A Phase I, Randomized, Placebo Controlled, Double-blind, Dose Escalation trial to Evaluate the Safety and Immunogenicity of an Andes Virus DNA Vaccine for the Prevention of Hantavirus Pulmonary Syndrome Using the PharmaJet Stratis® Needle-Free Injection System in Normal Healthy Adults

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Version Number: 4.0

Day Month Year
1 August 2019

STATEMENT OF COMPLIANCE

This trial will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions;
- The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule-Final Modification (45 CFR Parts 160 and 164);
- National Institutes of Health (NIH) Clinical Terms of Award, as applicable.

Compliance with these standards provides public assurance that the rights, safety and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed: _____ Date: _____

Name
Title

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase Test
ANC	Absolute Neutrophil Count
ANDV	Andes Virus
BUN	Blood Urea Nitrogen
CMS	Clinical Materials Services
CFR	Code of Federal Regulations
CRF	Case Report Form
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH,
eCRF	Electronic Case Report Form
EDC^{SM}	Electronic Data Capture System
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HBsAg	Hepatitis B Surface Antigen
HCPS	Hantavirus Cardio/Pulmonary Syndrome
HCV	Hepatitis C Virus
HFRS	Hemorrhagic Fever with Renal Syndrome
Hgb	Hemoglobin
HgbA1C	Hemoglobin A1C
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPS	Hantavirus Cardio/Pulmonary Syndrome
HTNV	Hantaan Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ICS	Intracellular Staining
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LPS	Lymphoproliferation Assay

MedDRA®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PMED	Particle Mediated Epidermal Delivery
PRNT	Plaque Reduction Neutralization Test
PsVNA	Pseudovirion Neutralization Assay
PUUV	Puumala Virus
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States
USAMRIID	Unites States Army Medical Research Institute of Infectious Diseases
WBC	White Blood Cell

PROTOCOL SUMMARY

Title:	A Phase I, Randomized, Placebo Controlled, Double-blind, Dose Escalation trial to Evaluate the Safety and Immunogenicity of an Andes Virus DNA Vaccine for the Prevention of Hantavirus Pulmonary Syndrome Using the PharmaJet Stratis® Needle-Free Injection System in Normal Healthy Adults
Phase:	I
Population:	48 males and non-pregnant females, 18-49 years old, inclusive, who are in good health and meet all eligibility criteria
Number of Sites:	One Vaccine and Treatment Evaluation Unit (VTEU) site
Study Duration:	Approximately 23 months
Subject Participation Duration:	Approximately 12 months
Description of Agent or Intervention:	Andes virus (ANDV) DNA vaccine, constructed from recombinant gene segments, plasmid pWRG/AND-M (opt2), was developed by the Department of Molecular Virology, US Army Medical Research Institute of Infectious Diseases and manufactured by Aldevron (Fargo, ND). ANDV DNA vaccine will be administered by the PharmaJet Stratis® Needle-Free Injection System in a 3 or 4 dose regimen on Days 1, 29, 57 and 169 at two doses, 2 or 4 mg. The placebo will be normal saline (0.9% Sodium Chloride, USP).
Objectives:	Primary: • Assess the safety and reactogenicity of the ANDV DNA vaccine by dosage cohort and treatment arm when administered using the PharmaJet Stratis® Needle-Free Injection system in normal, healthy adults. Secondary:

- Assess the immunogenicity of the ANDV DNA vaccine by dosage cohort and treatment arm.

Exploratory:

- Assess cellular immune response to ANDV DNA vaccine by dosage cohort and treatment arm.
- Assess immunogenicity of the ANDV DNA vaccine by dosage cohort and treatment arm at additional time points.

Outcome Measures:

Primary:

- Occurrence of vaccine-related Serious Adverse Events (SAEs) through approximately 6 months post last vaccination.
- Occurrence of vaccine-related unsolicited Adverse Events (AEs) through 28 days post last vaccination.
- Occurrence of Serious Adverse Events (SAEs) through approximately 6 months post last vaccination.
- Occurrence of unsolicited Adverse Events (AEs) through 28 days post last vaccination.
- Occurrence of solicited local and systemic AEs through 7 days after each study vaccination.
- Occurrence of clinical safety laboratory AEs through 7 days after each study vaccination.

Secondary:

- Incidence of an ANDV-specific titer of ≥ 20 on Day 57 (3 dose regimen), Day 85 (4 dose regimen) and Day 197 (3 and 4 dose regimen) as measured by:
    - Plaque reduction neutralization titers
    - Pseudovirion neutralization titers
- Incidence of seroconversion (defined as a post-vaccination ANDV-specific titer ≥40 if baseline titer <20 or a minimum four-fold rise compared to baseline if baseline titer ≥20) on Day 57 (3 dose regimen), Day 85 (4 dose regimen) and Day 197 (3 and 4 dose regimen) as measured by:

  - Plaque reduction neutralization titers
  - Pseudovirion neutralization titers

- Geometric mean titer (GMT) of neutralizing antibodies to ANDV on Day 57 (3 dose regimen), Day 85 (4 dose regimen) and Day 197 (3 and 4 dose regimen) as compared to baseline (Day 1) titer and measured by:
  - Plaque reduction neutralization titers
  - Pseudovirion neutralization titers

Exploratory:

