A Phase 1, Randomized Trial to Assess the Safety, Reactogenicity, and Immunogenicity of a Combination HTNV and PUUV DNA Vaccine Candidate Administered by Electroporation

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| **Sponsor** | The Surgeon General, Department of the Army |
| **Sponsor's Representative** | Mark S. Paxton JD, MS<br>US Army Medical Research and Development Command (USAMRDC)<br>1430 Veterans Drive<br>Fort Detrick, MD 21702-5009<br>Telephone: 301-619-0317; DSN: 343-0317<br>Fax: 301-619-0197<br>E-mail: usarmy.detrick.medcom-usamrmc.mbx.regulatory-affairs@mail.mil |
| **National Institutes of Allergy and Infectious Diseases Division of Microbiology and Infectious Diseases Funding Mechanism** | Contract No. HHSN272201200019C |
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| **Site Investigational Product Accountability** | UMMC Investigational Drug Service<br>University of Maryland Medical Center<br>IDS Pharmacy, Room N9E14<br>22 South Greene Street<br>Baltimore, MD 21201<br>Telephone: 410-328-5344/5468<br>Fax: 410-328-7326 |

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| **Delivery Device Supplier** | Drew Hannaman<br>Ichor Medical Systems, Inc.<br>6310 Nancy Ridge Drive, #107<br>San Diego, CA 92121<br>Telephone: 858-550-2022 ext. 103<br>Fax: 858-550-2092<br>E-mail: dhannaman@ichorms.com |
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# INVESTIGATOR'S AGREEMENT

**A Phase 1, Randomized Trial to Assess the Safety, Reactogenicity, and Immunogenicity of a Combination HTNV and PUUV DNA Vaccine Candidate Administered by Electroporation**

*"I have read this protocol and agree to conduct the study as outlined herein in accordance with US Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312); International Council for Harmonisation Good Clinical Practice Guideline (E6); 62 Federal Register 25691 (1997); National Institutes of Health Clinical Terms of Award; and FDA, DoD, and US Army Regulations. In addition, I will ensure that key personnel (individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training."*

_____ _____

Kirsten E. Lyke, MD
Principal Investigator
University of Maryland Center for Vaccine Development

Date

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Kirsten E. Lyke, MD
Principal Investigator
University of Maryland Center for Vaccine Development

03 Mar 2022
Date

# EMERGENCY CONTACT INFORMATION

**Table 1: Emergency Contact Information**

| Role in Study | Name | Address and Telephone Number |
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| Principal Investigator | Kirsten E. Lyke, MD | 685 W. Baltimore Street, HSF I, Room 480<br>Baltimore, MD 21201<br>Telephone: 410-706-5328/0462<br>Fax: 410-706-6205<br>E-mail: klyke@som.umaryland.edu |
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| Study Coordinator/Protocol Nurse | Wanda Somrajit, RN<br>Shirley George, Clinical Research Specialist | 685 W. Baltimore Street, HSF I, Room 480<br>Baltimore, MD 21201<br>Telephone: 410-706-6156<br>E-mail: wsomrajit@som.umaryland.edu<br>Shirley.george@som.maryland.edu |
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| **Role in Study** | **Name** | **Address and Telephone Number** |
|---|---|---|
| Institutional Review Board | Human Research Protections Office | University of Maryland, Baltimore<br>620 W. Lexington Street, Second Floor<br>Baltimore, MD 21201<br>Telephone: 410-706-5037<br>Fax: 410-706-4189<br>E-mail: HRPO@som.umaryland.edu |

# 1. SYNOPSIS

**Name of Sponsor:**
The Surgeon General, Department of the Army

**Name of Investigational Product(s):**
Hantaan Virus (HTNV) and Puumala Virus (PUUV) DNA Vaccines

**Name of Active Ingredients:**
pWRG/HTN-M(co) Plasmid (HTNV DNA Vaccine) and pWRG/PUUV-M(s2) Plasmid (PUUV DNA Vaccine)

**Title of Study:**
A Phase 1, Randomized Trial to Assess the Safety, Reactogenicity, and Immunogenicity of a Combination HTNV and PUUV DNA Vaccine Candidate Administered by Electroporation

**Study No.** HP-00081472; S-15-39; DMID 12-0100

**Study Center:**
University of Maryland Center for Vaccine Development

**Principal Investigator:**
Kirsten E. Lyke, MD

**Subinvestigator:**
Joel Chua, MD

| **Study Period (years):** | **Phase of Development:** 1 |
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| Approximately 5 to 11 months per subject, depending on Group assignment<br>Estimated date first subject enrolled: April/May 2019<br>Estimated date last subject completed: December 2022 | |

**Objectives:**

**Primary:**

- To evaluate the safety and reactogenicity of the hantaan virus (HTNV), puumala virus (PUUV), and combination HTNV/PUUV DNA vaccine candidates delivered to healthy adults either intramuscularly (IM) or intradermally (ID) by electroporation (EP).

**Secondary:**

- To obtain a preliminary assessment of the immunogenicity of the combination HTNV/PUUV DNA vaccine candidate relative to the monovalent formulations.
- To identify the HTNV and/or PUUV DNA vaccine combination and route of administration that elicits the most immunogenic response as determined by seroconversion and seropositivity rates, and geometric mean titers (GMT) as measured by PsVNA50.

**Methodology:**

A maximum of 82 subjects will be enrolled in the study. The study had initially planned to enroll 6 randomized groups of 12 subjects each, for a total of 72 subjects. Ten subjects in Cohort 6 may be replaced because vaccination and data collection were negatively impacted in this group by COVID-19 pandemic quarantine restrictions. This approach will ensure at least 60 subjects complete all vaccinations at around 10 subjects per group, taking possible attrition into account. Subjects will receive one dose of vaccine on each of Days 0, 28, and 56 and will be followed until Day 220.

- Group 1 (12 subjects): HTNV by ID EP
- Group 2 (12 subjects): HTNV by IM EP
- Group 3 (12 subjects): PUUV by ID EP
- Group 4 (12 subjects): PUUV by IM EP
- Group 5 (12 subjects): HTNV/PUUV by ID EP
- Group 6 (22 subjects): HTNV/PUUV by IM EP

**Estimated Number of Subjects Screened:**

It is estimated that up to 216 individuals will be screened to fill the desired enrollment of 82 subjects.

**Maximum Number of Subjects Enrolled:**

82

**Main Criteria for Inclusion/Exclusion:**

**Inclusion Criteria:**

1. Healthy adult male or nonpregnant, nonlactating female, ages 18-49 (inclusive) at time of screening
2. Have demonstrated adequate comprehension of the protocol by achieving a score of at least 80% correct on a short multiple-choice quiz. Individuals who fail to achieve a passing score on the initial quiz will be given the opportunity to retest after a review of protocol information. Individuals who fail the quiz for the second time will not be enrolled
3. Have provided written informed consent before screening
4. Subject is in good health[a] as determined by past medical history, medication use, and abbreviated physical examination[b]
5. Available and able to participate for all study visits and procedures
6. Sexually active men[c] and women of childbearing potential[d] must agree to use an effective method of contraception from 30 days prior to the first study vaccination until 6 months after the last study vaccination
7. Female subjects agree to not donate eggs (ova, oocytes), and male subjects agree to not donate sperm from the start of screening until at least 6 months after the last vaccination
8. Female subjects of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to each study vaccination
9. Negative hantavirus PsVNA test result at screening
10. Screening laboratory test values:
    a. Hemoglobin > 11.0 g/dL for women; > 12.9 g/dL for men

b. WBC with differential and platelets either within the normal range (provided by the laboratory performing the analysis) or a Grade 1 deviation from normal (per Appendix C) and deemed clinically insignificant

c. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin < 1.1x upper limit of normal (ULN) (per the normal range provided by the laboratory performing the analysis)

d. Serum creatinine < 1.35 g/dL

– For all other screening laboratory test values, results that do not meet the definition of a Grade 1-4 value within 56 days prior to enrollment (see Appendix C, "Clinical and Laboratory Toxicity Grading Scales") are eligible for enrollment

**Exclusion Criteria:**

1. History of prior infection with any hantavirus virus or prior participation in an HTNV, PUUV, or Andes virus vaccine trial
2. Has plans to travel to an area with endemic[e] Hantaan, Puumala, Seoul, and Dobrava virus transmission during the study
3. History of severe local or systemic reactions to any vaccine or vaccine products[f] or a history of severe allergic reactions
4. Is currently participating in or plans to participate in another study involving any investigational product (eg, vaccine, drug, or biologics) or a study that involves blood drawing, and/or an invasive procedure[g]
5. Receipt or planned receipt of any live vaccination, experimental or otherwise, within the period 30 days prior to or after each vaccination and receipt or planned receipt of an inactivated vaccination, experimental or otherwise, within the period of 14 days prior to or after each vaccination
6. Individuals, who based on clinical assessment by the investigator, have insufficient muscle mass to accommodate the 1 inch/25 mm penetration depth or have a skinfold thickness at eligible injection sites (deltoid region) that exceeds 40 mm
7. Individuals in whom the ability to observe local reactions at the eligible injection sites (deltoid region) is, in the opinion of the investigator, unacceptably obscured due to a physical condition or permanent body art
8. Presence of any surgical or traumatic metal implants at the injection site (medial deltoid muscles or overlying skin)
9. Subjects with autoimmune disorders or chronic inflammatory disorders with a potential autoimmune correlation
10. Receipt of immunoglobulins and/or any blood products within the 120 days preceding screening or planned administration during the study period
11. Donation of blood to a blood bank within 56 days prior to screening and at any time during the study period
12. Subject seropositive for hepatitis B surface antigen (HBsAg) or hepatitis C antibodies (anti-HCV)

13. Any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection, or use of anticancer chemotherapy or radiation therapy (cytotoxic) in the 3 years prior to screening
14. Administration of chronic (defined as more than 14 days) immunosuppressants or other immune-modifying drugs within 6 months of screening. For corticosteroids, this will mean prednisone, or equivalent, greater than or equal to 5 mg/day. Intranasal, inhaled (< 800 beclomethasone mcg/day), and topical steroids are allowed
15. Has current or past diagnosis of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations
16. Has been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to screening
17. Any chronic or active neurologic disorder, including Guillain-Barré syndrome, seizures, and epilepsy, excluding a single febrile seizure as a child
18. Syncopal episode within 12 months of screening
19. Suspected or known current alcohol and/or illicit drug abuse within the past 5 years based on self-reporting and physical exam
20. Any medical, psychiatric, social condition, occupational reason, or other responsibility that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a subject's ability to give informed consent or to comply with the protocol schedule
21. Pregnant or lactating female, or subject plans to father a child or become pregnant during the study period
22. Current employee or staff paid entirely or partially by the contract for this trial, or staff who are supervised by the PI or subinvestigators
23. Subjects with implanted electronic stimulation devices, such as cardiac demand pacemakers, automatic implantable cardiac defibrillator, nerve stimulators, or deep brain stimulators
24. Bleeding diathesis or condition associated with prolonged bleeding time, as determined through measurement of PT/PTT (prothrombin time/partial thromboplastin times) during screening labs, that would contraindicate ID or an IM injection
25. History of cardiac arrhythmia or palpitations or abnormal arrhythmia noted on ECG performed at screening (eg, supraventricular tachycardia, atrial fibrillation, frequent ectopy) prior to study entry. Measurement of sinus bradycardia (ie, < 50 beats per minute on exam) at screening
26. History of diabetes type 1 or 2

**Temporary Exclusion Criteria:**

1. The following criteria will result in a delay for vaccination of subjects:
    a. A febrile illness (oral temperature of > 38.0°C) within 48 hours before vaccination or other evolving acute illness
    b. Use of antibiotics for an acute illness within 24 hours or any antiviral within 72 hours of study vaccination
    c. Use of allergy treatment with antigen injections within 30 days prior to initial study vaccine administration

Investigational Product Dosage, Schedule, and Mode of Administration:

| Group | Product | Dose/Route | Dilution Preparation | Injection Volume | Administration Schedule |
|---|---|---|---|---|---|
| 1 | HTNV | 0.6 mg/ID EP | 0.1 mL 6.0 mg/mL HTNV DNA + 0.1 mL 0.9% saline | 0.2 mL | Days 0, 28, and 56 |
| 2 | HTNV[1] | 3.0 mg/IM EP | 0.5 mL 6.0 mg/mL HTNV DNA + 0.5 mL 0.9% saline | 1.0 mL | Days 0, 28, and 56 |
| 3 | PUUV | 0.6 mg/ID EP | 0.1 mL 6.0 mg/mL PUUV DNA + 0.1 mL 0.9% saline | 0.2 mL | Days 0, 28, and 56 |
| 4 | PUUV[1] | 3.0 mg/IM EP | 0.5 mL 6.0 mg/mL PUUV DNA + 0.5 mL 0.9% saline | 1.0 mL | Days 0, 28, and 56 |
| 5 | HTNV/PUUV[1] | 1.2 mg/ID EP (0.6 mg each) | 0.1 mL 6.0 mg/mL HTNV DNA + 0.1 mL 6.0 mg/mL PUUV DNA | 0.2 mL | Days 0, 28, and 56 |
| 6 | HTNV/PUUV[1] | 6.0 mg/IM EP (3.0 mg each) | 0.5 mL 6.0 mg/mL HTNV DNA + 0.5 mL 6.0 mg/mL PUUV DNA | 1.0 mL | Days 0, 28, and 56 |

The HTNV and PUUV DNA vaccines will be administered using the IM or ID TriGrid™ Delivery System (TDS-IM V1.0 or TDS-ID, respectively), which uses electroporation (the application of electrical pulses to improve the uptake of DNA into the cells). The system consists of 3 components: a pulse stimulator, an integrated applicator, and a single-use application cartridge. The device is manufactured by Ichor Medical Systems, Inc, San Diego, California. The TDS-IM device in use for this study as well as past HTNV and PUUV vaccine trials has been the first version of the device, therefore the name has been changed starting Version 5.0 of this protocol to adopt the nomenclature TDS-IM V1.0, such as it is termed in the IB.

Every subject will receive 1 injection on Days 0, 28, and 56 for a total of 3 injections, and subjects will be followed up to 6 months after the final vaccination (to Day 220).Sentinel subjects will not be used for Groups 1 and 3 because the IP dose is lower than the 2.0 mg/mL dose that was previously evaluated in human subjects.

Dosing for sentinels and main study subjects will progress as follows:

[1] Four of the 6 study groups include sentinel* subjects because the dose of investigational product (IP) has not been evaluated in human subjects, and we would like to evaluate the safety of the IP dose before the remainder of the subjects from the respective group are dosed (Appendix B).

- For Groups 2 and 4, the first dose will be given to 2 sentinel subjects (n = 2 per group, total of 4 subjects) on the same day, and those 4 subjects will be followed for 48 hours as per the Sentinel Group Halting Rules (Section 6). The SRC will meet and evaluate the safety data collected in the 48-hour period post-vaccination for sentinels in Groups 2 and 4. If the SRC finds that a halting rule(s) has been met (Section 6.6.1.1), the PI will notify ORA PSSO within 24 hours. A Safety Monitoring Committee (SMC) meeting will be held to review clinical and laboratory safety and reactogenicity data. After the meeting, the SMC will make its recommendations in writing regarding continuation, modification, or termination of the clinical trial. If the recommendation is to proceed, enrollment and randomization of the remaining volunteers in Groups 2 and 4 (n=10 per group, total of 20) for the first dose (Day 0) will commence. Subjects in Groups 2 and 4 will be evaluated for 48-hours post-vaccination for any halting criteria on the Day 2 clinic visit (Table 4).
- After the first dose for all subjects in Groups 2 and 4 has been successfully completed and none of the halting criteria are met, then the first dose will be given to 2 sentinel subjects in Group 6 (n=2 per group, total of 2subjects) on the same day, and those 2 subjects will be followed for 48 hours as per the Sentinel Group Halting Rules. The SRC will meet and evaluate the safety data collected in the 48-hour period post-vaccination for sentinels in Group 6. If the SRC finds that the halting rules have been met (Section 6.6.1.1), the PI will notify ORA PSSO within 24 hours. An SMC meeting will be held to conduct a review of clinical and laboratory safety and reactogenicity data. After the meeting, the SMC will make its recommendations in writing regarding continuation, modification, or termination of the clinical trial. If the recommendation is to proceed, enrollment, randomization, and vaccination (Day 0) of the remaining volunteers in Group 6 (n=10 per group). The first dose will be given to 2 sentinel subjects in Group 5 (n=2, total of 2 subjects) on the same day and those 2 subjects will be followed for 48 hours as per the Sentinel Group Halting Rules. The SRC will meet and evaluate the safety data collected in the 48-hour period post vaccination for sentinels in Group 5. If the SRC finds that the halting rules have been met (Section 6.6.1.1) the PI will notify ORA PSSO within 24 hours. A SMC meeting will be held to conduct a review of clinical and laboratory safety and reactogenicity data. After the meeting, the SMC will make its recommendations in writing regarding continuation, modification, or termination of the clinical trial. If the recommendation is to proceed, enrollment, randomization and vaccination (Day 0) of the subjects in Groups 1 and 3 (n=12 per group) will commence. Subjects in Groups 1 and 3 will be evaluated for the 48-hours post-vaccination for any halting criteria on the Day 2 clinic visit (Table 4). After completion of enrollment and first vaccination for Groups 1 and 3, the remaining subjects for Group 5 and ten Group 6 replacements (total of 20 subjects) may be enrolled, randomized and vaccinated (Day 0), provided that the enrollment of these 20 subjects can be completed within 4 months.

At this time, all groups will have received the first dose and the trial will advance through the prescribed visits and additional vaccinations.

**Duration of Treatment:**

Approximately 5 to 11 months, depending on Group assignment (8 months based on a 28-day month): Up to 56 day screening period, 3 doses over 2 months and a 6 month follow-up period.

**Criteria for Evaluation:**

**Safety Endpoints:**

1. The nature, frequency, and severity of adverse events (AEs) and/or serious adverse events (SAEs) associated with TDS-EP-based administration of HTNV and PUUV DNA vaccines.
2. The occurrence of solicited local and systemic AEs occurring from the time of each injection through 14 days following the procedure (memory aid to assist with the first 14 days)
3. The occurrence of vaccine-related unsolicited AEs from the time of the first injection through 28 days following each injection
4. The occurrence of SAEs from the time of the first injection through the final study visit for each subject (approximately 6 months post-last vaccination)
5. The occurrence of clinical safety laboratory AEs through 14 days following each study vaccination

**Immunogenicity Endpoints:**

1. Development and characteristics of an HTNV-specific antibody (PsVNA50 titer) and/or PUUV-specific antibody (PsVNA50 titer) of ≥ 1:20.
2. Determination of the proportion of seropositive subjects (defined as PsVNA50 ≥ 1:20) at each scheduled time point (eg, Days 0, 28, 56, 84 and 140)
3. Determination of the final overall rate of seroconversion over all scheduled time points (eg, Days 0, 28, 56, 84 and 140). Seroconversion is defined as a post-vaccination HTNV- or PUUV-specific titer of ≥ 1:40, or a minimum four-fold rise compared to baseline titer, and all study volunteers will begin the study with a baseline titer < 20 (eg, seronegative).
4. Determination of GMT of the PsVNA50 for HTNV- and PUUV-specific neutralizing antibodies at each scheduled time point (eg, Days 0, 28, 56, 84 and 140).

**Exploratory Endpoints:**

1. Exploratory endpoints, evaluated as part of the immunogenicity objectives, including calculation of PsVNA80 titers for HTNV and PUUV may be calculated from the collected data for informational purposes, and neutralization assays using other hantavirus PsV (eg, Seoul virus, Dobrava virus) may also be performed to evaluate levels of cross-neutralizing antibodies.
2. Determination of GMT of neutralizing of the PsVNA80 for HTNV- and PUUV-specific neutralizing antibodies at each scheduled time point for which blood samples are taken for each study group and over all time points for each study group
3. Determination of GMT of neutralizing of the PsVNA80 for Seoul- and Dobrava-specific neutralizing antibodies at each scheduled time point for which blood samples are taken for each study group and over all time points for each study group

**Statistical Methods:**

- Descriptive analysis of safety and reactogenicity outcomes will include all subjects who meet the eligibility criteria, receive at least one vaccination, and for whom safety data are available. Summary tables will be created in which incidence, gradation, and relationship to the use of investigational product of individual solicited local and systemic signs, symptoms, or unsolicited events are delineated by study group, severity, gender, and overall. Unsolicited AEs and SAEs will be analyzed in a similar fashion. To address the

primary objective of the study, safety and reactogenicity endpoints will include determining the nature, frequency, severity, and relatedness of AEs and/or SAEs associated with TDS-EP-based administration of HTNV, PUUV, and HTNV/PUUV DNA vaccines.

- To address the secondary objectives of the study, immunogenicity endpoints will be evaluated. Descriptive analysis of immunogenicity outcomes will include all subjects who meet the eligibility criteria, receive at least one vaccination, and for whom serological data are available. The primary analysis variable will be the proportion of seropositive subjects (defined as PsVNA50 ≥ 1:20) at each scheduled time point for which blood samples are taken (eg, Days 0, 28, 56, 84 and 140) and the final overall rate of seroconversion over all scheduled time points to study completion for each study group. Seroconversion is defined as a post-vaccination HTNV- or PUUV-specific titer of ≥ 1:40, or a minimum four-fold rise compared to baseline titer and all study volunteers will begin the study with a baseline titer < 20 (ie, seronegative). For each study group, a binomial proportion and 95% confidence interval (CI) will be calculated. The secondary analysis variable will be GMTs, with 95% CIs, of the PsVNA50 for HTNV- and PUUV-specific antibodies at each scheduled time point for which blood samples are taken for each study group and over all time points for each study group. GMTs, standard errors, and 95% CIs will be calculated using log-transformed titers. For titers below the lower limit of detection (< 20), the value will be transformed to a value equal to the lower limit of detection (ie, 20) divided by the square root of 2, which equals 14.1. Exploratory objectives may evaluate PsVNA80 titers, which may be calculated for informational purposes, and may evaluate PsVNA50 titers against related Seoul and Dobrava viruses.
- For hematology and serum chemistry tests, any clinically significant changes from baseline value will be identified. The median, interquartile range, and normal values for each laboratory value (as determined by the contract laboratory) will be reported for each treatment group for each specimen collection point.

**Sentinel Group Halting Rules:**

The following halting rules will be applied in the 48 hours following sentinel group vaccination (n = 2 subjects per cohort) for review by the designated members of the study Safety Review Committee (SRC) and if any are met, further vaccinations will be halted pending SRC and Safety Monitoring Committee (SMC) review:

1. Any SAE regardless of the relationship to the vaccine (with the exception of death or hospitalization that was the result of trauma or accident)
2. Type 1 hypersensitivity reaction (anaphylaxis or generalized urticarial)
3. Any Grade 3 systemic adverse event (solicited and unsolicited)
4. Any Grade 3 local adverse event (solicited and unsolicited) that has not reduced to a Grade 1 or 2 at 48 hours post vaccination[h]

**Study Halting Rules:**

Further enrollment and study vaccinations will be halted for SMC review/recommendation if any of the following are reported:

1. Any subject experiences a study product-related SAE from the time of the study product administration through the subject's last study visit.

2. Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
3. Two or more subjects experience generalized urticaria (defined as occurring at more than 2 body parts) within 3 days after administration of study product that is considered related to study product.
4. This trial will also be halted for SMC review/recommendation if, within 7 days after administration of any study vaccination, any of the following occurs:
5. Two or more subjects experience a Grade 3 unsolicited AE in the same MedDRA system organ class (captured by preferred term) after administration of study product that is considered related to study product and not resolved or improved to lower grade within 2 days.
6. Two or more subjects experience the same Grade 3 solicited local adverse event that is considered related to study product and not resolved or improved to lower grade within 2 days.
7. Two or more subjects experience the same Grade 3 solicited systemic adverse event that is considered related to study product and not resolved or improved to lower grade within 2 days.
8. Two or more subjects experience the same Grade 3 laboratory adverse event that is considered related to study product.

- Grading scales for solicited local and systemic AEs are included in Section 10.5 and Appendix C.
- Grading scales for clinical safety laboratory adverse events are included in Section 10.5 and Appendix C.

If any of the halting rules are met following any subject receipt of any study vaccination, then this trial will not continue with the remaining enrollments or study vaccinations without a review by and recommendation from the SMC. The SMC recommendation will be documented and provided in writing to the ORA PSSO. The sponsor's representative will then decide to terminate, modify, or continue the conduct of the study. The ORA PSSO will communicate the final decision to the appropriate parties involved in the study (ie, PI, RM/ISM, and DMID). The PI will in turn notify the IRB as appropriate. USAMRDC RA Scientist will communicate the final decision to FDA as appropriate.

