurs if the fold-increase at post-baseline visit is greater than or equal to 4 times the LLOQ/2.

The seroconversion rate of ELISA titers and PsVNT and their exact 95% CIs based on the Clopper-Pearson method will be presented by treatment group and combined PHV01 group on Days 8 (if tested), 15, 29, 85, 181. The comparison between each of the PHV01 vaccine dose levels, the combined PHV01 vaccine group, and the placebo arm will also be performed. Comparisons will be based on the 95% CI for differences in seroconversion rate and will use the Newcombe score method. The analysis will be repeated separately for males and females. These

differences will be used to indicate possible dose-related responses. A difference in seroconversion rate between a given PHV01 dose group and placebo that is greater than zero (i.e., if the lower bound of the 95% CI for difference in seroconversion rate is greater than zero) indicates the rate of seroconversion in the PHV01 dose group is larger than the rate in the placebo group.

Reverse cumulative distribution curves of ELISA titers and PsVNT will be generated by treatment group on Days 8 (if tested), 15, 29, 85, 181 respectively, where the x-axis will be the observed log-2 transformed titers and the y-axis will be the percentage of subjects having a log-2 transformed titer greater than or equal to each of the observed log-2 transformed titers on the x-axis.

Boxplots by study day and treatment group will present the mean, median, interquartile range, and 95% CI for the ELISA titers and PsVNT levels on the log-2 scale and will be overlaid with jittered dots representing individual datapoints. Spaghetti plots will also be plotted for ELISA titers and PsVNT levels on log-2 scale by days since dosing, treatment group and subject. Line plots will present GMTs for ELISA titers and PsVNT50 levels at each visit by treatment group.

Exploratory endpoints to examine aspects of both cell mediated and humoral immunity will be collected but not analyzed.

4.3.3. Seroresponse Rate of ELISA Titers and PsVNT

Seroresponse is defined as a $\geq$ 2-fold increase in the titer when compared to baseline (Day 1). If the baseline titer is <LLOQ or <LOD (ELISA only), a seroresponse is defined as titer greater than or equal to LLOQ.

The number, percent, and associated exact 95% CI of subjects whose baseline response is negative, i.e., less than the LLOQ, will be tabulated for each treatment group and the combined PHV01 vaccine group.

The number, percent, and exact 95% CI of seroresponse for Days 8 (if tested), 15, 29, 85 and 181 will be calculated. Comparisons between each PHV01 dose level with one another and with the placebo group will be performed using the Newcombe method.

Analyses of seroresponse rates will be performed separately for the ELISA IgG and neutralizing antibody titers.

4.3.4. Correlation between IgG ELISA and neutralizing antibody titers on Days 29, 85 and 181

Spearman's rank correlation will be used to estimate the association between IgG antibody titers and PsVNA titers for each PHV01 vaccine dose group including the combined PHV01 group at Days 29, 85, and 181.

4.4. Safety Analyses

Safety analyses will be conducted using the ASaT population.

4.4.1. Study Vaccine/Placebo Exposure

Within each vaccine group, dosing information for each subject will be presented in a data listing.

4.4.2. Adverse Events

AEs 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.

All AEs (including solicited AEs) will be coded using the MedDRA coding system and displayed in tables and data listings using system organ class (SOC) and preferred term (PT). Note that solicited and unsolicited AEs will be analyzed and presented separately. 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" described in Section 5.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined per protocol as any AE with onset after the administration of study medication.

All AEs occurring on-study will be listed in subject data listings.

By-subject listings also will be provided for the following: subject deaths, SAEs, AEs leading to withdrawal, solicited local site AEs, solicited systemic AEs, MAAEs, and AESIs.

Solicited AEs are predefined local events at the injection site and systemic events for which the subject is specifically questioned and facilitated by use of memory aides.

The following local AEs will be solicited:

- Arm Pain [CRF term: Pain at Site of Injection]
- Local Tenderness [CRF term: Tenderness at Site of Injection]
- Erythema (Redness) [CRF term: Redness at Site of Injection]
- Induration (Swelling/Firmness) [CRF term: Swelling at Site of Injection]

Note: CRF term in the square brackets refers to the term used on the Adverse Event Case Report Form to capture the local solicited AEs.