- Incidence of a >3 standard deviation increase in ANDV-specific, cytokine-secreting CD4+ and CD8+ cells on Day 29 (3 and 4 dose regimen), Day 57 (3 dose regimen), Day 85 (4 dose regimen), Day 169 (3 and 4 dose regimen), and Day 197 (3 and 4 dose regimen) compared to baseline (Day 1) as measured by flow-based intracellular staining (ICS).
- Incidence of a >3 standard deviation increase in ANDV-specific CD4+ and CD8+ cells on Day 29 (3 and 4 dose regimen), Day 57 (3 dose regimen), Day 85 (4 dose regimen), Day 169 (3 and 4 dose regimen), and Day 197 (3 and 4 dose regimen) compared to baseline (Day 1) as measured by a lymphoproliferation assay (LPA).
- Incidence of an ANDV-specific titer of ≥ 20 at additional time points as measured by:
  - Pseudovirion neutralization titers
  - Plaque reduction neutralization titers

Description of Study Design:

This is a Phase 1, randomized, placebo controlled, double-blind, dose escalation trial of 48 males and non-pregnant females, 18-49 years old, inclusive, who are in good health and meet all eligibility criteria. This trial is designed to assess the safety, reactogenicity and immunogenicity of an ANDV DNA vaccine for the prevention of Hantavirus Pulmonary Syndrome (HPS). ANDV DNA vaccine or placebo will be administered using the PharmaJet Stratis® Needle-Free Injection System. Subjects assigned to the 3 dose regimen will receive ANDV DNA vaccine on Days 1, 29 and 169, and placebo on Day 57. Subjects assigned to the 4 dose regimen will receive ANDV DNA on Days 1, 29, 57 and 169. Two doses (i.e., 2 or 4 mg) of ANDV DNA vaccine will be evaluated.

Potential subjects will be screened by history, physical exam, vital signs, and clinical laboratory tests. Height and weight will be collected. A urinalysis will be done for urine protein and drug screening for opiates. Potential female subjects of childbearing potential will have a serum pregnancy test. In addition, potential subjects will be screened for Human Immunodeficiency Virus (HIV) type 1 & 2 antibody, Hepatitis C Virus (HCV) antibody, and Surface Antigen for Hepatitis B Virus (HBsAg) prior to enrollment.

The first 24 participants in Cohorts 1 and 2 will include two sentinel subjects since this is a first-in-human Phase 1 study. Sentinel subjects will receive 2 mg of study vaccine in an open label manner in a 3 or 4 dose regimen. One sentinel subject will be vaccinated, followed for one day for safety and reactogenicity, and if no halting rules are met, then the second sentinel subject will receive study vaccine in an open-label manner. The two sentinels will be followed for safety through Day 8 (laboratory and solicited/unsolicited AEs) and if no pre-defined halting rules are met and no safety concerns are identified, enrollment of the 22 remaining subjects in Cohorts 1 and 2 will proceed, otherwise a Safety Monitoring Committee (SMC) meeting will be held to conduct an electronic review (E-Review) of clinical and laboratory safety and reactogenicity data. While safety data is being evaluated for the sentinel subjects, no new subjects will be enrolled, but screening may continue.

The 22 non-sentinel subjects in Cohorts 1/2 will be randomized in a 9:2:9:2 ratio (Treatment Arms 1b:1c:2b:2c) to receive either placebo or study vaccine (at a dose of 2 mg) in a 3 or 4 dose regimen, in double-blind fashion. The SMC will review all available safety data through 7 days post second vaccination for all 24 subjects in Cohorts 1 and 2 and provide recommendations to proceed to Cohorts 3 and 4 or, if necessary, modify the protocol before proceeding. While safety data is being evaluated by the SMC, no new subjects will be enrolled, but screening for Cohorts 3 and 4 and vaccination of the remaining doses in Cohorts 1 and 2 may continue.

The 24 participants in Cohorts 3 and 4 will follow the same schedule as outlined for Cohorts 1 and 2 above. Sentinel subjects will receive 4 mg of study vaccine open label in a 3 or 4 dose regimen and be followed for safety through Day 8. The remaining 22 non-sentinel subjects in Cohorts 3 and 4 will be randomized in a

9:2:9:2 ratio (Treatment Arms 3b:3c:4b:4c) to receive placebo or study vaccine at a dose of 4 mg in a 3 or 4 dose regimen, in double blind fashion.

Doses 2-4 for all treatment arms will be administered per the schedule outlined in Section 4,