DMID retains the authority to suspend additional enrollment and study interventions/administration of study product during the entire trial, as applicable.

**Safety Oversight:**

**Research Monitor/Independent Safety Monitor (RM/ISM):**

- The RM/ISM oversight position is required by the sponsor per Department of Defense Instruction 3216.02. The RM/ISM, provided by and local to the Center for Vaccine Development (CVD) at University of Maryland in Baltimore, is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely manner. The RM/ISM will review SAEs in real time and unanticipated problems involving risks to subjects or others.

- The RM/ISM is responsible for overseeing the safety of the research and reporting observations/findings to the IRB or a designated institutional official. The RM/ISM will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The RM/ISM may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the RM/ISM's report; and shall have the responsibility to promptly report his or her observations and findings to the IRB or other designated official.
- In addition to the responsibilities above the RM/ISM is required to review and provide an unbiased written report for all SAEs and subject deaths to the USAMRDC ORA PSSO (Safety Office) within 24 hours of awareness of the event. The report provided must include, at a minimum, a brief summary of the RM/ISM's review of the event and event outcome, relationship of the event to the investigational product, and whether or not the RM/ISM concurs with the details of the study investigator's report.

**Safety Review Committee (SRC):**

The SRC will be composed of:

- PI and/or subinvestigator
- RM/ISM
- DMID medical monitor
- USAMRDC pharmacovigilance physician or designee

Objective pre-defined halting rules and safety evaluations will be utilized. For sentinel subjects, the SRC will evaluate safety data at 48 hours post first dose. If none of the predefined halting rules are observed in the sentinel subjects, vaccination of the remainder of the cohorts will proceed. Should any of the study halting rules be met, the PI will notify ORA PSSO within 24 hours. The SMC will meet to evaluate data and make a recommendation regarding the trial. The SMC recommendation will be documented and provided in writing to the ORA PSSO. The sponsor's representative will then decide to terminate, modify, or continue the conduct of the study. The ORA PSSO will communicate the final decision to the appropriate parties involved in the study (ie, PI, RM/ISM, and DMID). The PI will in turn notify the IRB as appropriate. USAMRDC RA Scientist will communicate the final decision to FDA as appropriate.

**Safety Monitoring Committee (SMC):**

- Safety oversight will be conducted by an SMC, which is an independent group of experts that monitors subject safety and advises DMID. SMC members will be separate and independent of study personnel participating in this study and should not have scientific, financial, or other conflicts of interest related to the study. The SMC will consist of at least 3 voting members with appropriate expertise to contribute to the interpretation of safety data from this trial. The SMC will operate under the rules of a DMID-approved charter. The SMC will review applicable data to include but not limited to enrollment, demographics, dosing, and clinical and safety data, which may include solicited and unsolicited AEs/SAEs, reactogenicity, concomitant medications, clinical safety laboratory values, and any physical examinations at scheduled time points during the study as defined in the charter. The objective of the SMC is to make recommendations to the sponsor if the study should continue per protocol, be modified and then proceed, or be

terminated. After each meeting the SMC will make its recommendations in writing to the sponsor regarding continuation, modification, or termination of the clinical trial.

SMC meetings for data review are as follows:

1. Organizational meeting (prior to start of the study)
2. An SMC ad hoc meeting will be convened when a halting rule is met or at the request of the investigator and/or DMID if there are safety concerns during the course of the study
3. Annual review meetings.
4. End of study safety data review (after database lock and prior to final clinical study report)
5. Data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID.

[a] Good health is defined by the absence of any medical condition described in the exclusion criteria in a subject with a normal abbreviated physical examination including vital signs. If the subject has another current, ongoing medical condition, the condition cannot meet any of the following criteria: (1) first diagnosed within 3 months of enrollment, (2) is worsening in terms of clinical outcome in last 6 months, or (3) involves need for medication that may pose a risk to subject's safety or impede assessment of adverse events or immunogenicity if they participate in the study.

[b] An abbreviated physical examination differs from a complete physical examination in that it does not include a genitourinary and rectal examination.

[c] A sexually active man is defined as one whose partner is a woman of childbearing potential (see definition below) and has not had a vasectomy performed > 1 year prior to screening. They must agree not to father a child until 6 months after the last vaccination. These subjects must agree to use a barrier method of birth control (eg, either condom with spermicidal foam/gel/film/cream or partner usage of occlusive cap [diaphragm or cervical/vault caps] with spermicidal foam/gel/film/cream/suppository).

[d] Women of childbearing potential are defined as those who have not been sterilized via tubal ligation, bilateral oophorectomy, bilateral salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with history of documented radiological confirmation test at least 90 days after the procedure and are still menstruating or < 1 year of the last menses if perimenopausal. For this study, an effective contraceptive method is defined as one that results in a failure rate of less than 1% per year when it is used consistently and correctly

[e] Refer to the MOP for information on areas with endemic Hantaan, Puumala, Seoul, and Dobrava virus transmission

[f] This includes a known allergy to an aminoglycoside (eg, gentamicin, tobramycin, neomycin, and streptomycin)

[g] 1. An invasive procedure includes endoscopy, bronchoscopy, or procedure requiring administration of IV contrast or removal of tissue

[h] Four of the 6 study groups include sentinel* subjects because the dose of investigational product (IP) has not been evaluated in human subjects, and we would like to evaluate the safety of the IP dose before the remainder of the subjects from the respective group are dosed (Appendix B).

[h] Injection site pain (if resolved 48 hours post vaccination), the size (measured in mm) of erythema, and the occurrence of induration/swelling will not be used as halting criteria.

# 2. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF TABLES AND FIGURES

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# 3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations are used in this study protocol.

**Table 2: Abbreviations**

| Abbreviation | Explanation |
|---|---|
| AE | adverse event, adverse experience |
| ALT | alanine aminotransferase |
| AR | Army Regulation |
| AST | aspartate aminotransferase |
| BUN | blood urea nitrogen |
| C | Celsius |
| CBC | Complete blood count |
| CDC | Centers for Disease Control and Prevention |
| CFR | Code of Federal Regulations |
| cGMP | current good manufacturing practice(s) |
| cm | centimeter |
| Cr | creatinine |
| CVD | Center for Vaccine Development and Global Health, UMB |
| DA | Department of the Army |
| DMID | Division of Microbiology and Infectious Diseases, NIH |
| DNA | deoxyribonucleic acid |
| DoD | Department of Defense |
| eCRF | electronic case report form |
| EDC | electronic data capture |
| EP | electroporation |
| F | Fahrenheit |
| FDA | US Food and Drug Administration |
| GMT | geometric mean titers |
| HFRS | hemorrhagic fever with renal syndrome |
| GBS | Guillain-Barré Syndrome |
| GCP | good clinical practice(s) |
| GLP | good laboratory practices |
| Hg | mercury |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | human immunodeficiency virus |

| Abbreviation | Explanation |
|---|---|
| HRPO | Human Research Protection Office |
| HTNV | Hantaan virus |
| ICH | International Council for Harmonisation |
| ID | intradermal |
| IEC | Independent Ethics Committee |
| IM | intramuscular |
| IND | investigational new drug |
| IP | investigational product |
| IRB | institutional review board |
| mg | milligram |
| mL | milliliter |
| mm | millimeter |
| NIH | National Institutes of Health |
| OHRP | Office for Human Research Protections, Department of Health and Human Services |
| ORA | Office of Regulated Activities, USAMRDC |
| ORP, HRPO | Office of Research Protections, Human Research Protection Office |
| PI | principal investigator |
| PSSO | Product Safety Surveillance Office |
| PsVNA | pseudovirion neutralization assay |
| PT/PTT | prothrombin time/partial thromboplastin time |
| PUUV | Puumala virus |
| RM/ISM | research monitor/independent safety monitor |
| SAE | serious adverse event |
| SAP | statistical analysis plan |
| SMC | Safety Monitoring Committee |
| SOP | standard operating procedure |
| SRC | Safety Review Committee |
| SSP | study-specific procedures |
| TDS-ID | TriGrid™ Delivery System for intradermal delivery |
| TDS-IM V1.0 | TriGrid™ Delivery System for intramuscular delivery |
| TSG-DA | The Surgeon General, Department of the Army |
| ULN | upper limit of normal |
| UMB | University of Maryland, Baltimore |

| Abbreviation | Explanation |
|---|---|
| UPIRTSO | unanticipated problems involving risk to subjects or others |
| USAMMDA | US Army Medical Materiel Development Activity |
| USAMRIID | US Army Medical Research Institute of Infectious Diseases |
| USAMRDC | US Army Medical Research and Development Command |
| VEEV | Venezuelan equine encephalitis virus |

# 4. INTRODUCTION

The genus *Hantavirus* of the family *Bunyaviridae* includes more than 20 viruses known to cause human infections (Jonsson et al, 2010). The hantavirus family has a propensity for regional variation, although the viruses were evolutionarily likely derived from a common ancestor approximately 2000 years ago (Souza, 2014). Like other bunyaviruses, hantaviruses are spherical, lipid membrane-enclosed viruses containing 3 negative-sense RNA segments encoding a small number of proteins. In hantaviruses, these segments include the following: a large (L) segment that encodes the RNA-dependent RNA polymerase; a medium (M) segment that encodes 2 envelope glycoproteins (Gn and Gc, formerly G1 and G2, respectively); and a small (S) segment that encodes the nucleocapsid protein (N) (Schmaljohn and Nichol, 2007). Antibodies to both GN and GC have been demonstrated to be protective in passive antibody studies in rodents (Custer et al, 2003; Hooper et al, 2008; Schmaljohn 2009; Brocato et al, 2012; Hooper et al, 2013; Brocato et al, 2014).

Hantaviruses are distributed worldwide among various rodent host populations. Transmission to humans occurs primarily through the inhalation of aerosolized rodent urine or feces or through the bite of an infected animal. Infection with hantaviruses can result in a range of clinical presentations from asymptomatic to life threatening. Four of the hantaviruses, Hantaan virus (HTNV), Dobrava virus, Seoul virus, and Puumala virus (PUUV), have been described in association with the potentially life-threatening hemorrhagic fever with renal syndrome (HFRS) (Peters, 2010). While each of these viruses is endemic to different regions, there is some overlap. HFRS is a prolonged, multistage disease that can cause fever, thrombocytopenia, hypotension, and acute renal insufficiency. Mortality rates as high as 15% have been reported, and full recovery in survivors may require weeks to months (Jonsson et al, 2010; Peters, 2010).

Other hantaviruses, such as Sin Nombre virus in North America and Andes virus in South America, have been associated with hantavirus pulmonary syndrome, another potentially life-threatening condition characterized by a febrile prodrome followed by a severe but temporary increase in pulmonary vascular permeability and shock. Mortality rates for this presentation now range from 35% to 40% (CDC, 2012; Simpson et al, 2010).

Treatment for hantavirus infections includes primarily supportive care measures. Use of antivirals such as ribavirin has demonstrated mixed results (Peters, 2010). Preventive measures include avoidance of rodents and their excreta. In regions of the world where HFRS is endemic, virus-specific inactivated-virus vaccines have been developed. However, reports on efficacy of these vaccines have been mixed (Schmaljohn, 2009; Hammerbeck et al, 2009).

## 4.1. Military Relevance

The US Food and Drug Administration (FDA) has not licensed a vaccine for (virus-specific or generalized) HFRS. Vaccine efforts against hantaviruses have been under way since 1990, and an inactivated rodent brain or cell culture-derived HFRS vaccine is available in China with millions of doses administered (Zhang, 2010). An inactivated vaccine by the trade name Hantavax™ has some efficacy against Puumala and Dobrava-Belgrade viruses (Cho, 1999) although no licensed products exist in Europe or the United States. Hantaviruses are found worldwide in wild rodent populations. Combat and noncombat operations within these environs may place US military personnel at risk of exposure and disease, from one or more regional

hantaviruses, through interaction with and disruption of rodent habitats. Historical evidence confirms this supposition. HFRS was first recognized during the Korean War (the name Hantavirus derives from the Hantaan river in Korea) where it was a major cause of noncombat-related morbidity and mortality among US and allied forces. Specifically, over 1,000 United Nations soldiers developed HFRS during this conflict, with an overall mortality of 5% to 10%. From 2000 to 2009, US forces in Korea experienced 8 hospitalizations and 72 ambulatory visits for hantavirus infections. The risk posed by hantaviruses to soldiers has prompted the Military Infectious Diseases Research Program within the US Army Medical Research and Development Command (USAMRDC) to develop an effective combined-virus vaccine against the viruses that cause HFRS.

## 4.2. Rationale for Study

The development of DNA (deoxyribonucleic acid) vaccines, such as those to be utilized in this study, is based on the observation that antigen-encoding DNA plasmids can induce both cellular and humoral immune responses against various viral and bacterial pathogens. DNA vaccines are perceived as having a number of potential advantages over other types of vaccines. For example, DNA vaccines are easily constructed by recombinant technology; easily and inexpensively manufactured as a well-characterized molecule [DNA plasmid]; and at boost, not eliminated by prior immune response to the carrier or vector. Furthermore, as nonliving vaccines, they cannot lead to infection. The object of DNA vaccination is to deliver DNA into the nuclei of cells capable of presenting the encoded antigen to immune reactive cells that can elicit an immune response (Ingolotti et al, 2010; Ledgerwood and Graham, 2009).

Electroporation (EP) delivery was shown to be a very effective means of inducing antibody responses to HTNV and PUUV DNA vaccines in animals and has also been found to be effective in eliciting immune responses in humans with several other types of DNA vaccines (Hooper et al, 2008; Hooper et al, 2013; Spik et al, 2008). These data led to the Phase 1 clinical study at the Walter Reed Army Institute of Research (WRAIR), which demonstrated that administration of the HTNV and PUUV DNA vaccines both alone or in combination using an EP device to deliver intramuscular (IM) injections not only was safe but also provided improved seroconversion rates to both viruses (Hooper et al, 2014). This first IM-EP study was performed using the codon-optimized PUUV plasmid, but the HTNV plasmid had not yet been codon optimized.

Results from the Phase 1 IM-EP study prompted the decision to pursue a larger, Phase 2a study. This study evaluated the combination of HTNV and PUUV DNA vaccines, at 1.0 or 2.0 mg doses, as well as 2 different dosing schedules (2 doses versus 3, over a 2-month period, with a booster dose at Day 168). The study also used the Ichor EP device from the previous study performed at WRAIR to deliver vaccine by IM or intradermal (ID) inoculation. The HTNV vaccine was different than the iteration used in the Phase 1 study, as the HTNV plasmid (pWRG/HTN-M[co]) had an optimized open-reading frame and was predicted to produce a higher seroconversion rate when combined with the PUUV vaccine. The need for an improved HTNV vaccine was evidenced by our findings in hamsters, rabbits, and humans (from the first Phase 1 study), which indicated that delivery of a mixture of the HTNV and PUUV vaccines resulted in a reduced response to the HTNV vaccine as compared to when it was delivered by itself (Spik et al, 2008; Schmaljohn et al, 2014; Hooper et al, 2014). This interference was overcome by using an optimized version of the same HTNV DNA vaccine in which codons were

modified to reflect those used most frequently in humans and to eliminate motifs that are known to impact expression and/or messenger RNA stability (unpublished information). Preliminary results from the Phase 2a study confirm that a mixture of the codon-optimized HTNV DNA vaccine and the codon-optimized PUUV DNA vaccine delivered by IM EP is able to elicit strong responses to both HTNV and PUUV (unpublished results). This Phase 2a clinical study has been completed with follow-up ongoing. Study results have not been finalized, but there have been no related severe AEs to date, with preliminary findings consistent with what was seen in the earlier Phase 1 study using the same IM-EP device.

The rationale for dosages and plan of routes of IM compared to ID administration in the current Phase 1 study is based on prior work by the delivery device manufacturer (Ichor Medical Systems) and prior experience from the previous HTNV DNA and PUUV DNA vaccine clinical study in which candidate vaccines were shown to elicit antibodies in humans when administered by IM EP. This study affords the opportunity to compare the IM and ID routes of administration, at the highest possible doses, given the limitations of the volumes that can be administered by the IM and ID routes (1 mL and 0.2 mL, respectively). This study is being performed to determine which of IM or ID elicits a superior immune response, given the amount of DNA administered using these routes.

## 4.3. Name and Description of the Investigational Product

Under 21 CFR 3.2 (e) a combination product is a product composed of any combination of a drug and a device; a biological product and a device; a drug and a biological product; or a drug, device, and a biological product. The investigational product used in this study is a combination of a DNA vaccine and a device. The 2 investigational products, HTNV and PUUV DNA vaccines, were manufactured by Ajinomoto Althea, Inc., San Diego, California, from their respective drug substances, pWRG/HTN-M(co) and pWRG/PUUV-M(s2), in accordance with current good manufacturing practice (cGMP) guidelines, and were constructed on a well-characterized plasmid backbone, pWRG7077.

The HTNV and PUUV DNA vaccines will be administered individually and in combination by in vivo EP using either the intramuscular or intradermal TriGrid™ Delivery Systems (TDS-IM V1.0 or TDS-ID, respectively), manufactured by Ichor Medical Systems, San Diego, California. These devices are integrated, fully automated, and designed for delivering DNA-based vaccines and therapies to target tissues through temporary disruption of cellular membranes via electrical pulses.

Refer to Section 6.4 for additional information.

## 4.4. Summary of Nonclinical and Clinical Trials

### 4.4.1. Nonclinical Studies

The HTNV and PUUV DNA vaccines have been evaluated in several preclinical, good laboratory practice (GLP) toxicity studies. In support of a Phase 1 clinical study, the HTNV and PUUV DNA vaccines were administered to rabbits using the TDS-IM V1.0 device (Ichor, San Diego, California) (Hooper et al, 2014).

In support of this protocol, a repeat-dose toxicity study evaluated the immunogenicity, safety, and potential target organ toxicity of HTNV and PUUV DNA vaccines alone or in combination in New Zealand White rabbits when administered IM or ID with EP (unpublished results). Toxicity and reversibility of effects after a 4-week recovery period were also evaluated. Each group consisted of 10 rabbits/sex/group, as follows: Group 1, Saline (control), IM and ID without EP; Group 2, HTNV (3.0 mg), IM with EP; Group 3, HTNV (1.2 mg), ID with EP; Group 4, HTNV (3.0 mg) + PUUV (3.0 mg), IM with EP; Group 5, HTNV (1.2 mg) + PUUV (1.2 mg), ID with EP; and Group 6, PUUV (1.2 mg), ID with EP.

Overall, administration of the HTNV vaccine alone, the PUUV vaccine alone, or the HTNV + PUUV vaccines together by IM or ID EP was well tolerated in rabbits, as all findings were generally reversible and no longer seen at the end of the recovery period (except for the histopathologic findings at the injection sites), and none of these findings resulted in any limiting toxicity. The histopathologic findings at the injection sites were considered to be typical responses seen following either IM or ID administration with EP. These vaccines were also biologically active and immunogenic as all vaccine-treated rabbits regardless of dosing route and type of vaccine administered (either alone or in combination) developed neutralizing antibodies against the respective HTNV or PUUV viruses as measured by pseudovirion neutralization assay.

The plasmid vector backbone in the HTNV and PUUV DNA vaccines is pWRG7077. This is the same backbone used for several different DNA vaccine plasmids delivered by Ichor's EP delivery system. Thus, the vector backbone has been tested in multiple safety studies in addition to those with the HTNV and PUUV DNA vaccines; eg, studies supporting a Phase 1 clinical study of a Venezuelan equine encephalitis DNA vaccine which included escalating repeat-dose studies in rabbits to assess safety, toxicology, and biodistribution studies in rats to assess integration and persistence of vaccine DNA into host tissues and DNA (Hannaman et al, 2016).

In other safety and toxicology studies using different DNA vaccine vector backbones, notable findings associated with EP-mediated delivery of DNA vaccines were limited to localized inflammatory responses of mild to moderate severity at the site of administration (Dolter et al, 2011). The injection site findings were most prominent in tissue samples obtained at 48 to 72 hours after administration with progressive resolution of the local inflammatory responses over time (samples assessed at 14 and 43 days post-injection).

The results of the pWRG7077-based DNA vaccine biodistribution studies indicated negligible systemic uptake of vaccine DNA following IM EP delivery with no significant differences observed among the different DNA vaccine candidates tested to date. Persistence analysis indicated that the presence of vaccine DNA 30 to 90 days after administration was confined to the tissues at the site of administration (muscle and skin), and only at very low (ie, < 1,000 copies/µg) levels, suggesting minimal risk for potential integration of vaccine DNA into host DNA (Dolter et al, 2011).

### 4.4.2. Clinical Studies

#### 4.4.2.1. Phase 1 Study to Evaluate the Safety, Tolerability, and Immunogenicity of Hantaan and Puumala Virus DNA Vaccines, pWRG/HTN-M(x) and pWRG/PUU-M(s2), for Prevention of Hemorrhagic Fever with Renal Syndrome Administered to Healthy Adult Volunteers Using the TDS-IM Electroporation Delivery Device (S-11-12, WRAIR 1854)

The HTNV DNA and PUUV DNA vaccine products were previously administered to 31 healthy human subjects via the Ichor EP device in a Phase 1 study conducted at the Walter Reed Army Institute of Research, Silver Spring, Maryland, from January 2012 to January 2013 (Hooper, et al, 2014). In this study, 27 subjects received 3 doses at 2 mg/mL per dose of HTNV DNA vaccine, PUUV DNA vaccine, or a combination of HTNV DNA and PUUV DNA vaccines over a 2-month period by IM injection. Two other subjects received 2 doses of the HTNV DNA vaccines and 2 subjects received 1 dose of the HTNV/PUUV DNA vaccine.

##### 4.4.2.1.1. Immunogenicity Results

Subjects were vaccinated with an HTNV DNA vaccine, PUUV DNA vaccine, or combination of HTNV/PUUV DNA vaccines using TDS-IM V1.0. Both the HTNV DNA vaccine and PUUV DNA vaccine were immunogenic in humans when delivered alone or in combination. For subjects receiving at least 2 vaccinations, the overall seroconversion rate, as measured by plaque reduction neutralization test, was 71% (20 of 28). The seroconversion rates for HTNV DNA, PUUV DNA, and HTNV/PUUV DNA vaccines were 68%, 78%, and 44% (against both HTNV and PUUV), respectively. The lower seroconversion rate against both viruses in the combination group can be attributed to the lower frequency of anti-HTNV seroconversion in that group (44%) relative to the rate of anti-PUUV seroconversion in the same group (78%). For seroconverters, peak geometric mean titers (GMTs) for all 3 groups were between 100 and 1,000 regardless of whether the plasmid was delivered alone or in combination (the combination was half-dose for each plasmid). In general, peak titers occurred within 1 month of the last vaccination. Neutralizing antibodies were still detected in 70% (14/20) of the seroconverters 6 months after the last vaccination.

##### 4.4.2.1.2. Safety Results

During the performance of the TDS-IM V1.0 study, there were no significant safety concerns involving the investigational products. Overall, 30/31 (96.8%) subjects receiving at least 1 dose of HTNV DNA, PUUV DNA, or HTNV/PUUV DNA vaccines reported at least 1 AE. Of the 246 AEs reported, 96.7% were graded as mild to moderate. Seven out of 8 AEs identified as either Grade 3 or Grade 4 were considered unrelated to the study vaccine; 1 Grade 3 AE was considered unlikely to be related. There were 2 SAEs reported during this study, both involving transient Grade 4 laboratory abnormalities detected in otherwise asymptomatic individuals (hypoglycemia and hyperkalemia, respectively). Each event was evaluated and determined to be unrelated to study participation.

Seventy-six incidents of injection site pain were reported in 28/31 (90.3%) subjects, accounting for 30.9% of all AEs. The other most commonly reported AEs were headache (5.7%), injection site bruising (3.7%), muscle aches (3.3%), and fatigue (3.3%). Ninety-six/246 (39.0%) AEs were

considered related to injection, of which 87 (90.6%) were local reactions at the injection site (pain, bruising, or erythema).