The following systemic (non-injection site) AEs will be solicited:

- Subjective Fever [CRF term: Subjective Fever]
- Objective Fever [CRF term: Fever]
- Chills [CRF term: Chills]
- Sweats [CRF term: Sweats]
- Myalgia [CRF term: Myalgia]
- Arthralgia [CRF term: Arthralgia]
- Joint Swelling [CRF term: Joint Swelling]
- Joint Tenderness [CRF term: Joint Tenderness]
- Fatigue [CRF term: Fatigue]
- Gastrointestinal Symptoms (symptoms include nausea, vomiting, abdominal pain, and diarrhea) [CRF terms: Nausea, Vomiting, Abdominal Pain, Diarrhea]
- Mucosal Lesions [CRF terms: Mucosal Lesion, Mouth Ulcer]
- Skin Lesions [CRF terms: Skin Lesion]
- Neurological Symptoms (symptoms include 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) [CRF terms: 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]

Neurological symptoms described above will be considered as solicited neurological AEs. Specific joint related (arthralgia, joint swelling, and joint tenderness) will be considered as solicited rheumatologic AEs; and mouth ulcer or skin/mucosal lesions will be considered as dermatologic AEs.

All TEAEs that cause a subject to seek medical care (including extra visits to the clinical site) are considered to be MAAEs.

Additionally, certain neurologic/hematologic/immunologic diseases are considered as AESIs in the study. Such AESIs 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 ≥ Grade 2, anaphylaxis, and vasculitides.

Summary tables will be generated for the following:

- Solicited local site AEs within 7 days, and 8 to 14 days following vaccination by Solicited Term and severity
- Solicited systemic AEs within 7 days, and 8 to 14 days following vaccination by Solicited Term and severity
- Solicited neurological AEs within 7 days, 8 to 14 days, 15 to 28 days and up to day 181 following vaccination by Solicited Term and severity
- Unsolicited AEs within 7 days, 8 to 14 days, 15 to 28 days and up to day 181 following vaccination by SOC, PT, relatedness, and severity

- Solicited Rheumatologic AEs within 28 days and up to day 181 following vaccination by Solicited Term and severity
- Solicited Dermatologic AEs within 28 days and up to day 181 following vaccination by Solicited Term and severity
- SAEs up to day 181 by SOC, PT and relatedness
- MAAEs up to day 181 by SOC, PT and relatedness
- AESIs at baseline, and up to day 181 by SOC, PT and relatedness

The number and percentage of subjects with any AE type as described above will be summarized by treatment group and combined PHV01 group. Adverse events are summarized by subject incidence rates; therefore, in these tabulations, each subject will contribute once (i.e., the most related occurrence or the most intense occurrence) to each of the incidence rates in the analysis regardless of the number of episodes. Tabulation by relatedness to study vaccine will be categorized as Related (Definite, Probable, Possible) or Not related; while tabulation by severity will be graded as Grade 1 (Mild), Grade 2 (Moderate), Grade 3 (Severe), Grade 4 (Potentially life-threatening).

Additionally, a symptom score will also be used to summarize solicited local and systemic AEs. The symptom score is a scalar quantity that summarizes the overall severity of a subject's solicited AE experience. The symptom index for a given AE will be defined as individual unique toxicity grades reported for each TEAE multiplied by the duration of the TEAE. The individual symptom indices will then be summed for each subject to obtain their symptom score. Larger scores are indicative, overall, of more severe and/or AEs of longer duration; smaller scores are indicative, overall, of less severe and/or AEs of shorter duration.

If an AE is ongoing at the end of the observation period, the length of the observation period post vaccination will be used in the calculation of symptom index. If the severity is missing for any particular AE, then the corresponding symptom index will also be set to missing.

Symptom scores will be summarized descriptively by treatment group and the combined PHV01 treatment group. Symptom scores will be calculated separately for all solicited local and systemic AEs which started within 14 Days of vaccination. The mean, standard deviation, median, minimum, and maximum values will be tabulated.

4.4.3. Vaccine Viremia

The level of vaccine viremia and shedding will be assessed for all subjects by RT-qPCR in blood, urine, and saliva. For RT-qPCR results reported as < LLOQ, the result will be assigned a value of LLOQ/2 at such visit for computations. For RT-qPCR results reported as < LOD, the result will be assigned a value of 1 genome copies/mL for computations.