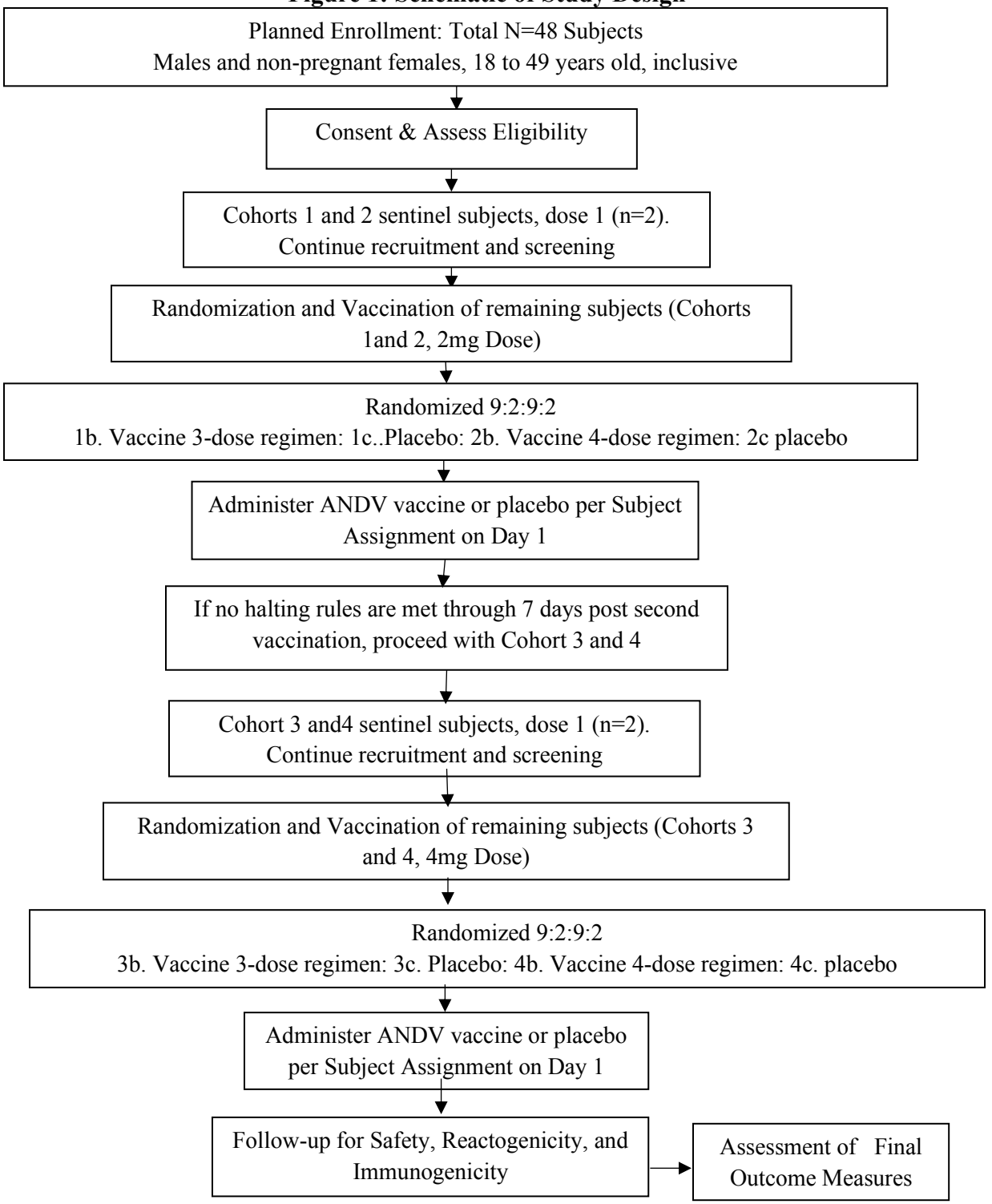
Table **1**, assuming no halting rules are met. As the vaccine is expected to be well-tolerated based on previously published studies in humans demonstrating the safety of Hantavirus DNA vaccines using the same plasmid backbone[1], more than one dose will be studied to best investigate safety and immunogenicity.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic AEs. Subjects will maintain a memory aid through 7 days post each vaccination, recording temperature, solicited local and systemic symptoms. Unsolicited non-serious AEs will be collected from the time of first study vaccination through approximately 28 days after last vaccination. SAEs occurring from the time of study vaccination through approximately 6 months after the last study vaccination will be collected. Clinical safety labs will be collected on Day 1 and 169, and 7 days after each vaccination; if labs are abnormal, they will be repeated at the next scheduled visit (sooner if medically indicated) and followed to normal or stabilization.

Immunogenicity testing will include evaluation of the cellular immune response to ANDV peptide pools and assessment of humoral immune response as measured by ANDV Pseudovirion Neutralization Assay (PsVNA) and by ANDV Plaque Reduction Neutralization Test (PRNT). PsVNA will be measured prior to study vaccination (Day 1) and approximately on Days 29, 57, 85, 169, 197, 253, and 337. ANDV PRNT will be measured immediately prior to study vaccination (Day 1) and approximately on Days 57, 85, 169 and 197. PBMCs to be used in the cellular assays, flow-based ICS and LPA, will be obtained at day 1, 29, 57, 85, 169 and 197. Blood will also be obtained at Days 85, 197 and 337 (PBMCs) for potential future exploratory assays. The duration of this trial for each subject will be approximately 12 months.

Estimated Time to Complete Enrollment: Approximately 6 months

Figure 1: Schematic of Study Design



1 KEY ROLES

Individuals:	**Protocol Champion:** Gabriele Feolo, RN,MSN Virology Branch NIAID, NIH **Principal Investigator:** Grant Paulsen, MD Cincinnati Children's Hospital Medical Center **Medical Officer:** Kay M. Tomashek, MD, MPH, DTM Division of Microbiology and Infectious Diseases NIAID, NIH **Medical Monitor:** Jorge Mejia-Galvis, MD, Office of Clinical Research Affairs Division of Microbiology and Infectious Diseases National Institute of Allergy and Infectious Diseases National Institutes of Health
Institutions:	Cincinnati Children's Hospital Medical Center
Local (Clinical) Laboratory:	Cincinnati Children's Hospital Medical Center CORE Laboratories
Cellular Assays Laboratory:	The Singh Laboratory Karnail Singh, PhD
ANDV Neutralization Assays Laboratory:	USAMRIID Jay Hooper, PhD
Study Agent Repository:	Fisher BioServices c/o DMID Clinical Materials Services Contract