#### 4.4.2.2. A Phase I, Randomized, Observer-blind, Placebo Controlled Study to Assess the Safety, Reactogenicity, and Immunogenicity of a Venezuelan Equine Encephalitis DNA Vaccine Candidate Administered Intramuscularly or Intradermally by Electroporation to Healthy Adults (NCT01984983)

In a Phase 1, randomized, double-blind, placebo-controlled study, a DNA-based Venezuelan Equine Encephalitis vaccine, pWRG/VEE, or placebo control was administered to 41 healthy, adult subjects using either the Ichor TDS-IM V1.0 or TDS-ID EP devices (Hannaman, et al, 2016). Subjects received up to 3 administrations of the pWRG/VEEV vaccine. Subjects in the TDS-IM V1.0 groups received 0.5 mg (N = 8) or 2.0 mg (N = 9) of pWRG/VEE or a saline placebo (N = 4) in a 1.0-mL injection. Subjects in the TDS-ID groups received 0.08 mg (N = 8) or 0.3 mg (N = 8) of pWRG/VEE or a saline placebo (N = 4) in a 0.15-mL injection. Subjects were monitored for a total of 360 days.

##### 4.4.2.2.1. Immunogenicity

VEEV-neutralizing antibodies in sera were measured by plaque reducing neutralization test (PRNT). All subjects (100%) in the high- and low-dose TDS-IM V1.0 groups developed detectable VEEV-neutralizing antibodies after 2 or 3 administrations of pWRG/VEE, respectively. After 2 doses, 5 of 8 subjects in the 0.3-mg TDS-ID group had measurable neutralizing antibodies by PRNT. There were no detectable responses after 2 doses in the 0.08-mg TDS-ID dose group. After 3 doses, 7 of 8 subjects in the 0.3-mg TDS-ID group and 5 of 8 subjects in the 0.08-mg TDS-ID EP dose group had developed neutralizing antibodies. Peak titers for both groups were observed 2 weeks after the third immunization (Study Day 70), with a geometric mean PRNT titer of 43.6 in the 0.3-mg TDS-ID group and 7.1 in the 0.08-mg TDS-ID group. The mean time to seroconversion was 43.5 days in the 0.3-mg TDS-ID group and 70 days in the 0.08-mg TDS-ID group. VEEV-neutralizing activity were also measured in serum samples collected at the final visit of each subject (Day 360), with5 of 8 subjects in the 0.3-mg TDS ID group and 1 of 8 subjects in the 0.08-mg TDS-ID group exhibiting detectable PRNT activity.

There was a correlation between the DNA dose and the magnitude of the resulting VEEV-neutralizing antibody responses for both IM and ID EP delivery. These results indicated that pWRG/VEE delivered by either TDS-IM V1.0 or TDS-ID EP is safe, tolerable, and immunogenic in humans at the evaluated dose levels.

##### 4.4.2.2.2. Safety

No vaccine- or device-related serious adverse events were reported for either EP device.

Adverse reactions associated with the use of the TDS-ID device included acute pain at the time of EP application in almost all subjects. Mild, transient bleeding at the sites of electrode penetration was commonly observed following removal of the device. These included injection site pain, erythema, induration, swelling, tenderness, bruising, punctures at the site of needle penetration, eschar formation at the site of needle penetration, and/or localized pigmentation changes. The associated injection site reactions typically resolved within 24-72 hours following administration. Systemic adverse events judged to be at least possibly related to the vaccine

candidate or administration procedure were all Grades 1 or 2 and included fatigue, headache, low-grade fever, dizziness, enlarged lymph nodes, and elevated blood pressure.

Based on the results of a subject questionnaire, the TDS-IM V1.0- and TDS-ID EP procedures were both considered to be generally acceptable for prophylactic vaccine administration, with the acute tolerability of TDS-ID EP delivery judged to be greater than that of TDS-IM V1.0 EP delivery.

#### 4.4.2.3. DNA Vaccine Administered to Healthy Adult Volunteers using the TDS-IM Electroporation Delivery Device for Prevention of Hemorrhagic Fever with Renal Syndrome (S-14-01, WRAIR 2085)

Results from the Phase 1 study prompted the decision to pursue a larger, Phase 2a study. This study evaluated the combination of HTNV and PUUV DNA vaccines, at 1.0 mg or 2.0 mg doses, as well as 2 different dosing schedules (2 doses versus 3, over a 2-month period, with a booster dose at Day 168). The study also used the Ichor EP device from the previous study performed at the Walter Reed Army Institute of Research. The HTNV vaccine was different than the iteration used in the Phase 1 study, as the HTNV plasmid (pWRG/HTN-M[co]) had an optimized open-reading frame and was predicted to produce a higher seroconversion rate when combined with the PUUV vaccine. This study has been completed. Study results have not been finalized, but there have been no related severe AEs to date, with preliminary findings consistent with what was seen in the Phase 1 study using the same EP device. No subjects withdrew from the study due to product-related reasons.

#### 4.4.2.4. Delivery Device

The TDS-IM V1.0 and TDS-ID devices are investigational. To date, the TDS-IM V1.0 device has been used as the means of administration in a total of 25 completed and ongoing clinical studies of DNA based vaccine candidates, with > 1000 subjects enrolled. These studies have been conducted in the United States, United Kingdom, European Union, Korea, and in East Africa. To date, a total of over 700 subjects have been dosed using the TDS-IM V1.0 device in these studies (including subjects receiving either the test DNA or placebo). Subjects have been administered regimens of up to 5 administrations at DNA doses of up to 8.0 mg per time point. In one study, allowing continuing dosing, subjects have received up to an additional 10 administrations delivered on a quarterly schedule. Doses have been administered in the medial deltoid and/or the quadriceps muscles either as a single injection in one muscle site (approximately half of subjects to date) or as 2 injections in 2 separate muscle sites (approximately half of subjects to date).

To date, the TDS-ID device has been used as the means of DNA vaccine administration in one completed and 2 ongoing clinical studies sponsored by Ichor. In the completed study, 20 subjects received the TDS-ID administration procedure either for delivery of a VEEV DNA vaccine candidate or placebo control. In the vaccine arm of the study, subjects received sequences of up to 3 administrations at DNA doses of either 0.08 mg or 0.3 mg per time point. Doses were administered in the skin in the deltoid region. Adverse reactions associated with the use of the device include acute pain at the time of electroporation application in almost all subjects. Mild, transient bleeding at the sites of electrode penetration was commonly observed following removal of the device. These included injection site pain, erythema, induration, swelling,

tenderness, bruising, punctures at the site of needle penetration, eschar formation at the site of needle penetration, and/or localized pigmentation changes. The associated injection site reactions typically resolved within 24-72 hours following administration.

## 4.5. Known and Potential Risks and Benefits to Human Subjects

### 4.5.1. Risks/Discomfort to Subjects and Precautions to Minimize Risk

The following safety profile is based on the aforementioned clinical studies with HTNV DNA and PUUV DNA vaccines, plus a VEEV DNA vaccine trial led by Ichor, and includes a brief description of possible procedures to ameliorate risks and symptoms. The potential risks of this trial are those associated with having blood drawn, use of the TDS-IM V1.0 and TDS-ID to deliver the study products, and possible reactions to the experimental vaccines. The TDS-IM V1.0 device also carries a theoretical risk that excessive energy could be delivered to the local tissues of the volunteer. There may be potential risk related to breach of confidentiality, as well as other unknown risks, discomforts, or side effects. All known risks and precautions described are explained in detail in the informed consent. In addition, it is important to note that this protocol investigates a combination product consisting of the DNA and the administration device, and that risks of local and systemic reactions for the 2 may necessarily need to be considered as a single phenomenon, and could be hard to attribute to either product or device.

#### 4.5.1.1. Local Reactions

Local reactions in previous studies have included pain, stinging, itching, bruising, tingling, numbness and redness at the injection site (deltoid muscle or associated skin in that area of the arm), and sometimes affecting the arm distal to the site. Information about local reactions are described below for each device type.

**TDS-IM V1.0 Device:**

Adverse reactions associated with the use of the TDS-IM V1.0 device for DNA vaccine delivery and reported in almost all subjects include acute pain with localized muscle contractions during the application of the electrical fields. In the TDS-IM V1.0 study of the hantavirus DNA vaccines, brief, but intense, muscle contractions and associated local pain/discomfort during the period of EP administration were observed in essentially all subjects. Additional AEs associated with the administration procedure included minor, transient cutaneous bleeding at the sites of electrode and injection needle penetration, and mild to moderate injection site soreness/bruising typically lasting for 24 to 72 hours, but in a small number of cases, up to 7 days post administration. Occasionally, subjects have also reported mild, transient paresthesia (tingling) or hypoesthesia (numbness) in the injected limb or fingers for a few seconds or minutes following electric field application. Over the 24-72 hours after administration, administration site soreness, erythema, induration and/or eschar formation of mild to moderate severity are commonly reported. Other injection site findings of mild severity including bruising and/or hematoma have been reported occasionally. The associated injection site reactions have typically been of mild to moderate severity and typically resolve within 24–72 hours following administration. However, in rare instances, subjects have reported severe local site soreness which resolved by 48 hours post administration.

There has been at least one reported instance of an aborted TDS-IM V1.0 administration procedure with observations consistent with injection needle contact with the underlying bone. This was associated with acute pain at the penetration site and increased difficulty in the withdrawal of the device. Subsequent inspection of the Cartridge revealed deformation of the needle consistent with impact on the bone. The TDS-IM V1.0 device registered an error code and no vaccine was delivered. This was believed to be due to an inaccurate measurement of skinfold thickness. Following the incident, associated adverse events were limited to mild transient soreness at the penetration site. Although the specific cause of this event could not be definitively established based on the available information, inspection of the device was sufficient to rule out device malfunction (ie, the needle did not penetrate beyond the specified depth setting). To ensure intramuscular injection, proper selection of the injection site as well as assessment and setting of the injection depth are important factors in procedure administration. In particular, care should be taken in specific subjects or subject populations with low muscle mass (eg, sarcopenia) to ensure that an administration site with adequate muscle thickness (eg, vastus lateralis) is selected for injection. Specifically, any selected administration site should have sufficient muscle mass to accommodate the 1-inch/25-mm maximum penetration depth of the device. To mitigate the risk of this happening again, only the PI or qualified, designated personnel with PI oversight will take all skinfold measurements and study staff training on skinfold measurement and device usage will be performed and documented by Ichor for implementation of this study. This should decrease the possibility of inter-rater variability errors in measurement and avoid overestimations of soft tissue girth. The measurement may be repeated, as needed, throughout the study or if any study team member has a concern about the accuracy of the measurement (eg, if there seems to be a discrepancy between the volunteer's body habitus and the indicated depth setting for the TDS-IM V1.0 device.

There have been no occurrences of excessive energy delivery in any of the nonclinical and clinical studies conducted with the pulse stimulator to date.

**TDS-ID Device:**

To date, the TDS-ID device has been used as the means of DNA vaccine administration in one completed and 2 ongoing clinical studies sponsored by Ichor. The results of the VEEV DNA vaccine study have been published (Hannaman et al, 2016). Adverse reactions associated with the use of the TDS-ID device include acute pain at the time of electroporation application in almost all subjects. Mild, transient bleeding at the sites of electrode penetration was commonly observed following removal of the device. These included injection site pain, erythema, induration, swelling, tenderness, bruising, punctures at the site of needle penetration, eschar formation at the site of needle penetration, and/or localized pigmentation changes. The associated injection site reactions typically resolved within 24-72 hours following administration.

Consistent with the clinical safety experience from the VEEV DNA vaccine study, interim safety reports from the 2 ongoing clinical studies utilizing the TDS-ID device (clinicaltrials.gov # NCT02589795 and EUDRA CT# 2014-001997-33) have included mild to moderate injection site reactions including minor bruising and hematoma at the sites of administration.

No serious adverse events attributed to the device or administration procedure have been observed to date during any of the clinical studies utilizing the TDS-ID device.

Tolerability of TDS-IM V1.0 compared to TDS-ID device:

In the VEEV DNA vaccine study, which compared IM and ID-EP delivery, the results of a subject questionnaire revealed that the IM- and ID-EP procedures were both considered to be generally acceptable for prophylactic vaccine administration, with the acute tolerability of ID EP delivery judged to be greater than that of IM-EP delivery published (Hannaman et al, 2016).

#### 4.5.1.2. Systemic Reactions

The systemic reactions that have been reported in previous studies using the investigational vaccines, together with these devices or similar devices, included fatigue, headache, tachypnea, and myalgia. Information about risks for systemic reactions are described below for each device.

**TDS-IM V1.0 Device:**

Experience with the TDS-IM V1.0 device indicates that mild to moderate vasovagal reactions are possible. Transient vasovagal reactions, comprising mild dizziness, skin pallor, diaphoresis, lightheadedness, and/or hypotension (consistent with pre-syncope) occurring immediately after device application, have been reported in approximately 1% of subjects. Vasovagal reactions including three instances of vasovagal syncope were also reported immediately after administration procedure in clinical testing to date. Two of the cases were described as vasovagal syncope associated with tonic-clonic or seizure-like muscle movements. Both cases of vasovagal syncope with tonic-clonic or seizure-like muscle movements manifested after the subjects had received sequential bilateral EP injections using the TDS-IM v1.0 device. In all cases of vasovagal syncope, the subjects recovered without sequelae. However, based on the judgment of the investigators, the subjects were withdrawn from the study.

At the time of enrolment, the subject with non-convulsive vasovagal syncope indicated a lifelong history of sinus bradycardia of unknown origin, which was confirmed by electrocardiogram during screening. Multiple electrocardiograms performed after the syncopic episode indicated no changes from pre-procedure baseline. Based on the judgment of the investigator, the subject was withdrawn from the study.

Systemic adverse events reported during the studies and judged to be possibly related to the study product and/or delivery device have been generally mild to moderate in severity and include flu-like symptoms, headache, fever, nausea, chills, dizziness, malaise, fatigue, arthralgia, myalgia, neuralgia, and aphthous stomatitis. Several subjects have reported individual instances of severe fatigue within 24 hours of dosing, all of which resolved by the following day. Mild to moderate transient hematological abnormalities have been observed in a small number of subjects during the studies and have included white blood cell count, red blood cell count, hemoglobin, absolute neutrophils, absolute monocytes, absolute lymphocytes, and platelets. Likewise, serum chemistry abnormalities have been generally mild to moderate in severity and transient in duration. These have included ALT/AST, creatine phosphokinase, creatinine, non-fasting glucose, estimated glomerular filtration rate (eGFR). Among these, elevation in serum creatine phosphokinase of mild to moderate severity in small minority of subjects has judged to be potentially associated with procedure administration. One instance of Grade 4 hypoglycemia was reported in one subject and was diagnosed as an unrecognized pre-existing condition.

**TDS-ID device:**

Systemic adverse events judged to be at least possibly related to the vaccine candidate or the TDS-ID administration procedure were all grade 1 or 2 and included fatigue, headache, low grade fever, dizziness, enlarged lymph nodes, elevated blood pressure. In rare cases, syncope has been reported in subjects receiving intramuscular injections with the TDS technology (TDS-IM V1.0). Monitoring for these and other systemic symptoms commonly seen in investigational vaccine studies (eg, fever, chills, rash, nausea, and allergic reaction) will be performed throughout the study and addressed as required to maximize subject safety.

No serious systemic adverse events attributed to the device or administration procedure have been observed to date during any of the clinical studies utilizing the TDS-ID device.

#### 4.5.1.3. DNA Vaccine Investigational Product Risks

It is important to note that this protocol investigates a combination product, which is the DNA vaccine product together with the TDS delivery device, and that risks for the 2 may necessarily need to be considered as a single phenomenon, and could be hard to attribute to either product or device.

The systemic reactions that have been reported in previous studies using the investigational vaccines with these and other devices included fatigue, headache, tachypnea, and myalgia. Light-headedness/dizziness, which in one case progressed to syncope, has also been reported. Monitoring for these and other systemic symptoms commonly seen in investigational vaccine studies (eg, fever, chills, rash, nausea, and allergic reaction) will be performed throughout the study and addressed as required to maximize subject safety.

Research from this program has shown that these DNA vaccines do not cause infection with the target viruses, nor do they cause integration of the vaccine DNA into the test subject.

The HTNV and PUUV DNA vaccines do not contain live virus and encode only a portion of the HTNV and PUUV viral genome, which is insufficient to support production of live virus. Therefore, there is no basis to expect that these candidates may cause infection. The FDA considered safety measurements appropriate based on results of preclinical and clinical studies performed using DNA vaccines with the same plasmid backbone (pWRG7077). Persistence analysis in a study using a similar DNA vaccine vector indicated that the presence of vaccine DNA 30 to 90 days after administration was confined to the tissues at the site of administration (muscle and skin), and only at very low (ie, < 1,000 copies/µg) levels, suggesting minimal risk for potential integration of vaccine DNA into host DNA (Dolter et al, 2011). The TDS-IB also describes acceptable biodistribution safety data for the VEE vaccine based in the pWRG-7077 backbone, which is the vector used in this study. In addition, the Center for Biologics Evaluation and Research (CBER) guidance entitled *Considerations for Plasmid DNA Vaccines for Infectious Disease*, dated November 2007, stipulates that "biodistribution studies may be waived for DNA vaccines produced by inserting a novel gene into a plasmid vector previously documented to have an acceptable biodistribution/integration profile."

#### 4.5.1.4. Pregnancy

Risks to unborn babies are unknown at this time; pregnant females will be excluded from this study.

Sexually active men[2] and women[3] of childbearing potential must be using an effective method of contraception from 30 days prior to the first study vaccination until 6 months after the last study vaccination.

All female subjects of childbearing potential must be abstinent or utilize effective contraceptive precautions. Non-childbearing potential is defined as either surgically sterilized or 1 year post-menopausal (defined as 12 consecutive months without menses). Study subjects should not become pregnant during the study and for at least 6 months after the last study vaccination.

Last, participants who are in non-male sexual relationships, abstaining from sexual intercourse with a male partner, or are in a monogamous sexual relationship with a vasectomized partner satisfy the study requirement to avoid pregnancy 30 days prior to the first study vaccination until 6 months after the last study vaccination (see inclusion criteria).

#### 4.5.1.5. Lactation

Risks to nursing infants are unknown at this time; lactating females will be excluded from this study.

#### 4.5.1.6. Venipuncture

Blood sampling carries a minimal risk of minor discomfort and the possibility of minor bruising at the site of the needle puncture and, rarely, the possibility of infection at the needle puncture site. Venipuncture causes transient discomfort and may result in fainting. Fainting is usually transient and managed by having the subject lie down and elevate his/her legs. Bruising at the site of the venipuncture may occur but may be prevented or lessened by applying pressure to the site for several minutes. Venipuncture may also cause infection. The use of aseptic (sterile) technique will make infection at the site where blood will be drawn extremely unlikely.

#### 4.5.1.7. Allergic Reaction

As with any Investigational New Drug (IND) product administration and no matter what precautions are taken, there is always the risk of a serious, or even life-threatening, allergic reaction. To mitigate this risk, potential subjects with a history of severe allergic reaction of any kind, or significant allergic reaction to a known component of the experimental products, will be excluded from participation. Medical emergency equipment is located at the University of

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[2] A sexually active man is defined as one whose partner is a woman of childbearing potential (see definition below) and has not had a vasectomy performed > 1 year prior to screening must agree not to father a child until 6 months after the last vaccination. These subjects must agree to use a barrier method of birth control (eg, either condom with spermicidal foam/gel/film/cream or partner usage of occlusive cap [diaphragm or cervical/vault caps] with spermicidal foam/gel/film/cream/suppository).

[3] Women of childbearing potential are defined as those who have not been sterilized via tubal ligation, bilateral salpingectomy, bilateral oophorectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with history of documented radiological confirmation test at least 90 days after the procedure (or with use of another birth control method if history of confirmation test not confirmed), still menstruating or < 1 year of the last menses if perimenopausal. For this study, an effective contraceptive method is defined as one that results in a failure rate of less than 1% per year when it is used consistently and correctly.

Maryland Center for Vaccine Development. This is available to handle emergencies, such as anaphylaxis, angioedema, bronchospasm, and laryngospasm.

#### 4.5.1.8. Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is a disorder in which the body's immune system attacks part of the peripheral nervous system. It afflicts only about one person in 100,000, and in rare instances vaccinations may increase the risk of GBS. The first symptoms of this disorder include varying degrees of weakness or tingling sensations in the legs. These symptoms can increase in intensity until certain muscles cannot be used at all. Most individuals recover from even the most severe cases of Guillain-Barré syndrome, although some continue to have a certain degree of weakness (NIH, 2011). To mitigate this risk, individuals with a history of GBS, other neurologic diseases per the physician investigator's discretion, or other significant reaction to vaccination will be excluded from participation.

#### 4.5.1.9. Unknown Risks

Furthermore, as with all research there is the 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. The long-term effects on EP-mediated delivery of DNA is not known.

### 4.5.2. Alternatives to This IND Product or Study

At this time, there is no known alternative to taking this product to afford the same potential protection from HFRS. An alternative is not to participate in this study.

### 4.5.3. Intended Benefit for Subjects

There is no intended direct benefit for study subjects.

### 4.5.4. Risks to the Study Personnel and the Environment

The principal risk in the clinical setting is (eg, in the handling of needles that may be contaminated and the attendant risks including hepatitis, human immunodeficiency virus (HIV), and other human pathogens). Adherence to standard operating procedures (SOP) for working with infectious agents and universal precautions will reduce the risk of exposure.

Injury to operators is possible if the electroporation devices are handled improperly; therefore, these devices are for use only by qualified medical personnel who have completed a training course on the set up and use of the TDS systems.

There are no known risks to the environment other than those associated with the generation of biohazardous waste attendant to HTNV and PUUV 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.

### 4.5.5. Risks to Subject Confidentiality

Efforts will be made to keep subject personal information confidential within the limits of the law. There is a small risk of loss of confidentiality by an unauthorized person viewing subject records. In order to maintain confidentiality, study physicians and the study team will store study

records in a limited-access, locked office. Subject personal information may be disclosed if required by law to authorized representatives of relevant organizations including the US Army Medical Research and Development Command USAMRDC, the National Institutes of Health (NIH), the Institutional Review Board (IRB) at the University of Maryland, and/or the Federal Drug Administration (FDA), who will be allowed to inspect sections of medical and research records related to the study. The risks of storing subject samples for use in possible future research studies or having them used in future research studies are those associated with possible loss of confidentiality. Subject samples will be labeled only by a code and will not be labeled with subject name or other personal identifying information.

## 4.6. Route of Administration, Dosage Regimen, Treatment Period, and Rationale

Subjects will receive HTNV DNA vaccine, pWRG/HTN-M(co), and PUUV DNA vaccine, pWRG/PUU-M(s2), administered separately and as a mixture using the TDS-IM V1.0 or TDS-ID devices developed by Ichor Medical Systems, Inc. Subjects will be randomized to the following 6 groups and will receive 1 dose on Days 0, 28, and 56 for a total of 3 doses. The vaccine mixing process will be outlined in a supplementary Pharmacy Manual. Table 3 shows the investigational product dosage, schedule, and mode of administration.

**Table 3: Investigational Product Dosage, Schedule, and Mode of Administration**

| Group | Product | Dose/Route | Dilution Preparation | Injection Volume | Dose Administration |
|---|---|---|---|---|---|
| Group 1 | HTNV | 0.6 mg/ID EP | 0.1 mL 6.0 mg/mL HTNV DNA + 0.1 mL 0.9% saline | 0.2 mL | Days 0, 28, and 56 |
| Group 2 | HTNV | 3.0 mg/IM EP | 0.5 mL 6.0 mg/mL HTNV DNA + 0.5 mL 0.9% saline | 1.0 mL | Days 0, 28, and 56 |
| Group 3 | PUUV | 0.6 mg/ID EP | 0.1 mL 6.0 mg/mL PUUV DNA + 0.1 mL 0.9% saline | 0.2 mL | Days 0, 28, and 56 |
| Group 4 | PUUV | 3.0 mg/IM EP | 0.5 mL 6.0 mg/mL PUUV DNA + 0.5 mL 0.9% saline | 1.0 mL | Days 0, 28, and 56 |
| Group 5 | HTNV/PUUV | 1.2 mg/ID EP (0.6 mg each) | 0.1 mL 6.0 mg/mL HTNV DNA + 0.1 mL 6.0 mg/mL PUUV DNA | 0.2 mL | Days 0, 28, and 56 |
| Group 6 | HTNV/PUUV | 6.0 mg/IM EP (3.0 mg each) | 0.5 mL 6.0 mg/mL HTNV DNA + 0.5 mL 6.0 mg/mL PUUV DNA | 1.0 mL | Days 0, 28, and 56 |

The rationale for dosages and plan of administration is based on prior work by the delivery device manufacturer and prior experience from a previous HTNV DNA and PUUV DNA

vaccine study in which candidate vaccines were shown to elicit antibodies in humans when administered by IM EP.