Vaccine viremia duration is defined as the time from first administration of study vaccine to vaccine viremia clearance detected by RT-qPCR. Subjects without vaccine viremia clearance will be censored at the last evaluable assessment.

The distribution for vaccine viremia duration by treatment group will be estimated by Kaplan-Meier methodology and the 25th percentile, median, 75th percentile, number and percentage of events and censored observations, and appropriate 95% CIs will be presented. If the 95% CIs of

three PHV01 groups overlap then the analysis will be repeated comparing the combined PHV01 group with the placebo group. The corresponding Kaplan-Meier curves will also be generated.

The proportion of subjects with vaccine viremia in blood, urine, and saliva, as well as the geometric mean of vaccine viremia level and the geometric mean of peak level, will be presented on Day 1 (at 0 and 6 hours), 2, 4, 8, 15, and 29 by specimen type, dose level and the combined PHV01 group.

Reverse cumulative distribution curves of rVSV RNA copies by specimen type will be generated by treatment group on Day 1 (at 0 and 6 hours), 2, 4, 8, 15, and 29, respectively, where the x-axis will be the observed log-10 transformed titers and the y-axis will be the percentage of subjects having a log-10 transformed titer greater than or equal to each of the observed log-10 transformed titers on the x-axis.

Line plots will present number of rVSV RNA copies by specimen type at each visit by treatment group.

4.4.4. Vaccine Viremia Area Under the Curve

Vaccine viremia area under the curve (AUC) will be estimated using the trapezoid rule. Calculations will be performed relative to the first vaccination for each subject.

Calculations relative to the first vaccination will be based on figures displaying the number of copies of rVSV RNA on the Y axis and the actual number of days after the first vaccination that the blood sample was taken on the X axis. The general formula used to calculate AUC will be:

$$AUC_i = \sum_{i=0}^{n[\ \ ]} \frac{1}{2} \times [(RNA\ Copies_k - RNA\ Copies_{k-1})\ ] \times [Days_k - Days_{k-1}\ ]$$

Where the index i represents subject, index k represents the time points measured in actual days in which the viremia sample was drawn, 'RNA Copies' represents the base-10 log transformed number of rVSV RNA copies, and Days represents the actual days the blood sample for viremia was taken.

Two sets of AUC calculations will be performed: AUC from first vaccination to Day 8, and AUC from first vaccination to Day 29. These quantities summarize viremia after the first vaccination.

AUC from first vaccination to Day 8 will be calculated using the above formula where k = Days 1 (at 0 and 6 hours), 2, 4, and 8.

AUC from first vaccination to Day 29 will be calculated using the above formula where k = where k = Days 1 (at 0 and 6 hours), 2, 4, 8, 15, 29.

Each set of AUC calculations will be summarized descriptively by the geometric mean AUC, geometric standard deviation, median, minimum, and maximum value, by treatment group. Descriptive statistics will also be presented for the combined PHV01 treatment group. Calculation of the geometric mean AUC and standard deviation will follow the approach outlined in Section 4.4.3.

Spearman's rank correlation will be used to estimate the association between viremia AUC and IgG ELISA antibody titers for each PHV01 vaccine dose group including the combined PHV01 group at Day 29. A similar analysis will be performed to estimate the correlation between viremia AUC and PsVNA titers.

Spearman's rank correlation will be used to estimate the association between the viremia AUC (Day 0 to 29) with the AE symptom index calculated for solicited local AEs that occurred from Day 0 to Day 29 for each vaccine dose group. The same analysis will be repeated using the AE symptom index calculated for systemic AEs.

4.4.5. Laboratory Data

Clinical laboratory values will be expressed in standard international (SI) units and results will be graded by the local laboratory as low, normal, or high. Additionally, select parameters will also 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" outlined in Table 5-4 and Table 5-5. For analyses purposes, results not meeting grading criteria will be graded as Grade 0.

Descriptive statistics for serum chemistry, hematology, and coagulation values at baseline, all scheduled post-baseline visits, and the last study visit will be summarized for each clinical laboratory parameter. In the event of repeat values, the last non-missing value per study day/time will be used. Change from baseline (Day 1) will be calculated for each subject as the value at the specified post-baseline study visit minus the baseline value. The descriptive statistics for actual and change from baseline will include: the mean, standard deviation, median, and range of all values for each test.