Statistical and Data Coordinating Center	The Emmes Corporation
Safety and Pharmacovigilance Contractor:	DMID Pharmacovigilance Group Clinical Research Operations and Management Support (CROMS) 6500 Rock Spring Dr., Suite 650 Bethesda, MD 20814 SAE Hot Line: 1-800-537-9979 (US) SAE Fax: 800-275-7619 (US) SAE Email: PVG@dmidcroms.com

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Hantaviruses are members of the Bunyaviridae family and are found worldwide. Hantaviruses are negative-sense, single-stranded RNA viruses with three segments: small (S), medium (M), and large (L). The M-segment encodes the envelope glycoproteins (Gn and Gc). Over 50 species of hantaviruses have been identified, causing a wide spectrum of disease. Some infections result in a mild disease, while others can be severe, ultimately leading to death. Pathogenic hantaviruses primarily infect vascular endothelial cells in humans causing dysfunction and increased vascular permeability[2]. The two primary disease presentations in humans are due to vascular leakage; hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardio/ pulmonary syndrome (HCPS or HPS). HPS was first described in 1993 in the southwestern United States, with subsequent identification of Sin Nombre virus (SNV) as the causative agent. HPS typically presents as a non-specific viral illness with cough, fever, chills, nausea/vomiting, diarrhea, myalgia and/or headache for 3-6 days, followed by abrupt onset of pulmonary edema and shock[3]. HFRS is more common (>20,000 cases/year), and is generally limited to Asia, Europe, Scandinavia, and Russia. HFRS presents with similar prodromal symptoms, and less commonly leads to hypotension, shock and acute renal failure[4,5].

In South America, the Andes virus (ANDV) is the predominant cause of HPS with the majority of cases occurring in Brazil, Chile and Argentina; there have been a few case reports from Bolivia, Paraguay and Uruguay.[6-8] HPS due to ANDV is primarily a zoonotic disease in humans, spread via rodent excreta. While rodents, shrews, moles and bats can all be reservoir hosts for hantaviruses, the long-tailed rat (*Oligoryzomys longicaudatus*) is the host of ANDV and the deer mouse (*Peromyscus maniculatus*) remains the reservoir of the SNV[2]. Both are relatively common rodents and can be trapped in the wild. Unlike the other known hantaviruses, ANDV remains the only one with persuasive evidence of person-to-person transmission[7,9].

While HPS is relatively uncommon (approximately 600 US cases & 4,000 South American from 1993-2012/13), it remains a serious infection, with mortality rates due to ANDV infection of 35-40%, despite modern critical care support[3,10,11]. Additionally, there is no vaccine or effective therapy for HPS and treatment remains supportive and symptomatic. In the United States, due to the relative infrequency of hantavirus in general and HPS in particular, seroprevalence of IgG antibodies to hantaviruses are uncommon. Among individuals tested in a Baltimore, MD area study[12,13] only 0.7 – 2.1% of the group had detectable serum antibodies to Hantaan, Seoul or

Puumala viruses, while no one had detectable antibodies against ANDV or SNV. Therefore, protective immunity against HPS is likely minimal in the North American population, and there is no evidence of cross protection between SNV and ANDV.[14]

Due to issues associated with both production and formulation of an inactivated HRFS vaccine, DNA vaccines against the virus have been developed.[4] A phase 1 clinical trial of Hantaan Virus (HTNV) and Puumala Virus (PUUV) DNA vaccines reported neutralizing antibodies of 30% and 40% to HTNV and PUUV, respectively.[4] In the combined vaccine group, 56% developed neutralizing antibodies to one or both viruses.[4] When the same vaccine was administered using a different delivery system, and administering 2 doses of vaccine, seroconversion was increased to 64% and 75% to HTNV and PUUV, respectively.[1] Currently, there is an ongoing phase 2a trial comparing two different dosing schedules of a mixed HTNV/PUUV DNA vaccine administered via IM-EP (clinicaltrials.gov, NCT02116205) as well as a phase 1 trial evaluating HTNV and PUUV DNA vaccines delivered by the PharmaJet Stratis® Needle-Free Injection System (clinicaltrials.gov, NCT02776761).

In order to develop a pan-hantavirus DNA vaccine, an ANDV vaccine has been developed using the same strategy and plasmid backbone as the HTNV and PUUV DNA vaccines. The development of the HTNV DNA, PUUV DNA, and the ANDV DNA vaccines from the same plasmid backbone, combined with the immunogenicity results of the previous hantavirus DNA vaccines, provides the rationale for evaluating the ANDV DNA vaccine administered by the PharmaJet Stratis® Needle-Free Injection System in humans. The plasmid, pWRG/AND-M (opt2), which is the active ingredient for the ANDV DNA vaccine, was constructed on a well-characterized plasmid backbone, pWRG7077, and synthesized elements of the ANDV DNA genome. The optimized ANDV M gene encodes viral GnGc envelope glycoproteins that have been shown to be the targets of neutralizing antibodies.[14] The DNA vaccine was developed by the Department of Molecular Virology, US Army Medical Research Institute of Infectious Diseases (USAMRIID; Fort Detrick, Maryland), and the final vaccine was manufactured by Aldevron (Fargo, ND). The proposed trial would be a first in man vaccine trial for the prevention of HPS.