## 4.7. Compliance Statement

The study will be conducted according to the US Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312), the study protocol and in compliance with International Council for Harmonisation (ICH) Good Clinical Practice (GCP), Belmont Principles, and other applicable regulatory and Department of Defense (DoD) requirements. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guideline and clinic site SOPs. Roles and responsibilities of study staff are presented in Appendix A.

## 4.8. Study Population

The study population may consist of as many as 82 healthy male and nonpregnant, nonlactating female subjects, ages 18 to 49 years (inclusive), who are HTNV and PUUV naïve.

Refer to Section 11.2 for a statistical justification of the sample size.

## 4.9. Study Site

The study will be performed at the University of Maryland Center for Vaccine Development.

# 5. TRIAL OBJECTIVES AND PURPOSE

## 5.1. Primary Objective

- To evaluate the safety and reactogenicity of the HTNV, PUUV, and HTNV/PUUV DNA vaccine candidates delivered to healthy adults either IM or ID by EP.

## 5.2. Secondary Objectives

- To obtain a preliminary assessment of the immunogenicity of the combination HTNV/PUUV DNA vaccine candidate relative to the monovalent formulations.
- To identify the HTNV and/or PUUV DNA vaccine combination and route of administration that elicits the most immunogenic response as determined by seroconversion and seropositivity rates, and geometric mean titers (GMT) as measured by PsVNA50.

# 6. TRIAL DESIGN

## 6.1. Study Endpoints

Primary endpoints, correlating to the primary objective of the study, will include determining the nature, frequency, and severity of AEs and/or SAEs associated with TDS-EP-based administration of HTNV, PUUV and HTNV/PUUV DNA vaccines.

- The occurrence of solicited local and systemic AEs occurring from the time of each injection through 14 days following the procedure
- The occurrence of vaccine-related unsolicited AEs from the time of the first injection through 28 days following each injection
- The occurrence of SAEs from the time of the first injection through the final study visit (approximately 6 months post-last vaccination)
- The occurrence of clinical safety laboratory AEs through 14 days following each study vaccination

Secondary endpoints, correlating to the secondary objectives of the study will be to determine the proportion of seropositive subjects (PsVNA50 ≥ 1:20) and the final overall rate of seroconversion over all scheduled time points to study completion for each study group. GMT will be calculated using PsVNA50.

- Determination of the proportion of seropositive subjects (defined as PsVNA50 ≥ 1:20) at each scheduled time point (eg, Days 0, 28, 56, 84 and 140)
- Determination of the final overall rate of seroconversion over all scheduled time points to study completion for each study group. Seroconversion is defined as a post-vaccination HTNV- or PUUV-specific titer of ≥ 1:40, or a minimum four-fold rise compared to baseline titer, and all study volunteers will begin the study with a baseline titer < 20 (ie, seronegative).
- Determination of GMT of the PsVNA50 for HTNV- and PUUV-specific neutralizing antibodies at each scheduled time point for each study group and over all time points for each study group.

Exploratory endpoints, evaluated as part of the immunogenicity objectives, including calculation of PsVNA80 titers for HTNV and PUUV may be calculated from the collected data for informational purposes, and neutralization assays using other hantavirus PsV (eg, Seoul virus, Dobrava virus) may also be performed to evaluate levels of cross-neutralizing antibodies.

- Determination of GMT of neutralizing of the PsVNA80 for HTNV- and PUUV-specific neutralizing antibodies at each scheduled time point for which blood samples are taken for each study group and over all time points for each study group
- Determination of GMT of neutralizing of the PsVNA80 for Seoul- and Dobrava-specific neutralizing antibodies at each scheduled time point for which blood samples are taken for each study group and over all time points for each study group

## 6.2. Overall Study Design

The trial design is shown in Table 3 and the study events schedule is shown in Table 4 and detailed in this section. This Version 6.0 amendment prioritizes vaccination of Groups 1 and 3, to meet the time limitations of the research contract. The new study design requires a modification to the randomization approach presented in Version 4.0. That version accommodated a change in the timing of new TDS-ID device manufacture; however, due to the impact of the COVID-19 pandemic on the timing of this overall study, and the timing of TDS-ID device manufacture is no longer a concern because that has been completed, the Version 4.0 randomization approach is no longer applicable and is considered suboptimal. The Version 6.0 randomization approach is presented in below and Appendix B.

The study design, including doses and schedule, is based on the experience of a team of investigators to include personnel at USAMRDC ORA, USAMRIID, and Ichor who have performed clinical trials with similar DNA vaccine constructs using Ichor's TDS-IM V1.0 EP device for DNA vaccinations. These TDS-IM V1.0 and TDS-ID EP devices have been tested in humans previously in a recent clinical trial to evaluate the safety and immunogenicity of a DNA vaccine for Venezuelan encephalitis virus (Hannaman et al, 2016). These same codon-optimized HTNV and PUUV DNA vaccine products have also been tested in humans previously, albeit only using the TDS-IM V1.0 EP device, and at lower dose levels than proposed in this study. Previous studies examined these vaccines in Phase 1 (Hooper et al, 2014) and 2a clinical trials at up to 2-mg dose levels per IM EP administration, whereas this study is testing up to 6 mg of DNA per administration using the 2 devices. The GLP repeat-dose rabbit toxicology and immunogenicity study performed in support of this study administered these same codon-optimized HTNV and PUUV DNA vaccines at up to 6 mg doses using the 2 devices and at an increased dosing frequency compared to this proposed Phase 1 clinical trial design. The rabbit toxicology study did not reveal safety concerns for the use of this higher dose level and with these 2 devices.

This study will be a single-center, randomized, study of the HTNV, PUUV, and combined HTNV/PUUV DNA vaccines delivered IM and ID by EP.

The study may enroll 6 randomized groups of 12 subjects each for a total of 82 subjects (This accounts for 10 replacement subjects for Group 6, impacted by COVID). The study had initially planned to enroll 6 randomized groups of 12 subjects each, for a total of 72 subjects. Ten subjects in Cohort 6 may be replaced because vaccination and data collection were negatively impacted in this group by COVID-19 pandemic quarantine restrictions. This approach will ensure at least 60 subjects complete all vaccinations at around 10 subjects per group, taking possible attrition into account. Subjects will receive one dose of vaccine on each of Days 0, 28, and 56 and will be followed until Day 220.

The HTNV and PUUV DNA vaccines will be administered to subjects as follows:

Group 1[4]: 0.6 mg HTNV by ID EP

[4] Sentinel subjects will not be included for Groups 1 and 3 because the dose is lower than the 2.0 mg/mL dose that was previously evaluated for these products.

Group 2: 3.0 mg HTNV by IM EP

Group 3: 0.6 mg PUUV by ID EP

Group 4: 3.0 mg PUUV by IM EP

Group 5: 1.2 mg HTNV/PUUV by ID EP

Group 6: 6.0 mg HTNV/PUUV by IM EP

Every subject will receive 1 injection on Days 0, 28, and 56 for a total of 3 injections.

The first study enrollment activities will involve selection and randomization of volunteers as sentinels for Groups 2 and 4, and then subjects for the remainder of the Group 2 and 4 cohort. The sentinel subjects for Groups 2 and 4 (n = 2 per group, or 4 total) will be vaccinated and evaluated for 48 hours prior to dosing the remainder of Group 2 and 4 (n = 10 per group, or 20 total). After the first dose for all subjects in Groups 2 and 4 has been successfully completed and none of the halting criteria are met, then the first dose will be given to 2 sentinel subjects in Group 6 (n=2 , total of 2 subjects) on the same day, and those 2 subjects will be followed for 48 hours as per the Sentinel Group Halting Rules. The SRC will meet and evaluate the safety data collected in the 48-hour period post-vaccination for sentinels in Group 6. If the SRC finds that the halting rules have been met (Section 6.6.1.1), the PI will notify ORA PSSO within 24 hours. An SMC meeting will be held to conduct a review of clinical and laboratory safety and reactogenicity data. After the meeting, the SMC will make its recommendations in writing regarding continuation, modification, or termination of the clinical trial. If the recommendation is to proceed, enrollment, randomization, and vaccination (Day 0) of the remaining volunteers in Group 6 (n=10 per group). The first dose will be given to 2 sentinel subjects in Group 5 (n=2, total of 2 subjects) on the same day and those 2 subjects will be followed for 48 hours as per the Sentinel Group Halting Rules. The SRC will meet and evaluate the safety data collected in the 48-hour period post vaccination for sentinels in Group 5. If the SRC finds that the halting rules have been met (Section 6.6.1.1) the PI will notify ORA PSSO within 24 hours. A SMC meeting will be held to conduct a review of clinical and laboratory safety and reactogenicity data. After the meeting, the SMC will make its recommendations in writing regarding continuation, modification, or termination of the clinical trial. If the recommendation is to proceed, enrollment, randomization, and vaccination (Day 0) of the subjects in Groups 1 and 3 (n=12 per group, total of 24 subjects) will commence. Subjects in Groups 1 and 3 will be evaluated for the 48-hours post-vaccination for any halting criteria on the Day 2 clinic visit (Table 4). After completion of enrollment and first vaccination for Groups 1 and 3, the remaining subjects for Group 5 and ten Group 6 replacements (total of 20 subjects) may be enrolled, randomized and vaccinated (Day 0), provided that the enrollment of these 20 subjects can be completed within 4 months (see randomization schedule in Appendix B). Subjects are expected to complete a total of 11 visits (not including screening). There will be a 6 month follow-up after the final injection (Day 220). Overall study duration is expected to be approximately 8 months.

**Table 4: Study Events Schedule**

| **Study Visit** | **Screen 1** | **Screen 2** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Month** | **-3.2** | | **0** | **0.1** | **0.5** | **1.0** | **1.1** | **1.5** | **2.0** | **2.1** | **2.5** | **3.0** | **5.0** | **8.0** |
| **Study Day** | **-90 to -7[a]** | | **0** | **2** | **14** | **28** | **30** | **42** | **56** | **58** | **70** | **84** | **140 [b,c]** | **220** |
| **Visit Window (Days)** | | | | **+ 1** | **± 2** | **± 4** | **± 1** | **± 2** | **± 4** | **± 1** | **± 2** | **± 4** | **± 7** | **± 14** |
| Obtain Informed Consent and Demographics | X | | | | | | | | | | | | | |
| Administration Procedure[b] | | | X | | | X | | | X | | | | | |
| Review Eligibility[c] | X | X[n] | X | | | X | | | X | | | | | |
| Medical History | X | X[n] | X | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant Medications | X | X[n] | X | X | X | X | X | X | X | X | X | X | X | X |
| Adverse Event Assessment | | | X | X | X | X | X | X | X | X | X | X | X | X[o] |
| Physical Examination[d] | X | X[n] | X | X | X | X | X | X | X | X | X | X | X | |
| Skinfold Thickness Measurement of All Possible Injection Sites and Weight | X | X[n] | X | | | X | | | X | | | | | |
| Reactogenicity Assessment[e] | | | X | X | X | X | X | X | X | X | X | X | X | |
| Collection of Written Memory Aids from Subjects | | | | | X | | | X | | | X | | | |
| Vital Signs[f] | X | X[n] | X | X | X | X | X | X | X | X | X | X | X | |
| Clinical Safety Laboratory Testing[g] | X | X[a,n] | X[h] | | X | X[h] | | X | X[h] | | X | | | |
| Serum Pregnancy Test (B-HCG) | X | | | | | | | | | | | | X | |
| Urine B-HCG | | X[n] | X | | | X | | | X | | | | | |
| HIV, HCV, HBsAg | X | | | | | | | | | | | | | |
| Pre-screening (PsVNA50)[i] | | X | | | | | | | | | | | | |
| Blood for Humoral Immune Response to Vaccination[j] | | | X | | | X | | | X | | | X | X | |

| Study Visit | Screen 1 | Screen 2 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Blood for Future Use Immunogenicity[j] | | | X | | | X | | | X | | | X | X | |
| Electrocardiogram (ECG)[k] | X | | | | | | | | | | | | X | |
| HLA Testing (buccal swab) | | | X | | | | | | | | | | | |
| Total Blood Sample Volume (mL) | 33 | 34.5 | 22.5 | 0 | 12.5 | 22.5 | 0 | 12.5 | 22.5 | 0 | 12.5 | 72.5 | 23.5 | 0[o] |

[a] All subject screen visit procedures will be performed on the first screening visit (Screen 1) to include clinical safety lab tests. The immunogenicity tests will be performed at a secondary screening visit (Screen 2) allowing sufficient time to process the sera while assuring subjects' safety lab values are still within accepted values within 56 days prior to first dose administration.

- Days calculated based on 28-day months.
- For subjects who withdraw prior to completion of all study visits, the subject is requested to return as soon as possible to the date of termination for completion of termination procedures detailed in Section 8.1.5 for final safety and immunogenicity assessments.

[b] Administration procedures will include a post-injection observation period/reactogenicity assessment at least 30 minutes after vaccine administration. Vital signs will also be rechecked.

[c] Safety lab results will not be reviewed prior to product administration, and inclusion/exclusion criteria will be applied, with the exception of exclusion criteria 9, which will not be applied for vaccinations 2 and 3 because it is for screening prior to enrollment.

[d] Physical examination will be completed at screening visit 1, and includes examination of general appearance, skin, neck (including thyroid), eyes, nose, throat, lungs, heart, abdomen, lymph nodes, extremities, and a basic neurological assessment; directed physical exams will be completed at all other visits. A directed physical exam is defined as a focus, complaint-driven physical examination. In the absence of a complaint, a physical exam will not be performed.

[e] Reactogenicity assessments will include subject completion of a memory aid each day for 14 days after a vaccination visit.

[f] Vital sign measurements include oral temperature, heart rate, respiratory rate, and systolic and diastolic blood pressure.

[g] Complete blood count (CBC [WBC, Hb, platelets] with differential), glucose, creatinine, AST, ALT, total bilirubin. Lab abnormalities will be followed up on an ad hoc basis as per the study investigators. At Screen 1, a collection of 2.7 mL blood will be used to perform the PT/PTT tests to determine any coagulation abnormalities for exclusion criterion 25.

[h] Blood will be drawn prior to study product administration. Safety lab results will not be reviewed prior to product administration.

[i] Approximately 10 mL of blood will be collected at Screen 2 to determine subject eligibility.

[j] Blood collected for the humoral immune response to vaccination will be approximately 10 mL on Days 0, 28, 56 and 140; any remaining blood will be stored for future use. Approximately 50 mL of blood will be collected on Day 84 for humoral immune response to vaccination and for future use.

For determination of any cardiac abnormalities, an ECG will be performed at Screen 1 and study visit 11.

[n] Procedures will be completed only if Screen Visit 2 falls outside of the 56-day screening window prior to vaccination. See Section 8.1.2 for further clarification

[o] Scripted telephone contact for collection of Serious Adverse Event information

## 6.3. Measures Taken to Minimize/Avoid Bias

### 6.3.1. Randomization

A randomization plan will be used to allocate subjects into experimental groups, per the study priorities described in Section 6.2. In addition, as of Version 5.0, ten replacement subjects will be enrolled into Group 6. A fixed random allocation will ensure that the groups are balanced in size at the end of experiment.

Designated study personnel (excluding the principal investigator [PI] and other investigators) will fill the vaccine cohorts as potential subjects successfully complete the screening process and are deemed eligible to participate by the PI (or another designated investigator). For the purposes of this study, the groups will be defined as follows, and each subject will receive one dose of vaccine on Days 0, 28, and 56.

Group 1 (12 subjects): HTNV by ID EP

Group 2 (12 subjects): HTNV by IM EP

Group 3 (12 subjects): PUUV by ID EP

Group 4 (12 subjects): PUUV by IM EP

Group 5 (12 subjects): HTNV/PUUV by ID EP

Group 6 (22 subjects): HTNV/PUUV by IM EP

### 6.3.2. Blinding

Blinding is not applicable and will not be used in this protocol.

## 6.4. Investigational Product

The investigational products that will be administered using the TDS-IM V1.0 or TDS-ID EP device are the HTNV, PUUV, and HTNV/PUUV DNA vaccines given as a single injection. These investigational products were manufactured by Ajinomoto Althea, Inc., San Diego, California. Table 5 presents a summary description of the investigational products. See the Investigator's Brochure and the Pharmacy Manual for further product information and for detailed vaccine preparation and administration instructions.

**Table 5: Investigational Product**

| Product Name | HTNV DNA Vaccine pWRG/HTN-M(co) | PUUV DNA Vaccine pWRG/PUU-M(s2) | HTNV/PUUV DNA Vaccines |
|---|---|---|---|
| Dosage Form | Liquid | Liquid | Liquid |
| Unit Dose/Injection Volume | ID: 0.6 mg/0.2 mL<br>IM: 3.0 mg/1.0 mL | ID: 0.6 mg/0.2 mL<br>IM: 3.0 mg/1.0 mL | ID: 1.2 mg (0.6 mg each)/0.2 mL<br>IM: 6.0 mg (3.0 mg each)/1.0 mL |
| Route of Administration | ID or IM injection deltoid region in alternate arms, if possible | ID or IM injection deltoid region in alternate arms, if possible | ID or IM injection deltoid region in alternate arms, if possible |
| Physical Description | Clear, colorless solution | Clear, colorless solution | Clear, colorless solution |
| Manufacturer | Ajinomoto Althea, Inc.<br>San Diego, CA 92121 | Ajinomoto Althea, Inc.<br>San Diego, CA 92121 | Ajinomoto Althea, Inc.<br>San Diego, CA 92121 |
| Lot Number | 1-FIN-2118 | 1-FIN-2117 | 1-FIN-2118/1-FIN-2117[a] |
| Product Indication | Intended for human use in adults as a prophylactic measure against HFRS | Intended for human use in adults as a prophylactic measure against HFRS | Intended for human use in adults as a prophylactic measure against HFRS |

[a] A pre-mixed lot of HTNV and PUUV does not currently exist. These will be mixed at the clinical site's pharmacy prior to administration.

### 6.4.1. Investigational Product Packaging and Labeling

Label information for the vaccines and saline are reproduced below (manufacturer information and/or a combination of additional over-label not to obscure the manufacturer label will be used):

Single use Hantaan vials are labeled as follows:

pWRG/HTN-M(co) Plasmid DNA in PBS Buffer
Part No.: 4-FF-1016 Lot No.: 1-FIN-2118
Vol.: 0.5 mL/vial Conc.: 6.0 mg/mL
Mfg. Date: 14Jan15 Store at -20°C ± 10°C
Caution: New Drug – Limited by Federal
(or United States) Law to Investigational Use
Manufactured by Ajinomoto Althea, Inc.,
11040 Roselle St., San Diego, CA 92121
The Geneva Foundation, Contract V-1674-01

Box ____ of ____ Vials Per Box _____

Single use Puumala vials are labeled as follows:

pWRG/PUUV-M(s2) Plasmid DNA in PBS Buffer
Part No.: 4-FF-1015 Lot No.: 1-FIN-2117
Vol.: 0.5 mL/vial Conc.: 6.0 mg/mL
Mfg. Date: 20Feb15 Store at -20°C ± 10°C
Caution: New Drug – Limited by Federal
(or United States) Law to Investigational Use
Manufactured by Ajinomoto Althea, Inc.,

11040 Roselle St., San Diego, CA 92121
The Geneva Foundation, Contract V-1674-01

Box _____ of _____ Vials Per Box _____

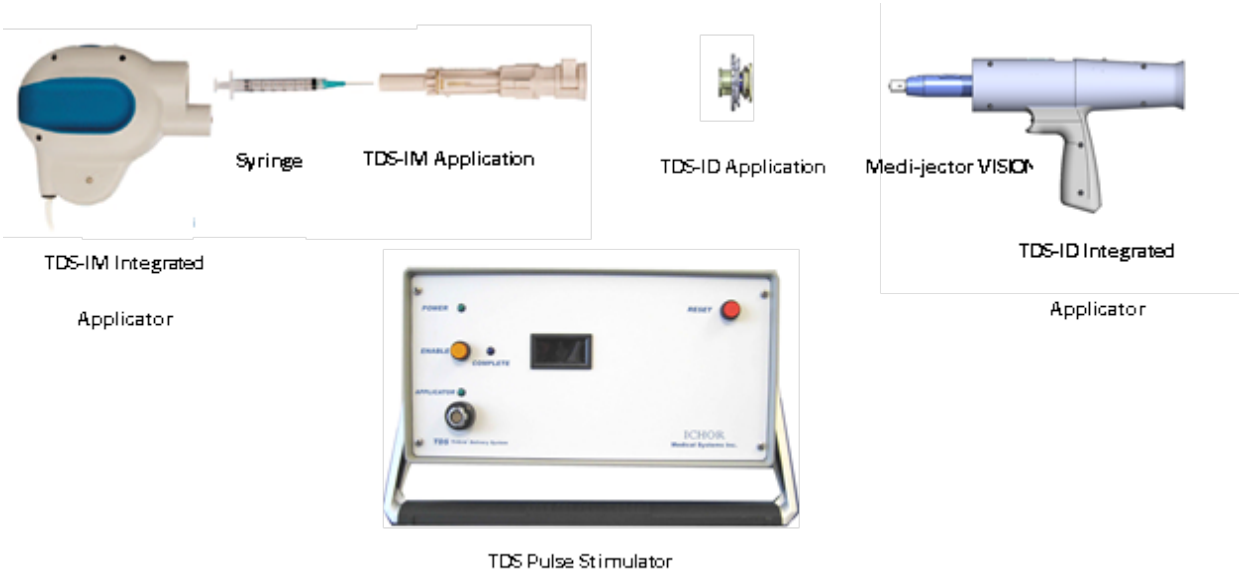
Saline material used as diluent, will be used and CoA, CoC will be provided for each lot utilized.

The vaccines may be transported to the University of Maryland's Investigational Drug Services Pharmacy using dry ice and other transport media whose average temperature is below the label ranges. Sponsor experience has shown that these vaccines may be transported and stored at temperatures below label (down to -80°C ± 10°C) for no more than 24 hours without compromising product potency or safety. Likewise, the vaccine manufacturer has successfully stored and transported plasmid vaccines at temperatures down to -80°C ± 10°C without issue.

#### 6.4.1.1. Ichor TriGrid™ Delivery System

The TDS-IM V1.0 and TDS-ID investigational devices are supplied by Ichor Medical Systems, Inc. The TDS devices utilize the in vivo application of electrical fields to enhance the intracellular delivery of agents of interest in a targeted region of tissue (EP). Before the study starts, study personnel will be trained on device usage by Ichor personnel, and this training will be documented. During this study, this device will be operated according to the TDS-IM V1.0 and TDS-ID Instructions for Use documents. Each TDS device consists of 3 parts: A pulse stimulator, an integrated applicator, and a single-use application cartridge (Figure 1). Further information on the devices can be found within the TDS-IM V1.0 and TDS-ID Investigator's brochure.

**Figure 1: TDS-IM V1.0 and TDS-ID Delivery Devices**




### 6.4.2. Investigational Product Storage

The HTNV DNA and PUUV DNA vaccines will be stored at -20°C ± 10°C. Saline will be stored at room temperature conditions, 22.5°C ± 2.5°C. Sponsor experience has shown that these vaccines may be transported and stored at temperatures below label (down to -80°C ± 10°C) for at least 24 hours without compromising product potency or safety. Likewise, the vaccine

manufacturer has successfully stored and transported plasmid vaccines at temperatures down to -10°C to -90°C (-80°C ± 10°C) without issue. Temperature excursions within these parameters during shipment/transportation of the vaccines will not incur a protocol deviation.

### 6.4.3. Investigational Product Preparation

The University of Maryland's Investigational Drug Services (IDS) will prepare the vaccines for administration. A supplementary document entitled "Manual of Procedures" has been supplied to the University of Maryland CVD describing the procedures to be followed when preparing the vaccines for administration. Briefly, the products to be administered in Groups 1-4 will be mixed with saline to bring them to the correct concentration for dosing; these groups are all single vaccine component groups. For the groups that are administered combination product (Groups 5 and 6), the HTNV and PUUV vaccines will be mixed with each other at a 1:1 ratio to bring them to the correct concentration. The supplementary document describes the products, their storage and management, procedures for product preparation, the volumes of each component to be combined, the supplies to be used (eg, syringes or other device-specific requirements), and any data collection forms for recording procedures as they are performed.