Shift tables of change in toxicity grade for select laboratory parameters from baseline to each scheduled post-baseline visit and from baseline to last value on study will also be presented by dose level, all PHV01 dose levels combined and placebo. The numbers and percentages of subjects in each treatment group who had values below, within, and above the normal range at baseline and at the last visit will also be presented.

The results of rVSV PCR from blood, urine, and saliva will be tabulated by visit and treatment group.

All laboratory data will be provided in data listings. A subset listing will be presented for all toxicity graded laboratory values and abnormal laboratory values.

4.4.6. Vital Signs and Physical Examination

The actual value and change from baseline (Day 1) to each on-study evaluation will be summarized for vital signs.

Vital sign measurements will be presented for each subject in a data listing.

All physical examination, dermatologic, joint assessment and neurologic exam findings will be presented in a data listing.

4.4.7. Concomitant Medications

Concomitant medications will be coded using the WHO Drug Dictionary. Results will be tabulated by anatomic therapeutic class (ATC) and PT.

Concomitant medications will be tabulated by treatment group, where any medications that did not end prior to vaccination will be included. If an end date is missing or the medication is ongoing, the medication will be included. Medication that ended prior to vaccination will be presented separately as prior medication.

The use of prior and concomitant medications will be included in a by-subject data listing.

Additional non-study vaccinations will be noted.

5. CLINICAL AND LABORATORY GRADING SCALES

Table 5-1 and Table 5-2 below illustrate toxicity grading scales based on FDA Guidance (Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials) that will be used to assess local and systemic solicited AEs.

Table 5-1 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 5-2 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

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
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 5-3, Table 5-4, and Table 5-5 show the grading scales used to assess vital signs, to assess toxicity of blood, chemistry, and urinalysis AEs, and to assess toxicity of hematology AEs.

Table 5-3 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 5-4 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	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma

Serum[a,b]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fasting – mg/dL Random – mg/dL				
Liver Function Tests – ALT, AST	1.1 – 2.5 × ULN	2.6 – 5.0 × ULN	5.1 – 10 × ULN	> 10 × ULN
Bilirubin – accompanied by any increased LFT	1.1 – 1.25 × ULN	1.26 – 1.5 × ULN	1.51 – 1.75 × ULN	> 1.75 × ULN
Bilirubin – when LFT is normal	1.1 – 1.5 × ULN	1.6 – 2.0 × ULN	2.0 – 3.0 × ULN	> 3.0 × ULN

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.

Table 5-5 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.

6. CHANGES TO PLANNED ANALYSES

All changes from procedures outlined in the protocol and procedures outlined in this SAP will be summarized in the study report. Decisions to deviate from planned analyses will be documented at the time they are made.

If any modifications in the experimental design, dosages, parameters, subject selection, or any other sections of the protocol are indicated or required, the Investigator will consult with the Sponsor before such changes are instituted. Modifications will be accomplished through formal amendments to this protocol by the Sponsor and approval from the appropriate Institutional Review Board (IRB) or Independent Ethics Committee (IEC).

7. REFERENCES

CDC. Marburg Virus Disease Outbreaks: CDC, 2023: [updated 9Jun2023; Accessed 13Oct2023]. Available from: https://www.cdc.gov/vhf/marburg/outbreaks/chronology.html.

Miraglia CM. Marburg viruses: An Update. Lab Med. 2019; 50(1):16-28.

8. REVISION HISTORY

8.1. Changes from Statistical Analysis Plan Version 1.0: 23 April 2024

Revisions were made to the SAP sections 1.2.5.2, 4.3, and 4.4.3 to reflect the handling of <LOD and <LLOQ results for immunogenicity and vaccine viremia analyses. The data handling rules were provided in the following memorandums from Battelle and PHV, respectively:

- Memo titled "Reporting of Test Sample Values, including LOD and LLOQ, for B06748 Data Transfer Files and Final Report Data Tables", dated 28 May 2024.
- Memo titled "PHV01-C-101 Translation of Test Sample Results and Definitions", dated 30 May 2024.