2.1.1 Safety/Toxicology of similar vaccines

The plasmid vector backbone, pWRG7077, has been used in several DNA vaccines, including HTNV and PUUV DNA vaccines and a Venezuelan Equine Encephalitis (VEE) DNA vaccine. In the VEE study, 41 subjects received one or more immunizations and there were no serious or unanticipated safety concerns identified.[15] There were 674 AEs recorded for all subjects over the

course of the study, the majority were local with resolution within 24 hours. Systemic AEs were also generally mild and transient; the only Grade 3 possibly vaccine related AE of severe fatigue resolved within 1 day. This study did report 3 SAEs, all of which were GI-related, occurred in the same patient with a recurrence of known ulcerative colitis and all concluded to be unrelated to the vaccine or study-related procedure.[15]

Investigators have previously evaluated plasmid DNA vaccine strategies for hantaviruses in both pre-clinical studies and human trials since 1999.[16] As referenced above, two prior hantavirus DNA vaccine trials have been published in humans. The first involved 3 cohorts of volunteers vaccinated with a DNA vaccine expressing M segments of HTNV and/or PUUV delivered via disposable gene gun devices (a.k.a. particle mediated epidermal delivery, PMED). Among the 28 subjects to receive at least one dose of vaccine, there were no SAEs related to the vaccine or study-related procedures and no subject withdrew due to adverse events.[4] Injection site pain was noted following 48-56% of doses and injection site tenderness following 79-89% of doses. All injection site pain was mild and moderate tenderness was documented only following the 3rd dose in 7% of subjects. The most common systemic AEs were fatigue, malaise, myalgia and, headache. Less common was oropharyngeal pain noted in 3 subjects. With the exception of one severe headache, all events were graded as mild or moderate in severity.

A subsequent human trial in 31 participants, receiving at least one immunization with HTNV DNA, PUUV DNA or mixture of both via intramuscular electroporation (IM-EP) was recently published.[1] No SAEs related to the vaccine or study-related procedures were observed. The most common solicited local AE was pain at the injection site which occurred in 28/31 participants. The next most common AEs were headache and myalgia, and all AEs were mild or moderate in severity. A single participant was found to have unrelated Grade 4 hypoglycemia, felt to be the result of an unrecognized pre-existing condition.[1]

2.1.2 Pre-Clinical Evaluations of ANDV vaccines

Several pre-clinical studies using variations of the ANDV vaccine and different delivery systems have been published. Studies of the same plasmid product as the current proposal (pWRG/AND-M (opt2)), demonstrated that a combined ANDV DNA and SNV DNA vaccine elicited neutralizing antibodies in rabbits and nonhuman primates[17]. In this study, significantly higher neutralizing titers were seen using a disposable syringe jet injection (DSJI) system as compared to needle/syringe delivery; anti-ANDV PRNT50 geometric mean titers (GMTs) were 349 versus 42 ($p = 0.01$). No safety issues or adverse events were reported.

Subsequent studies in geese and transchromosomal bovine also have demonstrated that the ANDV DNA vaccines administered with a DSJI system elicit neutralizing antibodies. Administration of these purified antibodies protected hamsters from lethal ANDV infection[18,19]. As in the nonhuman primate study above, there were no safety concerns or adverse events reported with respect to the vaccine product or delivery method.

2.1.3 Vaccine Delivery System

Historically, due to poor uptake of the DNA following injection, DNA vaccination has failed to raise a strong immune response in humans.[20] This has led to a progression of delivery systems to improved immunogenicity. The gene gun uses gold-coated DNA delivered with helium pressure and has shown promise. Electroporation (EP) was subsequently developed and it applies a current over cell membranes to increase cellular uptake. EP has been shown to increase uptake and immune response in humans, but can require a somewhat more complex apparatus[21]. DSJI systems have also been used successfully to administer DNA vaccines with good tolerability and effect (Hooper et al., unpublished data).[22]

Needle-free jet injection has a well-documented record of successful use for the delivery of conventional vaccines over the past 60 years, with estimates of hundreds of millions of doses delivered, including ANDV DNA vaccine delivery in nonhuman primates, as noted above[17]. The PharmaJet Stratis® Needle-Free Injection System is a US Food and Drug Administration (FDA) 510k-cleared for administering FDA- approved medicines and is the proposed delivery system in this trial.[17] This delivery system avoids both the use of gold-coated DNA and the complexity of administering a simultaneous electrical current with the immunization.

2.2 Rationale

ANDV is a Category A Bioterrorism Agent with high mortality and no effective prevention or specific treatment. An effective vaccine could protect against naturally occurring HPS as well as protecting soldiers from pathogens endemic to deployment areas as well as protection from a potential biologic weapon. This has spurred investigation into vaccine development.