### 6.4.4. Investigational Product Accountability

The sponsor's representative is responsible for overseeing the distribution of the investigational product to the study site. In this study, investigational product refers to both the vialed vaccine, diluent, and the single-use cartridges associated with the TDS-IM V1.0 and TDS-ID devices. The sponsor's representative has delegated drug accountability responsibility for this product to The University of Maryland's Investigational Drug Services (IDS) under the supervision of the PI; however, the sponsor's representative has ultimate responsibility for product accountability. After the investigational product is distributed, the IDS is responsible for and will maintain logs of investigational product receipt, storage, reconstitution, accountability by subject, and investigational product remaining before final disposition. At the UMB CVD, the logs will be maintained in the accountability files within the office of the CVD. The PI may delegate, in writing, this responsibility to another individual, but the PI is ultimately responsible for the investigational product and its proper storage upon receipt at the study site until it is transferred back to the sponsor's representative or designee, or is destroyed as directed by the sponsor's representative.

All vials (unused, partially used, and spent) will be retained by the study staff for accountability. Any IP that experiences a temperature excursion will be placed in quarantine status and the Sponsor will be notified of the temperature excursion. The sponsor will determine the disposition of the quarantined IP. No vials should be destroyed or disposed of without specific instructions from the sponsor's representative 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 investigational products.

All unused or partially used investigational product and empty vials will be destroyed when directed by the sponsor's representative and as stipulated by local, state, and Federal regulations. This occurs after the study monitor has completed the final accountability inspection.

The single-use application cartridges for the EP device will only be retained by the study product administrator for accountability if there is an error or issue at the time of administration,

otherwise the cartridge will be discarded appropriately. Any cartridge that is found to malfunction or otherwise function incorrectly will be recorded as such so that it can be assessed by the manufacturer for the cause of said dysfunction. The disposition records will account for all remaining single use cartridges.

Following completion of vaccinations, the TDS-IM V1.0 and TDS-ID reusable devices will be returned to Ichor.

## 6.5. Duration of Subject Participation

The expected duration of individual enrolled subject participation will be approximately 5 months including the screening period.

## 6.6. Study Halting Criteria

Further enrollment and study vaccinations will be halted for SMC review/recommendation if any of the following are reported:

- Any subject experiences a study product-related SAE from the time of the study product administration through the subject's last study visit.
- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- Two or more subjects experience generalized urticaria (defined as occurring at more than 2 body parts) within 3 days after administration of study product that is considered related to study product.

This trial will also be halted for SMC review/recommendation if, within 7 days after administration of any study vaccination, any of the following occurs:

- Two or more subjects experience a Grade 3 unsolicited AE in the same MedDRA system organ class (captured by preferred term) after administration of study product that is considered related to study product and not resolved or improved to lower grade within 2 days.
- Two or more subjects experience the same Grade 3 solicited local adverse event that is considered related to study product and not resolved or improved to lower grade within 2 days.
- Two or more subjects experience the same Grade 3 solicited systemic adverse event that is considered related to study product and not resolved or improved to lower grade within 2 days.
- Two or more subjects experience the same Grade 3 laboratory adverse event that is considered related to study product.

Grading scales for solicited local (application site) and systemic (subjective and quantitative) AEs are included in Section 10.5 and Appendix C.

Grading scales for clinical safety laboratory adverse events are included in Section 10.5 and Appendix C.

### 6.6.1. Sentinel Group Halting Rules

The following halting rules will be applied in the 48 hours following sentinel group vaccination (n = 2 subjects per cohort) for review by the SRC and if any are met, the PI will notify ORA PSSO within 24 hours and further vaccinations will be halted pending SMC review:

- Any SAE regardless of the relationship to the vaccine (with the exception of death or hospitalization that was the result of trauma or accident)
- Type 1 hypersensitivity reaction (anaphylaxis or generalized urticarial)
- Any Grade 3 systemic adverse event (solicited and unsolicited)
- Any Grade 3 local adverse event (solicited and unsolicited) that has not reduced to a Grade 1 or 2 at 48 hours post vaccination[5]

### 6.6.2. Study Termination Criteria

The PI, research monitor/independent safety monitor (RM/ISM), sponsor's representative, the UMB IRB, DMID, or the FDA may stop or suspend the use of this product at any time.

## 6.7. Trial Treatment Randomization Codes

A single document delineating the randomized assignment of subjects to vaccine groups will be generated and maintained in a secure location as part of the regulatory file. During the study, investigators, research coordinators, University of Maryland's IDS, representatives of the sponsor and applicable regulatory authorities will have access to this list if required by their duties.

## 6.8. Identification of Data to Be Recorded on the Case Report Forms

The electronic case report form (eCRF) data will be transcribed from source documentation. No source data will be recorded directly in the eCRF (ie, without prior written or electronic record of data). The transcribed data will be consistent with the source documents or the discrepancies will be explained.

For more information on data handling, refer to Section 15.

[5] Injection site pain (if resolved 48 hours post vaccination), the size (measured in mm) of erythema, and the occurrence of induration/swelling will not be used as halting criteria.

# 7. SELECTION AND WITHDRAWAL OF SUBJECTS

## 7.1. Recruitment of Subjects

Healthy adult men and women will be recruited from the Baltimore-Washington, DC area through the UMB clinical trial centers by use of advertisement in multiple media formats, to include, but not limited to: informational flyers, newspaper advertisements, websites, word of mouth, and e-mail. All recruitment materials will be prepared and submitted for review and approval by the UMB IRB prior to use. When a subject calls the study site and discloses an interest in the study, the recruitment staff will discuss the trial from an IRB-approved script. If the subject is still interested, contact information will be obtained and an appointment for briefing and/or screening will be arranged.

Informed consent will be obtained from each subject prior to any procedures being performed. The study briefing and informed consent process will be done within the respective clinical trial center, which may begin in a group setting, but then proceeds to individual discussion between an investigator and study subject prior to any procedures. Study individuals who are interested in learning about the trial will meet with a member of the study team and will undergo an informed consent process consisting of a detailed informational presentation of the study given by a study investigator or by using IRB-approved briefing slides in person or by means of a pre-recorded audio recording.

Following the briefing, the coordinator or designee will provide the subject ample time to read the informed consent document. A study investigator and/or designee will answer all questions raised during the session. The subject will be asked to sign the consent. The subject will be allowed to take the consent document home to consider and discuss it with others and return to the trial center at a later time to sign it. After signing the consent, the subject will take an assessment of understanding. The assessment is administered to aid the study personnel in identifying gaps in understanding. Subjects must score at least 80% correct on the 10-question multiple-choice questionnaire. Any questions missed will be explained to the subject, and the subject's questions will be answered. The subject will be given one additional opportunity to take the comprehension test. Any subject who, in the opinion of the study investigator, does not understand the study well enough to consider their consent truly informed will be excluded.

No study procedures will occur before the potential subject provides written informed consent. To minimize any possible coercion, supervisors or anyone in the chain of command or in a position of authority will not be permitted to consent the subject. However, they will be available to answer questions and provide additional information.

Consent for Future Use: As part of the informed consent process subjects will be asked to provide permission for the future use of their specimens (see Section 14.3). Refusal to allow samples to be stored for future use will not exclude subjects from participation.

Refer to Section 4.8 for a detailed description of the subject population.

## 7.2. Eligibility Screening

Each subject must meet all inclusion and no exclusion criteria. Subjects who have signed the informed consent form and successfully completed the assessment of understanding quiz will provide a medical history and undergo a physical examination and clinical safety laboratory tests

administered for screening. The PI or other study physician will make the final decision of the eligibility. Only eligible subjects will be given the investigational product.

Refer to Section 4.8 for a detailed description of the subject population.

## 7.3. Subject Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

1. Healthy adult male or nonpregnant, nonlactating female, ages 18-49 (inclusive) at time of screening.
2. Have demonstrated adequate comprehension of the protocol by achieving a score of at least 80% correct on a short multiple-choice quiz. Individuals who fail to achieve a passing score on the initial quiz will be given the opportunity to retest after a review of protocol information. Individuals who fail the quiz for the second time will not be enrolled.
3. Have provided written informed consent before screening.
4. Free of clinically significant health problems, in the opinion of study investigators, as determined by pertinent medical history and clinical examination before entry into the study. (Refer to Synopsis for full description of terms).
5. Available and able to participate for all study visits and procedures.
6. Sexually active men[6] and women of childbearing potential[7] must agree to use an effective method of contraception from 30 days prior to the first study vaccination until 6 months after the last study vaccination.
7. Female subjects agree to not donate eggs (ova, oocytes), and male subjects agree to not donate sperm from the start of screening until at least 6 months after the last vaccination.
8. Female subjects of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to each study vaccination.

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[6] A sexually active man is defined as one whose partner is a woman of childbearing potential (see definition below) and has not had a vasectomy performed > 1 year prior to screening. They must agree not to father a child until 6 months after the last vaccination. These subjects must agree to use a barrier method of birth control (eg, either condom with spermicidal foam/gel/film/cream or partner usage of occlusive cap [diaphragm or cervical/vault caps] with spermicidal foam/gel/film/cream/suppository).

[7] Women of childbearing potential are defined as those who have not been sterilized via tubal ligation, bilateral oophorectomy, bilateral salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with history of documented radiological confirmation test at least 90 days after the procedure (or with use of another birth control method if history of confirmation test not confirmed), still menstruating or < 1 year of the last menses if menopausal. For this study, an effective contraceptive method is defined as one that results in a failure rate of less than 1% per year when it is used consistently and correctly.

9. Negative hantavirus PsVNA test result at screening.
10. Screening laboratory test values:
    - Hemoglobin > 11.0 g/dL for women; > 12.9 g/dL for men
    - WBC with differential and platelets either within the normal range (provided by the laboratory performing the analysis) or a Grade 1 deviation from normal (per Appendix C) and deemed clinically insignificant
    - Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin < 1.1x upper limit of normal (ULN) (per the normal range provided by the laboratory performing the analysis)
    - Serum creatinine < 1.35 g/dL

For all other screening laboratory test values, results that do not meet the definition of a Grade 1-4 value within 56 days prior to enrollment (see Appendix C, "Clinical and Laboratory Toxicity Grading Scales") are eligible for enrollment.

## 7.4. Subject Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. History of prior infection with any hantavirus virus or prior participation in an HTNV, PUUV, or Andes virus vaccine trial.
2. Has plans to travel to an area with endemic[8] Hantaan, Puumala, Seoul, and Dobrava virus transmission during the study.
3. History of severe local or systemic reactions to any vaccine or vaccine product[9] or a history of severe allergic reactions.
4. Is currently participating or plans to participate in another clinical study involving any investigational product (including vaccines) or involves blood drawing, and/or an invasive procedure.[10]
5. Receipt or planned receipt of any live vaccination, experimental or otherwise, within the period 30 days prior to or after each vaccination and receipt of an inactivated vaccination, experimental or otherwise, within the period of 14 days prior to or after each vaccination.

[8] Refer to the MOP for information on areas with endemic Hantaan, Puumala, Seoul, and Dobrava virus transmission.

[9] This includes a known allergy to an aminoglycoside (eg, gentamicin, tobramycin, neomycin, and streptomycin).

[10] An invasive procedure includes endoscopy, bronchoscopy, or procedure requiring administration of IV contrast or removal of tissue.

6. Individuals, who based on clinical assessment by the investigator, have insufficient muscle mass to accommodate the 1 inch/25 mm penetration depth or have a skinfold thickness at eligible injection sites (deltoid region) that exceeds 40 mm.
7. Individuals in whom the ability to observe possible local reactions at the eligible injection sites (deltoid region) is, in the opinion of the investigator, unacceptably obscured due to a physical condition or permanent body art.
8. Presence of any surgical or traumatic metal implants at the site of administration (medial deltoid muscles or overlying skin).
9. Subjects with autoimmune disorders or chronic inflammatory disorders with a potential autoimmune correlation.
10. Receipt of immunoglobulins and/or any blood products within the 120 days preceding screening or planned administration during the study period.
11. Donation of blood to a blood bank within 56 days prior to screening and at any time during the study period.
12. Subject seropositive for hepatitis B surface antigen (HBsAg) or hepatitis C antibodies (anti-HCV).
13. Any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV infection or use of anticancer chemotherapy or radiation therapy (cytotoxic) in the 3 years prior to screening.
14. Administration of chronic (defined as more than 14 days) immunosuppressants or other immune-modifying drugs within 6 months of screening. For corticosteroids, this will mean prednisone, or equivalent, greater than or equal to 5 mg/day. Intranasal, inhaled (< 800 beclomethasone mcg/day), and topical steroids are allowed.
15. Has current or past diagnosis of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.
16. Has been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to screening.
17. Any chronic or active neurologic disorder, including Guillain-Barré syndrome, seizures and epilepsy, excluding a single febrile seizure as a child.
18. Syncopal episode within 12 months of screening.
19. Suspected or known current alcohol and/or illicit drug abuse within the past 5 years based on self-reporting and physical exam.
20. Any medical, psychiatric, social condition, occupational reason, or other responsibility that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a subject's ability to give informed consent or to comply with the protocol schedule.
21. Pregnant or lactating female, or subject plans to father a child or become pregnant during the study period.

22. Current employee or staff paid entirely or partially by the contract for this trial, or staff who are supervised by the PI or subinvestigators.
23. Subjects with implanted electronic devices stimulation device, such as cardiac demand pacemakers, automatic implantable cardiac defibrillator, nerve stimulators, or deep brain stimulators.
24. Bleeding diathesis or condition associated with prolonged bleeding time, as determined through measurement of PT/PTT (prothrombin time/partial thromboplastin times) during screening labs, that would contraindicate ID or an IM injection.
25. History of cardiac arrhythmia or palpitations or abnormal arrhythmia noted on ECG performed at screening (eg, supraventricular tachycardia, atrial fibrillation, frequent ectopy) prior to study entry. Measurement of sinus bradycardia (ie, < 50 beats per minute on exam) at screening.
26. History of diabetes type 1 or 2

### 7.4.1. Temporary Exclusion Criteria

The following criteria will result in a delay for vaccination of subjects:

1. A febrile illness (oral temperature of > 38.0°C) within 48 hours before vaccination or other evolving acute illness.
2. Use of antibiotics for an acute illness within 24 hours or any antiviral within 72 hours of study vaccination.
3. Use of allergy treatment with antigen injections within 30 days prior to initial study vaccine administration.

## 7.5. Termination of Dosing and/or Subject Withdrawal Criteria

Termination of dosing is defined as someone who elects to no longer receive doses but is willing to remain on the study for continued observations, immunogenicity assessment and monitoring of safety parameters. Withdrawal from study is defined as not participating in this study any longer, and not continuing to be observed by the study physician. Each subject may withdraw consent for continued dosing or from the study at any time without penalty. Counseling about the subject's health will be provided 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 PI may discontinue the subject's receipt of investigational product without the subject's consent if any of these criteria is met:

1. The discovery or development of any health condition within a subject that would make his or her continued participation in the protocol dangerous to him- or herself.
2. The failure of the subject to comply with the requirements of the protocol.
3. The scientific integrity of the study may be compromised by further participation.
4. 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.

For example:

- Pregnancy
- Receipt of disallowed licensed vaccine, experimental product or medication
- New onset of illness or condition that meets the Exclusion Criteria
- Medical condition or medication change for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would pose a risk to the subject or would likely confound interpretation of the results
- Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity
- Grade 3 solicited or unsolicited adverse event that is ongoing, whether or not it is improved or resolving. An unresolved or continuing Grade 1 or Grade 2 adverse event is permissible following the documented determination by the site principal investigator or appropriate sub-investigator, that it would not render study vaccination unsafe or interfere with the evaluation of adverse events or immunologic response
- Grade 3 solicited or unsolicited adverse event that occurs without alternative etiology in the 7 days following study vaccination
- Any laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product
- Any generalized urticaria within 3 days after administration of study product that is considered related to study product
- Serious adverse event related to the study vaccination
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons
- Subject refusal of further study vaccination
- Termination of this trial
- New information becomes available that makes further administration of the study vaccine unsafe

### 7.5.1. When and How to Terminate Dosing and/or Withdraw Subjects

While the preference will be that all subjects complete all study visits, 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 termination procedures as outlined in Section 7.5.5. Telephone calls, registered letters, and e-mail 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.

The investigator may elect to stop further dosing a subject for an adverse event (AE) or serious adverse event (SAE) resulting in a safety concern, for noncompliance with protocol requirements, or for circumstances that study personnel feel is in the best interest of the study participant. When a subject withdraws from the study due to an AE or further dosing is discontinued by the PI due to an AE, the following should be notified within 24 hours: the UMB

IRB; the sponsor's clinical trial monitor; and DMID. Investigators must follow specific policy regarding the timely reporting of AEs and SAEs to the UMB IRB (Section 10.7.1). In all cases, the PI will make a reasonable effort to complete study termination procedures per Section 7.5.5.

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.

### 7.5.2. Termination of Dosing

Subjects who do not receive all 3 vaccinations will be encouraged to remain in the study and complete study visits for follow-up, safety and immunogenicity assessments per the Study Events Schedule (Table 4). If the scheduled visit does not include collection of blood for safety or immunogenicity, the visit may be conducted by phone call/ electronic communication (eg, e-mail, text message) rather than in person.

### 7.5.3. Data Collected for Withdrawal 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 samples collected for storage and use in future research, will be kept and utilized as outlined in the protocol and consent form. The study termination eCRF will be completed, with the reason for withdrawal specified.

### 7.5.4. Replacement of Subjects

To ensure that a required minimum number of subjects (60) complete key study events for purposes of statistical analysis, 6 randomized groups of 12 subjects each will be enrolled, for a total of 82 subjects. If a subject is deemed ineligible or withdraws prior to enrollment/first dose (study Day 1) they will be replaced to ensure 82 subjects are enrolled. This approach will ensure at least 60 subjects complete all vaccinations at around 10 subjects per group, taking possible attrition into account from the start of the study.

### 7.5.5. Follow-up for Withdrawal Subjects

If a subject withdraws from the study, the investigator will make a reasonable effort to determine the reason for the subject's withdrawal and to have the subject return to the clinic to complete the planned activities/termination procedures detailed in Section 8.1.5. Withdrawing from the study for any reason will not impact the subject's medical care. Subjects who withdraw will be followed, as allowed by the subject, through resolution of any ongoing adverse events. Women who become pregnant and men who report a partner's pregnancy during the duration of the study will be encouraged to seek obstetric care and will be asked to provide follow-up information at the conclusion of the pregnancy. These pregnant subjects will be followed for safety and will not receive any additional immunizations. They will be followed for the duration of their pregnancy, and any evidence of fetal harm will be reported promptly to the UMB IRB and sponsor within 24 hours of study team knowledge.

# 8. TREATMENT OF SUBJECTS

## 8.1. Detailed Description of Study Visits

### 8.1.1. Screen 1/Briefing Visit (First Visit; Day -90 to Day -7)

Screening and enrollment procedures will be the same for all potential subjects. Potential subjects will undergo screening no more than 90 days prior to their planned initial injection (Day 0, Visit 1). All subject screen visit procedures will be performed on the first screening visit (Screen 1) to include clinical safety lab tests. The immunogenicity tests (HTNV and PUUV PsVNA) will be performed at a secondary screening visit (Screen 2) allowing sufficient time to process the sera while assuring subjects' safety lab values are still within accepted values within 56 days prior to first dose administration. A period of 56 days to complete screens 1 and 2 prior to enrollment is requested to accomplish not only the lengthy process of screening more than 200 people for the goal of enrolling 82 subjects, but to ensure the data from the immunogenicity tests will be returned for selection of subjects. A turnaround time of up to 4 weeks is required for results of the PsVNA immunogenicity tests (see Section 9.3). If more than 56 days has passed from the time of clinical safety lab test performance to the first vaccine dose, clinical safety lab tests will be rechecked on subjects who have passed the immunogenicity screen.

The following procedures will be performed during the initial screening/briefing visit (Screen 1):

- Brief potential subject and review of informed consent with subject
  - Obtain written informed consent for participation in the study and for HIV testing
  - Assess of subjects informed consent/protocol comprehension by administering a quiz
  - Provide copy of signed informed consent documents to subject
- Review inclusion and exclusion criteria to assess if subject is eligible for study
- Query subject for demographic information and medical history including concomitant medications
- Do a full physical examination to include the following organs and organ systems: general appearance, skin, head and neck, lungs, heart, liver, spleen, extremities, musculoskeletal, lymph nodes and nervous system. The exam will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator
- Take resting vital sign measurements including oral temperature, heart rate, respiratory rate, and systolic and diastolic blood pressure. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature
- Measure skinfold thickness of potential injection sites with a caliper
- Record subject's weight
- Collect approximately 33 mL venous whole blood for clinical safety laboratory tests to determine trial eligibility:

  - CBC (WBC, Hb, platelets) with differential
  - Glucose, Cr, AST, ALT, total bilirubin
  - Viral serologies (HBsAg, anti-HCV Ab, and anti-HIV Ab)
  - PT/PTT tests
- Collect blood for serum βHCG pregnancy test for all females of childbearing potential
- Perform ECG to determine trial eligibility. A baseline 12-lead ECG will be obtained on all subjects as part of the screening process. A study investigator will review all ECGs and a board-certified cardiologist will be available to review any questionable ECG readings. An abnormal ECG may be defined as, but not limited to, showing pathologic Q waves and significant ST-T wave changes; left ventricular hypertrophy unrelated to that of a healthy, athletic volunteer; any non-sinus rhythm excluding isolated premature atrial contractions; right or left bundle branch block; or advanced (secondary or tertiary) A-V heart block. Cardiology back-up may be obtained if questions arise as to the suitability of a candidate based upon the ECG results.

### 8.1.2. Screen 2 Visit (Second Visit; Day -90 to Day -7)

Screen 2 is required to determine if a potential subject, who had acceptable clinical laboratory screening results and met other eligibility criteria, has evidence of prior exposure to hantaviruses. Those who are seropositive, as defined by a Hantavirus PsVNA50 titer ≥ 20, will not be eligible to participate in the study. A turnaround time of up to 4 weeks is required for PsVNA50 results. (see Section 9). The following procedures will be performed during the second screening visit (Screen 2):

- Collect approximately 10 mL whole venous blood for the determination of:
  - Hantavirus PsVNA50 screen for prior humoral immunogenicity

If Screen 2 falls outside of the 56-day screening window prior to first vaccination, then the following tests will also be conducted:

- Check inclusion and exclusion criteria to ensure that subject is still eligible for the study
- Query subject about interim medical history including concomitant medication use
- Do a directed physical examination if indicated based on the subject's interim medical history. The exam will be done by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator
- Take resting vital sign measurement including oral temperature, heart rate, respiratory rate, and systolic and diastolic blood pressure
- Weigh and record subject's weight. If there is a > 15% change in body weight, remeasure skinfold thickness of potential injection sites with caliper
- Collect approximately 24.5 mL of whole venous blood for the determination of:

  - CBC (WBC, Hb, platelets) with differential
  - Glucose, Cr, AST, ALT, total bilirubin
  - Viral serologies (HBsAg, anti-HCV Ab, and anti-HIV Ab)
- Collect urine from all females of childbearing potential for urine pregnancy test

If deemed appropriate by an investigator, any or all of the above screening laboratories may be repeated at Screen 2 or a later (planned or unplanned) study visit to allow an accurate determination of a potential subject's eligibility. If screening lab values are out of the normal range (i.e. Grade 1-4 as outlined on toxicity table), with the exception of the values noted in Inclusion Criteria 10, but are expected to be temporary (eg, due to dehydration), they may be re-assessed onetime at the discretion of the investigator. If these screening procedures have occurred within the 56-day screening window prior to the first vaccination, the results and documents will not need to be repeated, as copies will be placed into the study file from the screening protocol file.

Potential subjects will be notified of their eligibility as soon as all lab 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 0, Visit 1).

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

Information on individuals with evidence of reportable infectious diseases (eg, HIV and hepatitis) will be transmitted to the required public health authorities as applicable. Potential subjects will be notified of this possibility during their informed consent process.

### 8.1.3. Vaccination Visits

There will be 3 primary vaccination visits: Day 0, Day 28 ± 4 days, and Day 56 ± 4 days. Subjects will receive up to 3 vaccinations in the outer aspect of the subject's upper arm (deltoid area; alternating arms, beginning with left, if possible), using the TDS-IM V1.0 or TDS-ID EP system during the course of the study. Vaccine composition, vaccine dose and schedules (per group), subject randomization, and the TDS EP system have been discussed earlier in this protocol.