Currently there are no FDA-licensed vaccines for HFRS or HPS, although there are inactivated cell culture-derived and rodent-brain derived vaccines available in other countries.[4,5] Due to issues associated with both production and formulation of an inactivated combination HFRS vaccine, hantavirus DNA vaccines have been developed. These include HTNV and PUUV, two of the predominant causes of HFRS. DNA vaccines were described in the early 1990s and have

since shown that by injecting a plasmid, expressing a viral antigen under the control of a promoter, an immune response to the viral antigen could be detected.[23] The current hantavirus DNA vaccine candidates utilize recombinant glycoprotein gene segments that are then subcloned into a plasmid backbone (pWRG7077). Given that the HTNV DNA, PUUV DNA, and ANDV DNA vaccines are all designed on the same plasmid backbone, the results of these ongoing clinical trials coupled with the immunogenicity of these DNA vaccines in humans and animals, provide the rationale for evaluating the ANDV DNA vaccine administered by the PharmaJet Stratis® Needle-Free Injection System in humans.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential risks of this trial are those associated with having blood drawn, intramuscular injections (IM), and possible reactions to the experimental ANDV vaccine. There may be potential risk related to breach of confidentiality, as well as other unknown risks, discomforts, or side effects.

The ANDV vaccine to be used in this clinical trial has never been administered to humans. It is administered intramuscularly and it is possible that vaccination may cause pain, tenderness, erythema (redness), induration (hardness/swelling), bruising and skin discoloration at the injection site. The vaccine may also cause systemic reactions such as fever, feverishness (chills, shivering, sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), nausea, dizziness, and headache. A less likely symptom is tachypnea (rapid breathing). In addition, vaccines can cause a severe allergic reaction. Such reactions are rare and would happen within a few minutes to a few hours after vaccination. Signs of a severe allergic reaction can include rash or hives, swelling of the face and throat, difficulty breathing, a fast heartbeat, dizziness, and weakness.

Based on the results of preclinical and clinical studies performed using DNA vaccines with the same plasmid backbone (pWRG7077), the likelihood of DNA integration or production of anti-DNA antibodies for the ANDV DNA vaccine is minimal.[24,25] These possibilities cannot be completely ruled out, however, and should be considered. In addition, long-term effects of repeated dosing with DNA vaccines have not been studied. The risk of a more virulent reaction or greater tendency toward a similar disease may be possible in immunized individuals.

It is unknown if the ANDV vaccine poses any risks to an unborn child. Women of childbearing potential must agree to practice effective contraception for a minimum of 30 days prior to study

product administration and agree to practice effective contraception until 90 days after the last study vaccination. If a female subject becomes pregnant while participating in this trial, we will ask permission to follow-up about her health through pregnancy outcome and the health of her baby. Men who are sexually active with a woman of childbearing potential and have not had a vasectomy performed more than 1 year prior to screening must agree not to father a child for 90 days after receipt of the last study vaccination. Female subjects must agree to not donate eggs (ova, oocytes) and male subject agrees not to donate sperm from the start of screening period until at least 90 days after receiving the last vaccination.

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down and elevate his/her legs. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure to the blood draw site for a few minutes after the blood is taken. Drawing blood may also cause infection. The use of aseptic (sterile) technique will make infection at the site where blood will be drawn extremely unlikely.

An IM injection may cause transient discomfort and fainting. Giving an IM injection may also predispose a subject to infection. However, the use of sterile technique will make an infection at the injection site extremely unlikely. The Pharmajet needle free delivery of vaccines has been associated with more local reactogenicity (e.g. pain, tenderness itching, redness or swelling) that was mild or moderate in nature than was observed with needle and syringe injections of vaccines. There were no differences in systemic adverse events

Subjects will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subjects' PHI. All study records will be kept in a locked file cabinet or maintained in a locked room at the VTEU site. Electronic files will be password-protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this trial will be allowed access to the PHI that is collected. Any publications from this trial will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the site for quality assurance and data analysis include groups such as the local Institutional Review Board (IRB), NIAID and their designees, USAMRIID and FDA.

There may be other risks, discomforts, or side effects that are unknown at this time.

2.3.2 Known Potential Benefits

There are no known benefits attributable to the receipt of the ANDV DNA vaccine. It is possible that the study will result in the availability of a vaccine against HPS due to ANDV and/or that vaccination will result in protection from HPS caused by ANDV. The duration of any such protection is currently unknown.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Study Objective

- Assess the safety and reactogenicity of the ANDV DNA vaccine by dosage cohort and treatment arm when administered using the PharmaJet Stratis® Needle-Free Injection system in normal, healthy adults.

3.1.2 Secondary Study Objectives

- Assess the immunogenicity of the ANDV DNA vaccine by dosage cohort and treatment arm.

3.1.3 Exploratory Study Objectives

- Assess cellular immune response to ANDV DNA vaccine by dosage cohort and treatment arm.
- Assess immunogenicity of the ANDV DNA vaccine by dosage cohort and treatment arm at additional time points.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

- Occurrence of vaccine-related Serious Adverse Events (SAEs) through approximately 6 months post last vaccination.
- Occurrence of vaccine-related unsolicited Adverse Events (AEs) through 28 days post last vaccination.
- Occurrence of Serious Adverse Events (SAEs) through approximately 6 months post last vaccination.
- Occurrence of unsolicited Adverse Events (AEs) through 28 days post last vaccination.
- Occurrence of solicited local and systemic AEs through 7 days after each study vaccination.
- Occurrence of clinical safety laboratory AEs through 7 days after each study vaccination.