The following procedures will be performed during each vaccination visit:

- Review subject's medical history including concomitant medication use since screening or previous vaccination
- Review eligibility criteria with subjects and safety criteria including AEs, prior to study vaccination to ensure continued eligibility (see Section 7.5)
- Do a directed physical examination if indicated based on the subject's interim medical history (see also Table 4). Assess injection site prior to study vaccination to establish a baseline.

- Take resting vital sign measurement including oral temperature, heart rate, respiratory rate, and systolic and diastolic blood pressure. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Weigh subject, and if there is a > 15% change in body weight, measure skinfold thickness at potential injection sites with a caliper
- Perform a buccal swab for human leukocyte antigen (HLA) testing (Day 0 only)
- Collect approximately 22.5 mL of whole venous blood for the determination of:
  - CBC (WBC, Hb, platelets) with differential
  - Glucose, Cr, AST, ALT, total bilirubin
  - HTNV and PUUV-specific antibody as measured by PsVNA, using approximately 10 mL of the total 22.5 mL blood
- Urine sample collection for determination of pregnancy (must be negative prior to administration of the vaccine)
- Injection with study product
- Post-injection observation period/reactogenicity assessment (Subjects will be kept under observation for at least 30 minutes after each inoculation to ensure their safety, and any reactions during this period will be documented. Appropriate medical equipment and emergency medications, including epinephrine [1:1000], will be available on site in the event of an anaphylactic or other immediate allergic reaction.)
  - Give subject ruler and thermometer and explain how to use (Day 0 only)
  - Distribute memory aid and teach them how to complete memory aid
  - Repeat examination of injection site
  - Do vital signs prior to discharge from clinic
  - Evaluate subject for occurrence of any immediate local and systemic reactogenicity and record AE(s) on appropriate form prior to discharge

### 8.1.4. Follow-Up Visits

There will be 9 follow-up visits: Day 2 + 1 day, Day 14 ± 2 days, Day 30 ± 1 day, Day 42 ± 2 days, Day 58 ± 1 day, Day 70 ± 2 days, Day 84 ± 4 days and Day 140 ± 7 days and D220 Phone Call ± 14 days

The following procedures will be performed during each follow-up visit (some exceptions):

- Review of subject's medical history (including concomitant medications and AE) since previous visit
- Directed physical examination (injection site) if indicated based on the subject's interim medical history

- Take resting vital sign measurement including oral temperature, heart rate, respiratory rate, and systolic and diastolic blood pressure. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Review and collect memory aids (Days 14, 42, 70)
- Collect approximately 12.5 to 72.5 mL (depending on the scheduled visit day, see Table 4) of whole venous blood for the determination of:
  - CBC (WBC, Hb, platelets) with differential (Days 14, 42, and 70 only)
  - Glucose, Cr, AST, ALT, total bilirubin, (Days 14, 42, and 70)
- Collect blood for determination of HTNV- and PUUV-specific antibodies as measured by PsVNA assay (Days 84 and 140)
- Collect approximately 50 mL of blood for future research on Day 84 only
- Collect blood sample for determination of pregnancy test (Day 140 only)
- Perform ECG as last assessment of cardiac normality (Day 140 only)

Day 140 will be the final scheduled in-clinic visit for all subjects

**Serious Adverse Event Final Telephone Follow Up Visit**

There will be a scripted telephone follow up visit conducted at Day 220 ± 14 days.

- Review and collection of serious adverse events occurring since last visit conducted at Day 140.

**Early Termination Visits**

If a subject leaves the study early (see also Section 7.5), procedures pertaining to Day 140 in the follow-up visit section (Section 8.1.4) will be followed:

- Review of subject's medical history (including concomitant medications and AE) since previous visit
- Directed physical examination (injection site) and resting vital signs check (oral temperature (no recent hot or cold beverages or smoking), heart rate, respiratory rate, and systolic and diastolic blood pressure)
- Review and collection of any remaining memory aids
- HTNV- and PUUV-specific antibodies (PsVNA50 for final visit)
- Serum sample collection for determination of pregnancy test
- Perform ECG

## 8.2. Biological Samples

Samples collected under this protocol will be used to conduct protocol-related safety and immunogenicity evaluations (HTNV and PUUV assays). All specimens for laboratory testing will be collected at the University of Maryland Center for Vaccine Development. The amount of

blood to be drawn, by visit, is provided in Appendix D. All samples will be collected using standard techniques and safety precautions.

General HLA testing will be performed on buccal swab samples on Day 0. This is exploratory in nature for the purpose of understanding genetics and immune responses. Future use research studies may include results of HLA testing to explore the impact of host genetics on immune responses to HTNV and PUUV DNA vaccine. Results of HLA testing will be part of research records and will not become part of a subject's medical record and will not be shared with their doctor.

If necessary, biological samples will be stored temporarily at the University of Maryland Center for Vaccine Development 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. Upon receipt of humoral immunogenicity samples at USAMRIID, any non-used/residual sera remaining after completion of the immunogenicity testing may be retained and used for future research.

Any study for the future use of these biological samples will have the appropriate human subjects protection regulatory review and 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 or her samples, any unused samples will be destroyed. Samples will be stored indefinitely after transport to USAMRIID.

## 8.3. Concomitant Medications

Concomitant medications will include all current medications and medications taken within 30 days prior to signing the ICF through approximately 28 days after the last study vaccination or early termination (if prior to 28 days after the last study vaccination), whichever occurs first. Medications will include prescription and over-the-counter drugs as well as herbals, vitamins, and supplements. Even though the medications of greatest concern are those taken 30 days prior to signing the ICF to 28 days post last vaccination, concomitant medications will be collected and recorded at each study visit.

Medications and non-study vaccines reported in the electronic case report form (eCRF) are limited to those received within 30 days prior to the first study vaccination through approximately 28 days after the last study vaccination.

Medications that might interfere with the evaluation of the investigational product(s) should not be used from time of study vaccination through 28 days post the last vaccination unless clinically indicated as part of the subject's health care. Medications in this category include the prohibited medications per the Subject Exclusion Criteria (see Section 7.4).

The only restrictions on the use of concomitant medications by subjects during this study are medications that alter the immune system. The following medications are disallowed during the study:

- Chronic (defined as more than 14 days) immunosuppressants or other immune-modifying drugs within 6 months of study entry. For corticosteroids, this will mean prednisone, or equivalent, greater than or equal to 5 mg/day. In contrast, intranasal,

inhaled (< 800 beclomethasone mcg/day), and topical steroids are allowed during the study.

- Antibiotics for an acute illness within 24 hours or any antiviral medication taken within 72 hours of each study vaccination.
- Allergy treatment with antigen injections within 30 days prior to initial study vaccine administration and during the study.

## 8.4. Subject Compliance and Memory Aid Completion

Subjects who are unable to follow the study requirements and arrive at study visits will be withdrawn from the study at the PI's discretion. Poor compliance with study visits would include the inability to come to a scheduled study visit within window on 2 or more occasions. Withdrawal procedures for such instances are detailed in Section 7.5.

Following each injection, subjects will be provided a memory aid (ie, fillable diary card) and instructed in its use. On a daily basis during the 14-day period after each injection subjects will evaluate and record any local or general symptoms (eg, fever, myalgia, and malaise) they experience (see Section 10.3.2). 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 daily for the first 14 days after each injection. Also, subjects will be asked to record local reactions at the injection site (such as erythema, edema, bruising, and pain) for 14 days after each injection. To more accurately account for the size of any such reactions, subjects will be given a ruler and instructed on how to use it. Memory aids will be reviewed by an investigator at follow-up visits during the 14-day post-injection period. Memory aids will be collected from the subjects at the 14-day post-injection visit. If memory aids are not completed or returned, an investigator will review the AEs that occurred during that time frame to the best of the subject's recollection. Subjects will be instructed on the use of the memory aid and encouraged to fill it out as directed; if it is not completed or not brought to a study visit by 28 days following each dose, then a protocol deviation will be documented. The maximum intensity reported by the subject whether recorded on the memory aid or verbally reported to the study staff will be recorded in the eCRF as an AE with a grade and relationship as determined by the assessing investigator.

# 9. IMMUNOGENICITY ASSESSMENTS

## 9.1. Immunogenicity Endpoints

The endpoint used to measure immunogenicity of the HTNV DNA and PUUV DNA vaccine components is the production of neutralizing antibody titers to HTNV and PUUV. The primary assay used to detect and quantify hantavirus neutralizing antibodies will be the PsVNA50. This assay will be used on samples collected during Screen 2 to measure any prior humoral immunogenicity, and throughout the rest of the study to assess immunogenicity in response to the vaccinations, which is the secondary endpoint of the study. A PsVNA50 titer ≥ 1:20 in 2 independent assays is considered positive. Initial immunogenicity results will be analyzed on the basis of intention-to-treat, in which the outcomes of all subjects who had at least one dose of vaccine, and contributed both pre- and at least one post-study vaccination venous blood sample for immunogenicity testing for which valid results were reported, will be analyzed with the group to which they were originally assigned, regardless of whether they completed the study. Subsequently, all subjects who completed the study and have serologic data will be included in the analysis of immunogenicity.

## 9.2. Sample Analysis

Blood will be collected, processed, aliquoted into samples, and shipped from the UMB CVD to USAMRIID per the laboratory manual instruction entitled “Specimen Collection, Handling/Processing and Transport for Research Blood Samples.” Samples will be stored at USAMRIID’s Nonclinical Development Division (NCDD). USAMRIID Molecular Virology Branch personnel will receive frozen sera. Samples will be thawed on wet ice and heat inactivated at 56°C (± 1°C) for 30 ± 1 minutes in a water bath. Samples will then be stored at 4°C (± 2°C) until assays are performed (< 5 weeks). Afterward, samples will be returned to -70°C (± 10°C) storage. Dates of serum thawing, heat inactivation, and re-freezing will be recorded. As a control for bias, beginning at the Day 0 sampling the labels on the sera will not identify the subject or the dose/regimen the subject received. All sera will be pre-screened at a 1:20 dilution in both the HTNV and PUUV PsVNA. Positive samples will be further analyzed by PsVNA to determine endpoint titers. Depending on resources and time availability, certain samples may be directly titrated for endpoint (ie, no pre-screen); this will be recorded and documented. For those samples, a second assay will be performed to confirm the results. If PsVNA50 titers are determined in 2 independent assays, then GMTs for the combined results will be calculated.

USAMRIID SOP AP-05-16, Pseudovirion Neutralization Assay, will be used for all PsVNA. If a pre-screened sera sample has a titer of ≥ 20, but if during re-titering for endpoint determination the sample is found to have a titer < 20, then the more conservative < 20 value will be used. If an assay fails to meet the SOP acceptance criteria or if there is assignable cause, then the assay will be repeated. For individual specimens, if questions of accuracy arise, then those specimens will be retested. Questions of accuracy include differences in duplicates greater than 4-fold in titer, or titers > 50,000. If a sample is retested and the assay passes acceptance criteria, then the new value will be reported but noted as a repeat.

Data will be reported as HTNV and PUUV PsVNA50 titers. Seroconversion rate, GMT, and peak titer, for the cohorts will be determined. A contributing scientist report summarizing the

neutralizing antibody titers for the samples will be prepared after the titers for the final blood draw have been determined. Laboratory personnel can receive the list of subject treatment assignments following database lock.

In this study, up to 10 mL of blood on Days 0, 28, 56 and 140 and approximately 50 mL of blood on Day 84 will be collected for future use purposes. Any blood remaining after immunogenicity studies are conducted will be retained for future hantavirus-related research for subjects who agreed to future use of these samples. These samples will be stored at USAMRIID. The future research purposes for which this blood will be utilized are yet to be determined, but are likely to be additional immunogenicity assays; exact assays are yet to be determined.

### 9.2.1. Primary Immunogenicity Endpoint

The primary immunogenicity endpoint is the production of hantavirus neutralizing antibodies after vaccination as measured by PsVNA50. This information will allow calculation of seroconversion rates. Seropositivity is defined as a post-vaccination PsVNA50 titer ≥ 20. The primary analysis variable will be the proportion of seropositive subjects (defined as PsVNA50 ≥ 1:20) at each scheduled time point (eg, Days 0, 28, 56, 84 and 140), and the final overall rate of seroconversion over these time points. Seroconversion is defined as a post-vaccination HTNV- or PUUV-specific titer of ≥ 1:40, or a minimum four-fold rise compared to baseline titer, and all study volunteers will begin the study with a baseline titer < 20 (eg, are seronegative). The PsVNA that will be used in this trial is a well-characterized, investigational assay that has been used in multiple research studies. This assay is based on vesicular stomatitis virus (VSV) that expresses a luciferase reporter protein. The VSV-luciferase particles are pseudotyped with hantavirus envelope glycoproteins. When the PsV successfully attach to and then enter cells, the luciferase reporter protein is expressed. If sera contain antibodies that prevent the PsV from attaching to and/or entering cells, then the reporter activity is neutralized. The reciprocal of the dilution that results in a 50% decrease is the PsVNA50 titer. The assay will be performed under GLP conditions in accordance with an SOP and will be monitored for quality by USAMRIID's quality assurance unit.

### 9.2.2. Secondary Immunogenicity Endpoints

The secondary analysis variable will be GMT, with 95% CIs, of the PsVNA50 for HTNV- and PUUV-specific antibodies at each scheduled time point (eg, Days 0, 28, 56, 84and 140). GMTs will be calculated using PsVNA50.

### 9.2.3. Exploratory Immunogenicity Endpoints

PsVNA80 titers may be calculated from existing data for information. The reciprocal of the dilution that results in an 80% decrease in luciferase activity is the PsVNA80 titer. PsVNA using other hantavirus PsV (eg, Seoul virus, Dobrava virus) may also be performed to evaluate levels of cross-neutralizing antibodies.

## 9.3. Methods/Timing for Assessing, Recording, and Analyzing Immunogenicity Endpoints

### 9.3.1. Pre-screening Prior to Enrollment

Subjects will be screened to ensure they are negative for hantavirus neutralizing antibodies prior to enrollment as defined by HTNV- or PUUV-specific titer <20 as measured by PsVNA50. Serum samples collected at the study site will be sent to USAMRIID. Serum samples will be stored at USAMRIID NCDD. USAMRIID Molecular Virology Branch personnel will receive frozen sera from USAMRIID NCDD. The labels on the serum aliquots will be verified to ensure they include the correct information for each sample. Samples will be thawed on wet ice and then heat-inactivated at 56°C (± 1°C) for 30 ± 1 minutes in a water bath. Samples will then be stored at 4°C (± 2°C) until assays are performed. The pre-screening assays will be performed approximately 2 weeks after arrival at USAMRIID. Puumala virus and Hantaan virus PsVNA will be performed in accordance with USAMRIID SOP AP-05-16 "Pseudovirion Neutralization Assay." Screening results will be reported to CVD in a timely manner to assess subject inclusion/exclusion, but it has been communicated that the results can take up to 4 weeks to be made available. If pre-screening samples are positive for Puumala or Hantaan neutralizing antibodies as measured by PsVNA in 2 independent assays, then that subject would be excluded from the study.

### 9.3.2. Determining Neutralizing Antibody Titers of Study Subjects

Sera for immunogenicity testing will be collected on Days 0, 28, 56, 84and 140. Samples will be analyzed as described in Section 9.2. All PsVNA50 titers will be reported to the PI after the final titer has been determined and have completed all USAMRIID quality assessments.

# 10. SAFETY ASSESSMENT

Safety monitoring will be conducted throughout the study; therefore, safety concerns will be identified by continuous review of data by the PI, clinic staff, clinical monitor, RM/ISM, DMID medical monitor, and USAMRDC ORA PSSO.

## 10.1. Study Safety Management

The IRB, DMID, RM/ISM, USAMRDC ORA PSSO, and PI will review any safety concern. A Safety Review Committee and a DMID Safety Monitoring Committee will be established.

### 10.1.1. Research Monitor/Independent Safety Monitor

The RM/ISM oversight position is required by the sponsor per Department of Defense Instruction 3216.02. The RM/ISM, provided by and local to the CVD, is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely manner. The RM/ISM will review SAEs in real time and unanticipated problems involving risks to subjects or others.

The RM/ISM is responsible for overseeing the safety of the research and reporting observations/findings to the IRB or a designated institutional official. The RM/ISM will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The RM/ISM may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the RM/ISMs report; and shall have the responsibility to promptly report his or her observations and findings to the IRB or other designated official.

In addition to the responsibilities above the RM/ISM is required to review and provide an unbiased written report for all SAEs and subject deaths to the USAMRDC ORA PSSO within 24 hours of awareness of the event. The report provided must include, at a minimum, a brief summary of the RM/ISM's review of the event and event outcome, relationship of the event to the investigational product, and whether or not the RM/ISM concurs with the details of the study investigator's report.

### 10.1.2. USAMRDC ORA PSSO

The USAMRDC ORA PSSO is responsible for coordinating and integrating the review of safety data regarding The Surgeon General, Department of the Army (TSG-DA)-sponsored products. The PSSO reviews each SAE report for medical consistency, accuracy, and completeness and follows each event until it is satisfactorily resolved.

### 10.1.3. Safety Review Committee

The SRC will be composed of:

- PI and/or subinvestigator
- RM/ISM
- DMID medical monitor

- USAMRDC pharmacovigilance physician or designee

Objective criteria and safety evaluations will be utilized. For each group of sentinel subjects, the SRC will evaluate safety data at 48 hours post first dose. If none of the events described (in sentinel group halting rules) are observed in the sentinel subjects, vaccination of the remainder of the cohorts will proceed. Should any of the study halting criteria be met, the PI will notify ORA PSSO within 24 hours. The SMC will meet to evaluate data and make a recommendation. The SMC recommendation will be documented and provided in writing to the ORA PSSO. The sponsor's representative will then decide to terminate, modify, or continue the conduct of the study. The ORA PSSO will communicate the final decision to the appropriate parties involved in the study (ie, PI, RM/ISM, and DMID). The PI will in turn notify the IRB as appropriate. USAMRDC RA Scientist will communicate the final decision to FDA as appropriate.

### 10.1.4. DMID Safety Monitoring Committee (SMC)

Safety oversight will be conducted by an SMC, which is an independent group of experts that monitors subject safety and advises DMID and the study team. SMC members will be separate and independent of study personnel participating in this study and should not have scientific, financial, or other conflicts of interest related to the study. The SMC will consist of at least 3 voting members with appropriate expertise to contribute to the interpretation of safety data from this trial. The SMC will operate under the rules of a DMID-approved charter. The SMC will review applicable data to include but not limited to enrollment, demographics, dosing, and clinical and safety data, which may include solicited and unsolicited AEs/SAEs, reactogenicity, concomitant medications, clinical safety laboratory values, and any physical examinations at scheduled time points during the study as defined in the charter. The objective of the SMC is to make recommendations to the sponsor if the study should continue per protocol, be modified and then proceed, or be terminated. After each meeting the SMC will make its recommendations in writing regarding continuation, modification, or termination of the clinical trial and provide to ORA PSSO.

The SMC meetings for data review are as follows:

- Organizational meeting (prior to start of the study)
- An SMC ad hoc meeting will be convened when a halting rule is met or at the request of the investigator and/or DMID if there are safety concerns during the course of the study.
- Annual review meetings.
- End of study safety data review (after database lock and prior to final clinical study report).

Data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID.

## 10.2. Specification of Safety Endpoints

Safety will be assessed by evaluating reactogenicity (local reactions and systemic reactions such as fever, headache, and fatigue) during specified periods of the study. The following endpoints will be evaluated:

- The occurrence of solicited local and systemic AEs occurring from the time of each injection through 14 days following the procedure (memory aid to assist with the first 14 days)
- The occurrence of vaccine-related unsolicited AEs from the time of the first injection through 28 days following each injection
- The occurrence of SAEs from the time of the first injection through the final study visit for each subject (approximately 6 months post-last vaccination)
- The occurrence of clinical safety laboratory AEs through 14 days following each study vaccination

## 10.3. IND Safety Reporting

The following terms, as defined by 21 CFR 312.32, apply to IND safety reporting.

### 10.3.1. Adverse Event or Suspected Adverse Reaction

ICH E6 Good Clinical Practice Guidelines define an adverse event (AE) as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product.

An AE is considered to be any adverse change or exacerbation from a baseline condition that occurs following the initial administration of an investigational product whether or not the event is considered to be related to the investigational product. 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 an increased intensity of a condition
- Concomitant disease with onset or increased severity after the start of study product administration
- A new pattern in a preexisting condition, occurring after the receipt of investigational product that may signal a clinically meaningful change
- Clinically significant changes in laboratory values

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Adverse events will be documented in terms of a medical diagnosis. When this is not possible, the adverse event will be documented in terms of signs and/or symptoms observed by the investigator or reported by the subject at each study visit. AEs occurring while on study will be documented appropriately regardless of relationship. Information to be collected include event

description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of resolution/stabilization of the event. All AEs will be followed to adequate resolution or stabilization.

All AEs must be graded for severity and relationship to study product.

### 10.3.2. Solicited Adverse Event

A solicited AE is a predetermined event, identified in the Investigator's Brochure, which may reflect safety concerns related to the investigational product. The solicited AEs for this study include:

- Redness, swelling, bruising, pain, or tenderness at the injection site
- Fever
- Myalgia (general muscle aches)
- Headache
- Lymphadenopathy
- Axillary pain or discomfort
- Tachypnea
- Fatigue

Adverse events that are considered related to the TDS-ID and TDS-IM V1.0 devices include eschar formation, bleeding, tingling or numbness at injection site or hand/arm distal to site, and/or pigmentation changes. If any of these should occur, they will be captured as unsolicited adverse events.

### 10.3.3. Serious Adverse Event or Serious Suspected Adverse Reaction

An adverse event 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 adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

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 patient or subject 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.

All SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site PI or subinvestigator Day 1 through Day 220.
- Recorded on the appropriate SAE form and eCRF
- Followed through resolution or end of study by a licensed study physician listed on the Form FDA 1572 as the site PI or subinvestigator
- Reviewed and evaluated by an SMC, RM/ISM, DMID, PSSO PVG physician, and the IRB, if indicated

### 10.3.4. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

### 10.3.5. Unanticipated Problems Involving Risks to Subjects or Others

Federal regulations require that unanticipated problems involving risks to subjects or others be promptly reported to the IRB. 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 investigational product; adverse psychological reactions; or breach of confidentiality. Risks to others (eg, 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, investigators brochure or informed consent document; and (b) the characteristics of the subject population.

- Related or possibly related to a subject's participation in the study; and
- Suggests that the study places subjects or others at a greater risk of harm than was previously known or recognized.

The PI will determine whether a given incident, experience or outcome constitutes an unanticipated problem involving risk to subjects or others and ensure upward reporting of the unanticipated problems involving risk to subjects or others to the appropriate regulatory offices.

## 10.4. Relationship to Investigational Product

The investigator must assign a relationship of each AE to the receipt of the investigational product. 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 investigational product, 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 PHYSICIAN CAN MAKE THIS DETERMINATION.**

- Not related: No relationship to investigational product. Applies to those events for which evidence exists that there is an alternate etiology.
- Unlikely: Likely unrelated to the investigational product. Likely to be related to factors other than the investigational product but cannot be ruled out with certainty.
- Possible: An association between the event and the administration of investigational product 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 investigational product 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 investigational product and the event. An association to other factors has been ruled out.

The sponsor uses 5 categories of relatedness (defined above), which are to be used for this clinical trial. The category of "Not Related" maps to DMID category of "Not Related," while the categories of "Unlikely," "Possible," "Probable," and "Definite" map to the DMID category of "Related."

## 10.5. 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, potentially life threatening/life threatening, or fatal. Refer to the grading scale in Appendix C for further guidance in the assignment of severity. The criteria below may be used

for any symptom not included in the grading scale. Any potentially life threatening/life threatening, or fatal AE must be reported as an SAE.

The eCRF for AEs will reflect only the highest severity for continuous days an event occurred.

| | | |
|---|---|---|
| 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/life threatening | Grade 4 | Hospitalization or ER visit or urgent intervention indicated |
| Fatal | Grade 5 | Death |

FDA guidelines for toxicity will be followed; however, if a subject is evaluated in an emergency room for nonlife threatening illness or symptoms (ie, 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 adverse event 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.

## 10.6. Recording Adverse Events

The PI will report all AEs to the sponsor's representative (USAMRDC ORA) and the UMB CVD 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. The sponsor's representative will be responsible for reporting any required information to the delivery device supplier (Ichor Medical Systems, Inc.) and DMID.

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

AEs, solicited AEs, 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. 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 investigational product, severity, and other possible etiologies. When an adverse event has not resolved by study closure, it will be documented on the AE eCRF as "ongoing."

The time frame for the collection of AEs and SAEs begins at the first administration of investigational product through the end of the study.

The timeframe for the collection of solicited AEs begins at the first administration of investigational product through 14 days following each dose of investigational product (the first 14 days will be assisted with a memory aid).

### 10.6.2. Duration of Follow-Up of Subjects after Serious Adverse Events

Investigators are required to follow SAEs to resolution or end of study. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. The SAE outcomes will be reported to the sponsor's representative using the Serious Adverse Event Report Form. If the follow-up information requires an update to the SAE Report Form, a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report should be e-mailed or faxed to usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil. The follow-up information should describe the outcome of the event, if and how it was treated, and whether the subject continued or withdrew from study participation.

Investigators are not obligated to actively seek SAEs in former subjects; however, if an SAE, considered to be related to the investigational product is brought to the attention of the investigator *at any time* following completion of the study, the event will be reported to the USAMRDC ORA PSSO as defined in Section 10.7.1.1.

## 10.7. Reporting Adverse Events

The PI will report all AEs to the sponsor, the UMB IRB, and DMID 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 clinical data manager to the USAMRDC ORA Data Manager at USAMRDC ORA.

The study site will provide data files to the sponsor's representative for preparation of annual and final reports to the FDA. The sponsor's representative will be responsible for reporting any required information to the delivery device supplier (Ichor Medical Systems, Inc.).

### 10.7.1. Reporting Serious Adverse Events and Unanticipated Problems Involving Risks to Subjects or Others

Contact information for reporting SAEs is provided in Table 6.

#### 10.7.1.1. Reporting to the Sponsor

**All SAEs** must be reported promptly (within 24 hours of discovery of the event) to the USAMRDC ORA PSSO as per 21 CFR 312.64, whether or not the event is considered related to study product. All notification will be provided to the USAMRDC ORA PSSO. The completed SAE Report Form must be e-mailed to usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil **or faxed within 24 hours of discovery of the event**. Further, the investigator should comply with relevant study site SOPs on reporting SAEs. The minimum

information that the investigator will provide to the USAMRDC ORA PSSO is specified in Table 7. The USAMRDC ORA PSSO may request the investigator to provide additional follow-up information, which may include a discharge summary or extracts from the medical record. Information provided about the SAE must be consistent with that recorded on the event CRF. SAEs will be forwarded to the DMID Pharmacovigilance Group by the USAMRDC ORA PSSO for DMID medical monitor review in accordance with the Safety Management Plan.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) to the USAMRDC ORA PSSO on an SAE form (Table 7) to the address provided in Table 6. Other supporting documentation of the event may be requested by the USAMRDC ORA PSSO and will be provided as soon as possible. The USAMRDC ORA PSSO PVG physician will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the USAMRDC ORA PSSO.

**Table 6: Study Contacts for Reporting Serious Adverse Events and Unanticipated Problems Involving Risk to Subjects or Others**

| | |
|---|---|
| USAMRDC ORA PSSO[a]<br>(Sponsor's Safety Office) | US Army Medical Research and Development Command<br>ATTN: MCMR-UMR<br>1430 Veterans Drive<br>Fort Detrick, MD 21702-5009<br>Fax: 301-619-0197<br>Telephone: 301-619-1005<br>E-mail: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil |
| Institutional Review Board | Human Research Protections Office<br>University of Maryland, Baltimore<br>620 West Lexington Street, 2nd floor<br>Baltimore, MD 21201<br>Telephone: 410-706-5037<br>Fax: 410-706-4189<br>E-mail: HRPO@som.umaryland.edu |
| USAMRDC Office of Research Protections (UPIRTSOs only) | Human Research Protection Office<br>US Army Medical Research and Development Command<br>ATTN: MCMR-RPH<br>810 Schreider Street<br>Fort Detrick, MD 21702-5000<br>Fax: 301-619-7803<br>Telephone: 301-619-2165<br>E-mail: usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil |
| Research Monitor/Independent Safety Monitor | Justin R. Ortiz, MD, MS, FACP, FCCP<br>Center for Vaccine Development<br>University of Maryland School of Medicine<br>685 W. Baltimore Street, Room 480<br>Baltimore, MD 21201<br>Telephone: 410-706-3502<br>Fax: 410-706-6205<br>E-mail: jortiz@som.umaryland.edu |

PSSO[a] SAEs will be forwarded to the DMID Pharmacovigilance Group by the USAMRDC ORA PSSO in accordance with the Safety Management Plan.

**Table 7: SAE Information to Be Reported to the USAMRDC ORA PSSO**

| Notification Method | Information to Be Provided |
|---|---|
| **E-mail or Telephone (within 24 hours of site awareness)** | **IND number, sponsor study number, name of the investigational product, and investigator name and contact number** |
| | Subject identification number |
| | SAE, onset date, date of investigational product administration, severity, relationship, and subject's current status |
| **AND** | |
| **E-mail or Fax** | **Cover sheet or letter** |
| | Adverse event case report form |
| | Serious adverse event 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 e-mail, the subject line of each email notification will read as follows: **SAFETY REPORT – IND # _____, Sponsor Study #_____, Subject# _____, Event Term: _____** | |

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 will report unexpected SAEs associated with the use of the product to the FDA as specified at 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy regarding the timely reporting of SAEs to the RM/ISM, USAMRDC ORA PSSO, and the UMB IRB.

Reporting to the USAMRDC ORA PSSO does not fulfill the investigator's duty to report all unanticipated problems involving risk to subjects or others (UPIRTSOs) to the IRB. The PI will notify the UMB IRB, DMID, and the RM/ISM.

#### 10.7.1.2. Reporting to the IRB

UPIRTSOs, SAEs related to participation in the study, and all subject deaths related to participation in the study should be promptly reported within 24 hours of site awareness by telephone, email, or fax to the UMB IRB. A complete written report should follow the initial notification.

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

All UPIRTSOs must be promptly reported to the USAMRDC ORP HRPO.

### 10.7.2. Reporting Additional Immediately Reportable Events to the USAMRDC ORA PSSO, UMB IRB, DMID, and the USAMRDC ORP HRPO

#### 10.7.2.1. Pregnancy

Each pregnancy must be reported immediately using the Pregnancy Report Form (**within 24 hours of identification**) by e-mail or fax to the USAMRDC ORA PSSO. Report the incident to UMB IRB in accordance with IRB policy. USAMRDC ORA PSSO will be responsible for notifying DMID within 48 hours of notification by site.

Subjects who become pregnant after Day 0 will be followed until 30 days after delivery to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The following information will be gathered for outcome, date of delivery, 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. While pregnancy is not per se an AE/SAE, the product of the pregnancy could potentially be an AE/SAE. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion, an elective termination for medical rationale, or the infant has a congenital anomaly/birth defect.

Follow-up information should be e-mailed to usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil, using the Pregnancy Report Form stating that this is a trimester update or final report to the previously reported Pregnancy Report and giving the date of the original report.

#### 10.7.2.2. AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported *immediately* (**within 72 hours of identification**) by e-mail or fax to the sponsor's representative. USAMRDC ORA will be responsible for notifying DMID within 48 hours of notification by site.

#### 10.7.2.3. Pending Inspections/Issuance of Reports/Serious or Continuing Noncompliance/Suspensions, Clinical Holds, or Terminations/Subject Becomes a Prisoner

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 by the principal investigator immediately to the UMB IRB, and the sponsor's representative. USAMRDC ORA will be responsible for notifying ORP HRPO, DMID and Geneva within 24 hours of notification by site. Additionally, all suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies must be reported to the USAMRDC ORP HRPO, DMID and Geneva.

Change in subject status when a previously enrolled human subject becomes a prisoner must be promptly reported to the USAMRDC ORP HRPO. The report must include actions taken by the institution and the IRB.

### 10.7.3. IND Annual Report to the FDA

The PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the sponsor's representative (USAMRDC). Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. The sponsor's representative will notify the PI of the due date with sufficient time for the PI to assemble the required information.

### 10.7.4. Final Report

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications" and ICH E3 Guideline "Structure and Content of Clinical Study Reports" and provided to the sponsor's representative for review and approval. The sponsor's representative will use this report to prepare the final clinical study report for submission to the FDA.

The PI will report all AEs to the sponsor (USAMRDC ORA) and the UMB 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 clinical data manager at the UMB CVD.

The final study report submitted to the IRB, including a copy of any acknowledgement documentation and any supporting documents, will be submitted to the HRPO as soon as all documents become available.

# 11. STATISTICS

Detailed statistical procedures, listings, table shells and figures will be provided in a separate statistical analysis plan (SAP) written shortly after protocol approval but before any subject enrollment. The SAP will be finalized before study close-out and database lock. The following key statistical components will be considered and a detailed description will be documented in the SAP:

- Primary, secondary, and exploratory endpoints and how they will be measured,
- Statistical methods and tests that will be used to analyze the endpoints,
- Strategy that will be used if the statistical test assumptions are not satisfied,
- Indication of whether the comparisons will be one-tailed or two-tailed (with justification of the choice) and the level of significance to be used,
- Identification of whether any adjustments to the significance level or the overall p value will be made to account for any planned or unplanned subgroup analyses or multiple testing,
- Specification of potential adjusted analyses and a statement with which covariates or factors will be included,
- Planned exploratory analyses and justification of their importance, and
- Any subgroup effects with biological justification and support from within and outside the study.

## 11.1. Description of Statistical Methods

Descriptive analysis of safety and reactogenicity outcomes will include all subjects who meet the eligibility criteria, receive at least one vaccination, and for whom safety data are available. Summary tables will be created in which incidence, severity, and relationship to the use of investigational product of individual solicited local and systemic signs, symptoms, or trending unsolicited events are delineated by study group, severity, gender, and overall. Unsolicited AEs and SAEs will be analyzed in a similar fashion.

Descriptive analysis of immunogenicity outcomes will include all subjects who meet the eligibility criteria, receive at least one vaccination, and for whom serological data are available. The primary analysis variable will be the proportion of seropositive subjects (defined as PsVNA50 $\geq$ 1:20) at each scheduled time point for which blood samples are taken (eg, Days 0, 28, 56, 84and 140), and the final overall rate of seroconversion over all scheduled time points to study completion for each study group. Seroconversion is defined as a post-vaccination HTNV- or PUUV-specific titer of $\geq$ 1:40, or a minimum four-fold rise compared to baseline titer and all study volunteers will begin the study with a baseline titer < 20 (ie, seronegative). For each study group, a binomial proportion and 95% confidence interval (CI) will be calculated. The secondary analysis variable will be geometric mean titers (GMT), with 95% CIs, of the PsVNA50 for HTNV- and PUUV-specific antibodies at each scheduled time point for which blood samples are taken for each study group and over all time points for each study group. GMT, standard errors, and 95% CIs will be calculated using log-transformed titers. For titers below the lower limit of detection (< 20), the value will be transformed to a value equal to the lower limit of detection (ie,

20) divided by the square root of 2, which equals 14.1. Exploratory objectives may evaluate PsVNA80 titers, which may be calculated for informational purposes, and may evaluate PsVNA50 titers against related Seoul and Dobrava viruses.

For hematology and serum chemistry tests, any clinically significant changes from baseline value will be identified. The median, interquartile range, and normal values for each laboratory value (as determined by the contract laboratory) will be reported for each treatment group for each specimen collection point.

### 11.1.1. Safety Analyses

Adverse events will be classified by MedDRA system organ class and preferred term, severity, and relationship to study treatment. Serious adverse events will be described. A complete listing of adverse events for each subject will provide details including severity, relationship to study product, onset, duration, and outcome. Laboratory results will be summarized by laboratory parameter, severity, study day, and study arm.

Safety analysis will include data collected from all subjects. Adverse event data will be listed individually (including intervention and outcome) and summarized by body system and preferred terms within a body system for each treatment group. Serious and/or unexpected AEs will also be discussed on a case-by-case basis. For the tabulation of the AEs by body system, a subject will be counted only once in a given body system. For example, a subject reporting nausea and diarrhea will be reported as one subject, but the symptoms will be listed as 2 separate AEs within the class. Therefore the total number of AEs reported within a body system may exceed the number of subjects within the body system reporting AEs.

The total number of AEs reported within body systems between each treatment group will be compared using Fisher's exact test.

Changes in pulse rate, systolic and diastolic blood pressure, weight, and respiratory rate will be compared within each group and among groups using analysis of variance procedures.

Depending upon the sample size, the more common adverse events that may be drug related should be examined for a dose, duration, or demographic relationship, or using other analyses such as standard life-table methods.

### 11.1.2. Clinical Laboratory Data Analyses

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.

These tables will be reviewed by the PI or RM/ISM to evaluate whether any significant trends in laboratory values occurred. The PI or RM/ISM will also review the urinalysis data by inspecting the laboratory data tabulations, but no summary tables of these will be prepared.

## 11.2. Planned Enrollment and Reason for Sample Size

The study will enroll 6 randomized groups of 12 subjects each, for a total of 72 subjects. Due to COVID restrictions, 10 subjects from Group 6 may be replaced to ensure completion of the 3 series dose of vaccine. This will account for a total of 82 subjects. This approach will ensure at least 60 subjects complete all vaccinations at around 10 subjects per group, taking possible attrition into account. Subjects will receive one dose of vaccine on Days 0, 28, and 56 and will be followed until Day 220.

The proposed study size is appropriate for a Phase 1 study. A Phase 1 study is designed to evaluate preliminary human safety data but not designed to show statistically significant differences between the groups.

Thus at least 7/10 or 8.4/12 subjects must seroconvert to establish a true seroconversion rate of no less than 30% with 95% confidence.

## 11.3. Level of Significance to Be Used

All statistical analyses will be made using a two-tailed $\alpha = 0.05$.

## 11.4. Statistical Criteria for the Termination of the Trial

There are no statistical criteria for study termination in this clinical trial.

### 11.4.1. Interim Analysis and Stopping Rules

No interim analyses are planned.

## 11.5. Accounting for Missing, Unused, and Spurious Data

Missing data will be documented as such in the database. Missing data will not be imputed or replaced for analytic purposes.

## 11.6. Procedures for Reporting Deviations from the Original Statistical Plan

Non-analyzable data will be documented in the deviations. Any deviation(s) from the original statistical plan 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 UMB CVD SOPs.

## 11.7. Selection of Subjects to Be Included in Analyses

All subjects who receive at least one dose of the investigational product will be included in the safety and immunogenicity analysis.

# 12. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Subjects will be identified on eCRFs and source documents by a unique subject identification number. No personal identifier will be used in any publication or communication used to support this research study. The subject identification number will be used if it becomes necessary to identify data specific to a single subject. Representatives of USAMRDC, the sponsor's representative, the UMB IRB, USAMRDC ORP HRPO, DMID, The Geneva Foundation, 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.

## 12.1. Study Monitoring

Study monitoring will be the responsibility of the USAMRDC ORA. Upon successful approval of the protocol and establishment of the regulatory file, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor 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 clinical monitor 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 GCPs.

Monitoring visits by a sponsor's representative-designated clinical monitor 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. A report of monitoring observations will be provided to the PI (for corrective actions), USAMRDC ORA, and the product manager.

## 12.2. Audits and Inspections

Authorized representatives of the sponsor, DMID, The Geneva Foundation, and the FDA, the independent ethics committee or institutional review board 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, GCP guideline of the ICH, and any applicable regulatory requirements.

The investigator should contact the sponsor's representative and USAMRDC ORP HRPO immediately if contacted by a regulatory agency about an inspection.

## 12.3. Institutional Review Board

As the IRB of record, the UMB IRB will serve as the responsible IRB and will review the protocol, informed consent, and progress reports on a continuing basis in accordance with all applicable regulations, including Title 21, Code of Federal Regulations (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 patient consent form and recruitment materials, must be maintained by the protocol physician and made available for inspection.

The PI will be responsible for preparing and submitting continuing review reports per institution and IRB requirements. The PI or a designee will submit the approved continuing review reports and the local IRB approval notifications to the USAMRDC ORP HRPO as soon as the documents are available.

# 13. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor's representative and/or The Geneva Foundation may conduct quality assurance audits. Refer to Section 12.2 for more details regarding the audit process.

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

The Quality Management plan will comply with DMID Clinical Quality Management Plan (CQMP) policy; and the implementation of that plan benefits the internal site audits by:

- Supporting substantive performance measurements/findings/corrective actions, as required, and
- Providing data to support reporting requirements, as applicable.

The University of Maryland Center for Vaccine Development core Quality Management (QM) Plan is accepted by DMID quality management oversight and is in place onsite, and available upon request. As defined in the core QM plan, a separate protocol-specific clinical research QM plan outlining the sample size, priority of protocol review, frequency of quality assurance audits, communication of findings, and annual review will be prepared for DMID QMP reviewers.

Clinical site monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. These monitoring visit reports will be submitted to the sponsor's representative, The Geneva Foundation, and DMID.

The investigational sites will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

# 14. ETHICS

The investigators will ensure that this study is conducted in full conformity with the Declaration of Helsinki, or with the ICH GCP regulations and guidelines, whichever affords the greater protection to the participant.

The investigators will also ensure that this study is conducted in full conformity with the principles of the *Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research* (April 18, 1979) and codified in 45 CFR 50 and 56. The institution will hold a current FWA issued by OHRP for federally funded research.

## 14.1. Ethics Review

The study is based on adequately performed laboratory and animal experimentation and will be conducted under a protocol reviewed by the UMB 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 all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

### 14.1.1. 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 in the order listed for review and/or approval, with the final review by the FDA:

- Integrated Product Team
- Sponsor's Representative Team (Senior Regulatory Affairs Advisor; ORA, USAMRDC)
- Commander, Subordinate Command, if applicable
- DMID
- UMB IRB
- USAMRDC ORP HRPO
- Sponsor's Representative (acting for The Surgeon General of the Army)
- USAMRDC Commanding General, if applicable

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.

### 14.1.2. Protocol Modifications

All modifications to the protocol and supporting documents (informed consent, study-specific procedures, SOPs, recruitment materials, etc) must be reviewed and approved prior to implementation. Any protocol amendment will be agreed upon and approved by the sponsor's

representative and DMID prior to submission to the UMB IRB and prior to implementation of said change or modification. Any modification that could potentially increase risk to patients must be submitted to the FDA prior to implementation. The informed consent document must be revised to concur with any amendment as appropriate and must be reviewed and approved with the amendment. Any patient already enrolled in the program will be informed about the revision and asked to sign the revised informed consent document if the modification directly affects the individual's participation in the program. A copy of the revised, signed, and dated informed consent document will be given to the patient. All original versions of the informed consent document will be retained in the protocol regulatory file, and a copy will be retained in the protocol regulatory file.

Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the USAMRDC ORP HRPO for approval prior to implementation. The USAMRDC ORP HRPO defines a substantive modification as a change in PI, change or addition of an institution, elimination or alteration of the consent process, change in the IRB of Record, change to the study population that has regulatory implications (adding children, adding active-duty population, etc.), significant change in study design (ie, would prompt additional scientific review), or a change that could potentially increase risks to subjects.

### 14.1.3. Protocol Deviation Procedures

All subject-specific deviations from the protocol (eg, 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 study-specific procedure. Deviations will be reported annually in the continuing review report to the UMB IRB and, if appropriate, in the final study report. 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 non-significant.

Any protocol deviation that adversely affects the safety or rights of a subject or scientific integrity of the study, will be reported immediately to the sponsor's representative and the UMB IRB. The sponsor's representative is responsible for notifying The Geneva Foundation and DMID.

## 14.2. Ethical Conduct of the Study

This study will be conducted in accordance with all applicable Federal and DoD human research protections 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.

### 14.2.1. Confidentiality

HIPAA requires that researchers obtain the subject's permission (HIPAA Authorization) to use and disclose health information about the subject that is either created by or used in connection with this research. The information includes the entire research record and supporting

information from the subject's medical records, results of laboratory tests, and both clinical and research observations made during the individual's participation in the research.

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 investigational product; 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 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.

Representatives of the TSG-DA as the IND sponsor, the sponsor's representative, the UMB IRB, DMID, The Geneva Foundation, the DoD, 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's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

This research is covered by a Certificate of Confidentiality from the NIH. The investigators and their staff may not disclose or use information, documents, or biospecimens that may identify the subjects in any federal, state, or local civil, criminal, administrative, legislative, or other action, or be used as evidence unless the subject has consented. This does not apply to requests for information from the NIH or its representatives that are needed to monitor or audit the study, or for information that must be disclosed in order to meet FDA requirements.

### 14.2.2. Compensation for Participation

Compensation will occur at the time of a pre-designated payment visit and reflect the interim amount of compensation earned (Table 8). Per PI discretion, compensation will also be provided for necessary unscheduled visits, or if a subject is delayed from returning to his or her regular, daily activities due to unanticipated delays with study-related visits/procedures. Subjects who serve as alternates but who are not immunized/challenged will be compensated for the screening visit and for the day when they serve as an alternate ($100). A study completion bonus will be provided ($200) upon completion of the study. This is seen by the study staff as an amount that is fair and non-coercive compensation for the subject's contribution to the study.

**Table 8: Compensation Plan for Participation**

| Activities | Number of Visits | Compensation per Visit |
|---|---|---|
| Screening<br>(Day -90 to Day -7) | 2[a] | $75 |
| Immunization<br>(Days 0, 28, 56) | 3 | $150 |
| Return of Completed Memory Aid<br>(Days 14, 42, 70) | 3 | $50 |

| Activities | Number of Visits | Compensation per Visit |
|---|---|---|
| Post-Immunization (Days 2, 14, 30, 42, 58, 70, 84, 140) | 8 | $75 |
| Day 220 Phone Visit | 1 | $10 |
| Completion Bonus (Day 220) | 1 | $200 |
| **Total Potential Compensation** | | **Up to $1560** |

[a] Subjects that screen between days -90 and -56 will be required to return for a second screening visit.

### 14.2.3. Medical Care for Research-Related Injury

If a subject suffers an injury directly related to participation in this project, UMB and/or one of its affiliated institutions or health care groups will help obtain medical treatment for the specific injury and provide referrals to other health care facilities, as appropriate. The Federal Government, UMB and/or its affiliated institutions or health care groups will not provide financial compensation or reimbursement for the cost of care provided to treat a research-related injury or for other expenses arising from a research-related injury. The institution or group providing medical treatment will charge the subject's insurance carrier, the subject, or any other party responsible for treatment costs. If the subject incurs uninsured medical costs, they are the responsibility of the subject.

## 14.3. Future Use of Stored Specimens and Data

Through the informed consent form, permission will be sought from subjects to keep any remaining blood indefinitely to use in possible future research studies. In this study, up to 10 mL of blood on Days 0, 28, 56 and 140 and approximately 50 mL of blood on Day 84 will be collected for future use purposes. Any blood remaining after immunogenicity studies are conducted will be retained for future hantavirus-related research for subjects who agreed to future use of these samples. Future use studies may test for antibodies against hantaviruses and/or other infectious diseases using assays or methods that are yet to be determined.

Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality. The samples will be stored at a repository at the US Army Medical Research Institute of Infectious Diseases and may be shared with investigators at this institution and with other investigators at other institutions. Electronic files associated with these coded samples will be password protected. Only people who are involved in the conduct, oversight, or auditing of this study will be allowed access.

If these stored samples are tested in the future, the results may be published. Such publication will not contain any information about the subjects that would enable subject identification. The results of any future testing will be kept confidential in the same way as the results of testing done for this study. Results from this future research will not be reported to the subject, his or her doctor, or placed in his or her medical record.

Although the results from this future research, including donated samples, may be patentable or have commercial value, subjects will have no legal or financial interest in any commercial development resulting from the research. There are no benefits to subjects in the collection, storage, and subsequent use of their specimens for future research.

Subjects may change their decision to participate in the study at any time by notifying the study doctors or nurses in writing. However, any data from a previously collected sample prior to the withdrawn consent will not be removed. Any future use of residual samples or collection of samples specifically for future research not yet collected/stored will not be stored for future/residual use. Any data from a previously collected sample prior to the withdrawn consent will not be removed.

## 14.4. Written Informed Consent

The informed consent process and document will be reviewed and approved by the UMB IRB, DMID, and sponsor's representative 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 50. The consent document indicates that by signature, the subject, or where appropriate, legal guardian, permits witnessing of applicable study procedures by the sponsor's representative, as well as access to relevant medical records by the sponsor's representative, DMID, and The Geneva Foundation and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the UMB IRB.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, and the Belmont Principles and HIPAA Authorization 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 informed consent document. 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, and
- The individual 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 informed consent document. 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 http://www.ClinicalTrials.gov, as required by US law.