3.2.2 Secondary Outcome Measures

- Incidence of an ANDV-specific titer of ≥ 20 on Day 57 (3 dose regimen), Day 85 (4 dose regimen) and Day 197 (3 and 4 dose regimen) as measured by:
    - Plaque reduction neutralization titers
    - Pseudovirion neutralization titers
- Incidence of seroconversion (defined as a post-vaccination ANDV-specific titer ≥40 if baseline titer <20 or a minimum four-fold rise compared to baseline if baseline titer ≥20) on Day 57 (3 dose regimen), Day 85 (4 dose regimen) and Day 197 (3 and 4 dose regimen) as measured by:
    - Plaque reduction neutralization titers
    - Pseudovirion neutralization titers
- Geometric mean titer (GMT) of neutralizing antibodies to ANDV on Day 57 (3 dose regimen), Day 85 (4 dose regimen) and Day 197 (3 and 4 dose regimen) as compared to baseline (Day 1) titer and measured by:
    - Plaque reduction neutralization titers
    - Pseudovirion neutralization titers

3.2.3 Exploratory Outcomes

- Incidence of a >3 standard deviation increase in ANDV-specific, cytokine-secreting CD4+ and CD8+ cells on Day 29 (3 and 4 dose regimen), Day 57 (3 dose regimen), Day 85 (4 dose regimen), Day 169 (3 and 4 dose regimen) and Day 197 (3 and 4 dose regimen) compared to baseline (Day 1) as measured by flow-based ICS.
- Incidence of a >3 standard deviation increase in ANDV-specific CD4+ and CD8+ cells on Day 29 (3 and 4 dose regimen), Day 57 (3 dose regimen), Day 85 (4 dose regimen), Day 169 (3 and 4 dose regimen) and Day 197 (3 and 4 dose regimen) compared to baseline (Day 1) as measured by a LPA.
- Incidence of an ANDV-specific titer of ≥20 at additional time points as measured by:
    - Pseudovirion neutralization titers
    - Plaque reduction neutralization titers

4 STUDY DESIGN

This is a Phase 1, randomized, placebo controlled, double-blind, dose escalation trial of 48 males and non-pregnant females, 18-49 years old, inclusive, who are in good health and meet all eligibility criteria. This trial is designed to assess the safety, reactogenicity and immunogenicity of an ANDV DNA vaccine for the prevention of Hantavirus Pulmonary Syndrome (HPS). ANDV DNA vaccine or placebo will be administered using the PharmaJet Stratis® Needle-Free Injection System. Subjects assigned to the 3 dose regimen will receive ANDV DNA vaccine on Days 1, 29 and 169, and placebo on Day 57. Subjects assigned to the 4 dose regimen will receive ANDV DNA on Days 1, 29, 57 and 169. Two doses (i.e., 2 or 4 mg) of ANDV DNA vaccine will be evaluated.

Potential subjects will be screened by history, physical exam, vital signs, and clinical laboratory tests including white blood cells (WBC), hemoglobin (Hgb), platelet count, absolute neutrophil count (ANC), Hemoglobin A1C (HgbA1C), total bilirubin, alanine aminotransferase (ALT), sodium, potassium, and creatinine. Blood urea nitrogen (BUN) will be obtained only if creatinine is above the normal range. Height and weight will be obtained. A urinalysis will be done for urine protein and drug screening for opiates. Potential female subjects of childbearing potential will have a serum pregnancy test. In addition, potential subjects will be screened for HIV – 1/2 antibody, HCV antibody, and HBsAg prior to enrollment.

The first 24 participants in Cohorts 1 and 2 will include one sentinel subjects in each cohort since this is a first-in-human Phase 1 study. Sentinel subjects will receive 2 mg of study vaccine in an open label manner but will be randomized and blinded to the dose regimen. Subjects randomized to the 3 dose regimen (Cohort 1) will receive ANDV DNA vaccine on Days 1, 29 and 169, and placebo on Day 57. Subjects randomized to the 4 dose regimen (Cohort 2) will receive ANDV DNA on Days 1, 29, 57 and 169. One sentinel subject will be vaccinated, followed for one day for safety and reactogenicity, and if no halting rules are met, then the second sentinel subject will receive study vaccine in an open-label manner. The two sentinels will be followed for safety through Day 8 (laboratory and solicited/unsolicited AEs) and if no pre-defined halting rules are met and no safety concerns are identified, enrollment of the 22 remaining subjects in Cohort 1 and 2 will proceed, otherwise a Safety Monitoring Committee (SMC) meeting will be held to conduct an electronic review (E-Review) of clinical and laboratory safety and reactogenicity data. While safety data is being evaluated for the sentinel subjects, no new subjects will be enrolled, but screening may continue.

The 22 non-sentinel subjects in Cohorts 1 and 2 will be randomized in a 9:2:9:2 ratio (Treatment Arms 1b:1c:2b:2c) to receive either study vaccine (at a dose of 2 mg) or placebo in a 3 or 4 dose

regimen, in double-blind fashion. The SMC will review all available safety data through 7 days post second vaccination for all 24 subjects in Cohort 1 and 2 and provide recommendations to proceed to Cohort 3 and 4 or, if necessary, modify the protocol before proceeding. While safety data is being evaluated by the SMC, no new subjects will be enrolled, but screening for Cohorts 3 and 4 and vaccination of the remaining doses in Cohorts 1 and 2 may continue.