# 15. DATA HANDLING AND RECORDKEEPING

The primary source document for this study will be the subject's medical record. If separate research records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study. Applicable source data will be manually transcribed to approved eCRFs. The investigator is ultimately responsible for the accuracy of the data transcribed on the forms.

All research data will be entered manually, into a computerized database, designed in accordance with 21 CFR Part 11 and based on protocol requirements defined by the sponsor's representative in association with the PI and clinical data manager. The database will be managed by the University of Maryland's Bioinformatics Department.

A detailed data management plan will be written and approved by the study team and the PI prior to study start, with approval by the sponsor's data manager in the USAMRDC ORA. All updates to the data management plan must be approved before study close-out and database lock.

## 15.1. Inspection of Records

The sponsor's representative or designee, DMID, and The Geneva Foundation 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, investigational product stocks, drug accountability records, subject charts, study source documents, and other records relative 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, DMID, The Geneva Foundation, and by representatives of the FDA.

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.

## 15.2. 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 investigational product for investigation. If it becomes necessary for the sponsor's representative or designee, DMID, The Geneva Foundation, 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. A copy of each completed eCRF will be retained by the investigator.

The PI will be responsible for retaining sufficient information about each subject, ie, name, address, telephone number, and subject identifier in the study, so that the sponsor's representative, the UMB IRB, the FDA, employees of USAMRDC, or other regulatory authorities may have access to this information should the need arise.

It is the policy of the USAMRDC that data sheets are to be completed for all subjects participating in research with an FDA-regulated product for which The Surgeon General, Department of the Army is the sponsor. (Form 60-R, Volunteer Registry Data Sheet). The data sheets will be entered into this Command's Volunteer Registry Database. The information to be entered into this confidential data base includes the subject's name, address, and Social Security Number; study title; and dates of participation. The intent of this data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRDC; and second, to ensure that USAMRDC can exercise its obligation to ensure research subjects are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDC for a minimum of 75 years. The Volunteer Registry Database is a separate entity and is not linked to the study database.

# 16. PUBLICATION POLICY

All data collected during this study will be used to support this IND. USAMRDC will post the trial on www.clinicaltrials.gov. All data may be published in the open medical or military 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 DMID, The Geneva Foundation, Ichor, USAMRIID, and UMB policies and then forward the publication for review to the USAMRDC ORA at usarmy.detrick.medcom-usamrmc.mbx.regulatory-affairs@mail.mil and to the USAMRDC Public Affairs Office at usarmy.detrick.medcom-usamrmc.list.clearances@mail.mil prior to submission.

# 17. LIST OF REFERENCES

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# APPENDIX A. STUDY PERSONNEL ROLES AND RESPONSIBILITIES

**Principal Investigator:** The principal investigator (PI) will have overall responsibility for the study and for compliance with the GCP guideline. The PI will conduct the study according to the investigational plan, institutional policies, and all applicable regulations. The PI will comply with the investigator's agreement (FDA 1572), supervise the use of the investigational product, and maintain accurate study records. The PI will permit and comply with audits and monitoring requirements. The PI will report all serious adverse events (SAEs) to appropriate regulatory bodies, as outlined in Section 10.7.

**Subinvestigators:** Subinvestigators at the clinical site designated by the PI will be responsible for protocol adherence and execution, passage of protocol through local protocol committees, data integrity, applying for and receiving approval for any modifications to the protocol or consent form, ensuring safety of the study subjects, managing and reporting of any AEs, briefing potential subjects, obtaining proper informed consent, determining study eligibility based on screening data and the exclusion criteria, and recording all observations and data in the individual subject records.

**Clinical Research Coordinator:** The study-site clinical research coordinator is responsible for quality control on all aspects of the study, to include sample collection, volunteer records, source documents and eCRFs, regulatory binders, and datasheets. The coordinator will maintain regulatory files, host visits of auditors, monitor all inspections, coordinate routing of all protocol activity, and maintain and update files of study staff curricula vitae and GCP training certificates.

**Research Staff:** The research staff will assist in the preparation of the protocol, eCRFs, and other associated documents as needed, monitor various aspects of the study, review information on eCRFs to ensure data are complete and correct, and assist in rectifying discrepancies on eCRFs; maintain study records and logs; assist in evaluating study results and preparing reports; ensure that volunteers have read and understand the informed consent document and have all questions appropriately answered and that informed consent documents are properly signed and dated; collect pre- and post-transfusion vital signs, conduct blood draws, and collect and ensure the integrity of blood samples; collect and record AEs and follow up and consult with physician investigators on all moderate and severe AEs; assist protocol administrator with maintenance of regulatory files and update all study staff curriculum vitae and GCP training certificates.

**Clinical Monitor:** Study clinical monitoring will be the responsibility of the USAMRDC Clinical Operations and Quality Branch's designated monitor. The clinical monitor will conduct initial, periodic, and termination study site visits; check protocol adherence including adherence with information presented in the informed consent documents, SOPs, and applicable regulations; oversee study file and regulatory documents, cross-check source documents and eCRFs; and observe study procedures.

**Clinical Trial Manager:** The clinical trial manager coordinates development of the protocol and protocol amendments, coordinates review of the protocol and all protocol-related documents; and coordinates completion of the study start and study close out checklists.

**Pharmacovigilance Physician:** The pharmacovigilance physician (PVG physician), part of the USAMRDC ORA staff, will serve as the final decision maker in cases where the PI and RM/ISM's review of data results in discordant determinations. In addition, the PVG physician evaluates all SAEs and provides the final determination on relatedness to the product, and

whether expedited reporting is warranted, per current FDA regulation and guidance. The PVG physician is responsible for integrating the review of safety data regarding SAEs and reviewing each SAE report.

**Research Monitor/Independent Safety Monitor:** The research monitor/independent safety monitor (RM/ISM) oversight position is required by the sponsor per Department of Defense Instruction 3216.02. The RM/ISM, provided by and local to the CVD, is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely manner. The RM/ISM will review SAEs in real time and unanticipated problems involving risks to subjects or others.

The RM/ISM is responsible for overseeing the safety of the research and reporting observations/findings to the IRB or a designated institutional official. The RM/ISM will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The RM/ISM may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the RM/ISM's report; and shall have the responsibility to promptly report his or her observations and findings to the IRB or other designated official.

In addition to the responsibilities above the RM/ISM is required to review and provide an unbiased written report for all SAEs and subject deaths to the USAMRDC ORA PSSO (Safety Office) within 24 hours of awareness of the event. The report provided must include, at a minimum, a brief summary of the RM/ISM's review of the event and event outcome, relationship of the event to the investigational product, and whether or not the RM/ISM concurs with the details of the study investigator's report.

Reports of events determined by either the investigator or the RM/ISM to be possibly or definitely related to participation, reports of events definitely related to participation, and reports of events resulting in death should be promptly forwarded to appropriate regulatory bodies; as outlined in Section 10.7.

**Clinical Data Manager (sponsor):** The sponsor's clinical data manager is responsible for oversight of study eCRF development, database design and validation, and the data management plan.

**Clinical Data Manager (study site):** The study site clinical data manager is responsible for the development of study eCRFs, database design and validation, data management plan, and data entry into the study database.

**Biostatistician:** The biostatistician will assist in protocol design and development, design and development of the study database, prepare the study SAP, perform statistical analyses, and review of the study final report.

**Product Manager:** Responsible for the overall management of the product development effort.

**Regulatory Affairs Scientist:** Coordinates protocol development and amendments. Conducts regulatory review and obtains IND sponsor representative's approval for study execution. Responsible for submission of the protocol and amendments to the FDA. Responsible for all communications with the FDA.

**DMID Medical Officer:** The DMID medical officer is a physician who reviews inclusion/exclusion criteria, assesses study design and appropriateness of outcome measures to meet objectives, and provides input from a medical perspective.

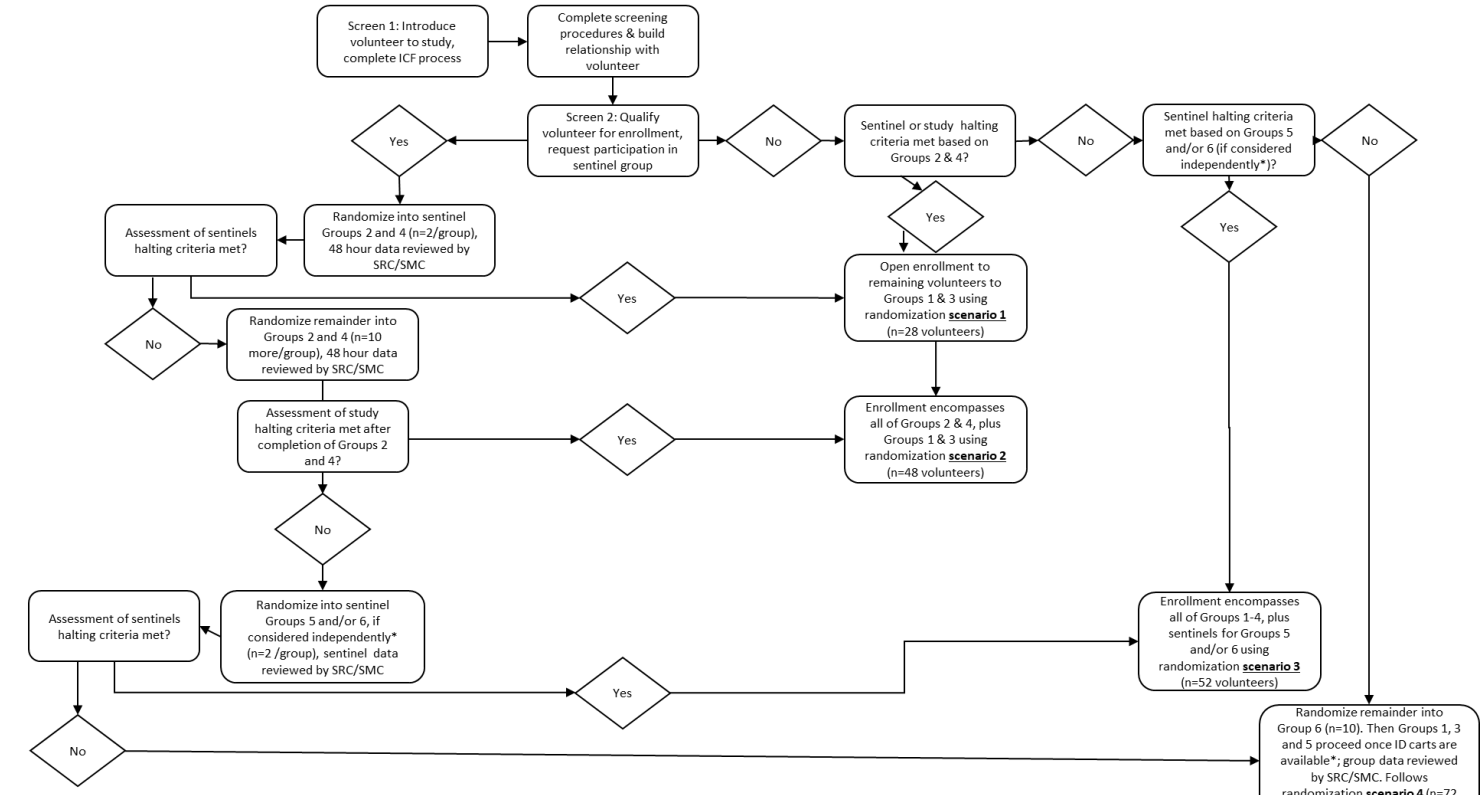
**DMID Medical Monitor:** The DMID medical monitor is the physician responsible for reviewing halting rules and safety reports per DMID criteria. The medical monitor generally provides advice regarding safety aspects of the protocol. The medical monitor reviews all serious adverse events and provides input to the Safety Monitoring Committee and Safety Review Committee.

**DMID Clinical Project Manager:** The clinical project manager ensures that DMID policies are adhered to by the IND holder as it prepares and conducts the trial. This includes meeting DMID and project requirements, facilitating protocol development, and review and/or implementation of the study protocol to promote timely completion.

**DMID Regulatory Affairs Specialist:** The regulatory affairs specialist reviews concepts, protocols, and supporting documentation as required by DMID and the FDA (informed consents, chemistry, manufacturing, and controls section of the IND, etc.) for study feasibility. The regulatory affairs specialist serves as a consultant for product development.

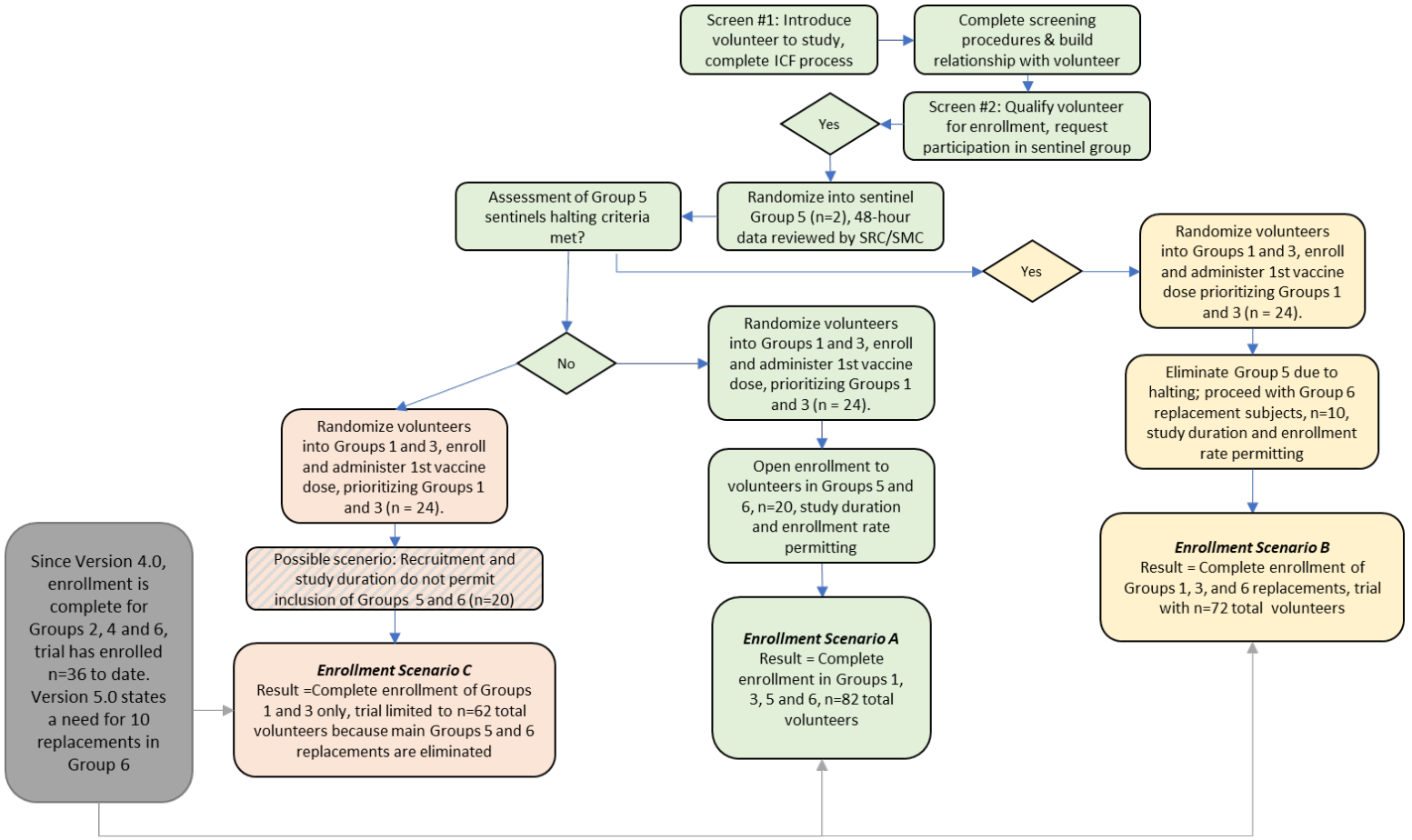
# APPENDIX B. SENTINEL SUBJECT ENROLLMENT PROCESS

Randomization Figure pertaining to Amendments 1.0-4.0 (maintained in this document for reference).




*Study schedule in Amendment 4.0 is decoupling sentinel enrollment and vaccination activities for Groups 5 and 6 due to complications with ID injection device, see protocol page 42. Ultimate enrollment in Groups 5, then 1 and 3 will still occur with the same number of volunteers, but at a delay upon availability of ID cartridges.

Randomization Figure pertaining to Protocol Version 6.0 and future versions These activities will proceed since completion of enrollment and vaccinations for Groups 2 and 4 and sentinels for Group 6 (n=36 already enrolled by March of 2020). The Randomization Scheme describes plan for enrollment in Nov 2021 of sentinel participants in Group 5 (n=2), then enrollment of Groups 1 and 3 (n=24) and Groups 5 and 6 (n=20).



# APPENDIX C. CLINICAL AND LABORATORY TOXICITY GRADING SCALES

All toxicity grading scales are based upon *FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials* (September 2007). The toxicity grading scale for lymphadenopathy is based upon *National Institutes of Health, Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0* (November 27, 2017).

## C.1 TABLES FOR CLINICAL ABNORMALITIES

### Table C-1: FDA Toxicity Grading Scale – Local Injection Reactions

| Local Reaction to Injectable Product | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Pain | 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 | Mild discomfort to touch | Discomfort with movement | Significant discomfort at rest | ER visit or hospitalization |
| Bruising | Barely perceptible difference compared to surrounding skin | Clearly discernable difference compared to surrounding skin | Unsightly difference when compared to surrounding skin | Not applicable |
| Erythema/Redness[a] | 2.5-5 cm | 5.1-10 cm | > 10 cm | Necrosis or exfoliative dermatitis |
| Induration/Swelling[b] | 2.5-5 cm and does not interfere with activity | 5.1-10 cm or interferes with activity | > 10 cm or prevents daily activity | Necrosis |

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
[b] Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

### Table C-2: FDA Toxicity Grading Scale – Vital Sign Abnormalities

| Vital Signs[a] | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Fever (°C)[b]<br>(°F)[b] | 38.0-38.4<br>100.4-101.1 | 38.5-38.9<br>101.2-102.0 | 39.0-40<br>102.1-104 | > 40<br>> 104 |
| Tachycardia – beats per minute | 101-115 | 116-130 | > 130 | ER visit or hospitalization for arrhythmia |

| Vital Signs[a] | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| 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 C-3: FDA Toxicity Grading Scale – Systemic Abnormalities

| Systemic (General) | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| 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 |
| Diarrhea | 2-3 loose stools/24 hours | 4-5 stools or 400-800 gms/24 hours | 6 or more watery stools or > 800 gms/24 hours or requires outpatient IV hydration | ER visit or hospitalization |
| 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 | 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 |

| Systemic (General) | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Illness or clinical adverse event (as defined according to applicable regulations) | No interference with activity | Some interference with activity not requiring medical intervention | Prevents daily activity and requires medical intervention | ER visit or hospitalization |

**Table C-4: Common Terminology Criteria for Adverse Events – Lymphadenopathy**

| CTCAE[a] Term | Grade 1 | Grade 2 | Grade 3 | Grade 4 | Grade 5 |
|---|---|---|---|---|---|
| Vaccination site lymphadenopathy[b] | Local lymph node enlargement | Localized ulceration; generalized lymph node enlargement | - | - | - |

[a] National Institutes of Health, Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 27, 2017.
[b] Defined as a disorder characterized by lymph node enlargement after vaccination.

## C-2 TABLE FOR LABORATORY ABNORMALITIES

**Table C-5: FDA Toxicity Grading Scale – Serum and Hematology Laboratory Abnormalities**

| Laboratory Test[a] | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4)[b] |
|---|---|---|---|---|
| Glucose – Hypoglycemia (mg/dL) | 65-69 | 55-64 | 45-54 | < 45 |
| Glucose – Hyperglycemia<br>Fasting (mg/dL)<br>Random (mg/dL) | <br>106-110<br>121-125 | <br>111-125<br>126-200 | <br>> 125<br>> 200 | Insulin requirements or hyperosmolar coma |
| Creatinine (mg/dL) (Female) | 1.15-1.7[c] | 1.8-2.0 | 2.1-2.5 | > 2.5 or requires dialysis |
| Creatinine (mg/dL) (Male) | 1.35-1.7 | 1.8-2.0 | 2.1-2.5 | > 2.5 or requires dialysis |
| Liver Function Tests – ALT, AST<br>Increase by factor | 1.1-2.5 x ULN[d] | 2.6-5.0 x ULN | 5.1-10 x ULN | > 10 x ULN |
| Bilirubin – When accompanied by any increase in liver function test<br>Increase by factor | 1.1-1.25 x ULN | 1.26-1.5 x ULN | 1.51-1.75 x ULN | > 1.75 x ULN |
| Bilirubin – when liver function test is normal; increase by factor | 1.1-1.5 x ULN | 1.6-2.0 x ULN | 2.0-3.0 x ULN | > 3.0 x ULN |
| Hemoglobin (Female) (gm/dL) | 11.0-11.9[c] | 9.5-10.9 | 8.0-9.4 | < 8.0 |
| Hemoglobin (Female) | Any decrease-1.5 | 1.6-2.0 | 2.1-5.0 | > 5.0 |

| **Laboratory Test**[a] | **Mild (Grade 1)** | **Moderate (Grade 2)** | **Severe (Grade 3)** | **Potentially Life Threatening (Grade 4)**[b] |
|---|---|---|---|---|
| change from baseline value (gm/dL) | | | | |
| Hemoglobin (Male) (gm/dL) | 12.5-13.4[c] | 10.5-12.4 | 8.5-10.4 | < 8.5 |
| Hemoglobin (Male) change from baseline value (gm/dL) | Any decrease-1.5 | 1.6-2.0 | 2.1-5.0 | > 5.0 |
| WBC Increase (cell/$mm^3$) | 10,100-15,000[c] | 15,001-20,000 | 20,001-25, 000 | > 25,000 |
| WBC Decrease (cell/$mm^3$) | 2,500-3,950[c] | 1,500-2,499 | 1,000-1,499 | < 1,000 |
| Lymphocytes Decrease (cell/$mm^3$) | 750-1000 | 500-749 | 250-499 | < 250 |
| Neutrophils Decrease (cell/$mm^3$) | 1,500-1,559 | 1,000-1,499 | 500-999 | < 500 |
| Eosinophils (cell/$mm^3$) | 501-1,500 | 1,501-5000 | > 5,000 | Hypereosinophilic |
| Platelets Decreased (cell/$mm^3$) | 125,000-139,500 | 100,000-124,000 | 25,000-99,000 | < 25,000 |

[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 might result in characterization of the laboratory abnormalities as potentially life threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mE/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.
[c] Modified
[d] ULN = Upper limit of the normal range.

# APPENDIX D. BLOOD SAMPLING TABLE

| | Visit | Screen 1 | Screen 2 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Day** | **-90 to -7** | | **0** | **2** | **14** | **28** | **30** | **42** | **56** | **58** | **70** | **84** | **140** |
| | **Volume (mL)** | | | | | | | | | | | | | |
| Clinical Safety Labs (CBC[a], glucose, Cr, AST, ALT, Total Bilirubin) | 12.5 | X | X[b] | X | | X | X | | X | X | | X | | |
| PT/PTT Sample | 2.7 | X | | | | | | | | | | | | |
| Serologies (HBsAg, anti-HCV Ab, anti-HIV Ab) | 12 | X | X[b] | | | | | | | | | | | |
| Serum Pregnancy (B-HCG) | 3.5 | X | | | | | | | | | | | | X |
| Pre-screening (PsVNA50) | 10 | | X | | | | | | | | | | | |
| Blood for Humoral Immune Response to Vaccination[c] | 10 | | | X | | | X | | | X | | | X | X |
| Future Research - Serum | 50 | | | | | | | | | | | | X | |
| Total per Study Visit (mL) | - | 33 | 34.5 | 22.5 | 0 | 12.5 | 22.5 | 0 | 12.5 | 22.5 | 0 | 12.5 | 72.5 | 23.5 |
| Total for Study (mL) | 268.5 | | | | | | | | | | | | | |

[a] CBC = (WBC, Hb, platelets) with differential

[b] Samples may not be required if collected within the 56-day screening window.

[c] Upon receipt of humoral immunogenicity samples at USAMRIID, any non-used/residual sera remaining after completion of the immunogenicity testing may be retained and used for future research.