The 24 participants in Cohorts 3 and 4 will follow the same schedule as outlined for Cohorts 1and 2 above. Sentinel subjects will be randomized to a dose regimen and will receive 4 mg of study vaccine open label and be followed for safety through Day 8. Subjects assigned to the 3 dose regimen will receive ANDV DNA vaccine on Days 1, 29 and 169, and placebo on Day 57. Subjects assigned to the 4 dose regimen will receive ANDV DNA on Days 1, 29, 57 and 169. The remaining 22 non-sentinel subjects in Cohorts 3 and 4 will be randomized in a 9:2:9:2 ratio (Treatment Arms 3b:3c:4b:4c) to receive study vaccine or placebo at a dose of 4 mg in a 3 or 4 dose regimen, in double blind fashion.

Doses 2-4 for all treatment arms will be administered per the schedule outlined in the table below, assuming no halting rules are met.

Table 1: Treatment Arms

Cohort	Subjects Enrolled	Randomization/ Treatment Arm*#	Day 1	Day 29	Day 57	Day 169
Cohort 1 2mg^	12	1a* n=1 Sentinel	ANDV DNA	ANDV DNA	Placebo	ANDV DNA
		1b# N=9 ANDV DNA	ANDV DNA	ANDV DNA	Placebo	ANDV DNA
		1c# n=2 Placebo	Placebo	Placebo	Placebo	Placebo
Cohort 2 2mg^	12	2a* n=1 Sentinel	ANDV DNA	ANDV DNA	ANDV DNA	ANDV DNA
		2b# N=9 ANDV DNA	ANDV DNA	ANDV DNA	ANDV DNA	ANDV DNA
		2c# n=2 Placebo	Placebo	Placebo	Placebo	Placebo
Cohort 3 4mg+	12	3a* n=1 Sentinel	ANDV DNA	ANDV DNA	Placebo	ANDV DNA
		3b# N=9 ANDV DNA	ANDV DNA	ANDV DNA	Placebo	ANDV DNA
		3c# n=2 Placebo	Placebo	Placebo	Placebo	Placebo
Cohort 4 4mg+	12	4a* n=1 Sentinel	ANDV DNA	ANDV DNA	ANDV DNA	ANDV DNA
		4b# N=9 ANDV DNA	ANDV DNA	ANDV DNA	ANDV DNA	ANDV DNA
		4c# n=2 Placebo	Placebo	Placebo	Placebo	Placebo
TOTAL	48 subjects					

* All sentinel subjects and study personnel will be unblinded to dose and blinded to treatment schedule.
# All non-sentinel subjects will be blinded to dose and treatment schedule.
^ 1mg ANDV DNA administered into the left and right deltoid
+ 2mg ANDV DNA administered into the right and left deltoid.

As the vaccine is expected to be well tolerated based on previously published studies in humans demonstrating the safety of hantavirus DNA vaccines using the same plasmid backbone, more than one dose will be studied to best investigate safety and immunogenicity.

On vaccination days, all females of childbearing potential will have a urine pregnancy test done. All subjects will have vital signs and a targeted physical examination pre-vaccination. Blood will be collected pre-vaccination for immunogenicity testing. On the first and fourth vaccination days, clinical safety laboratory tests will be collected pre-vaccination. Vaccine and/or placebo will then be administered by intramuscular injection using the PharmaJet Stratis® Needle-Free Injection System, into both the left and right deltoid muscle one after another or simultaneously. In this way, subjects will be blinded to cohort (i.e., dosage group) and treatment arm within a cohort.

Subjects will be observed for 30 minutes after the last injection is given. Reactogenicity will be measured by the occurrence of solicited injection site and systemic AEs. Subjects will maintain a memory aid through 7 days post each vaccination recording temperature, solicited local and systemic symptoms. Unsolicited non-serious AEs will be collected from the time of first study vaccination through approximately 28 days after the last vaccination. SAEs occurring from the time of study vaccination through approximately 6 months after the last study vaccination will be collected. Clinical safety labs will be collected on Days 1 and 169, and 7 days after each vaccination; if labs are outside the clinical laboratory reference range, they will be repeated at the next scheduled visit (sooner if medically indicated) and, followed to normal or stabilization.

Immunogenicity testing will include evaluation of the cellular immune response to ANDV neutralizing antibodies peptide pool and assessment of humoral immune response as measured by ANDV Pseudovirion Neutralization Assay (PsVNA) and by ANDV Plaque Reduction Neutralization Test (PRNT). PsVNA will be measured prior to study vaccination (Day 1) and approximately on Days 29, 57, 85, 169, 197, 253, and 337. PRNT will be measured immediately prior to study vaccination (Day 1) and approximately on Days 57, 85, 169 and 197. PBMCs to be used in the cellular assays (ICS+ LPA) will be obtained at day 1, 29, 57, 85, 169 and 197. Blood will also be obtained at Days 85, 197 and 337 (PBMCs) for potential future exploratory assays. The duration of this trial for each subject will be approximately 12 months.

For additional details on study procedures and evaluations by study visits/days, see Appendix A: Schedule of Events .

5 STUDY ENROLLMENT AND WITHDRAWAL

Forty-eight males and non-pregnant females, 18-49 years old, inclusive, who are in good health and meet all eligibility criteria, will be enrolled from a single VTEU site. The target population should reflect the community at large at the VTEU site. Estimated time to complete enrollment in this trial is approximately 6 months. Information regarding this trial may be provided to potential subjects who have previously participated in vaccine trials conducted at the VTEU site. Recruitment materials may be used however, the Institutional Review Board (IRB) will approve all materials prior to use.

Subject Inclusion and Exclusion Criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

No exemptions are granted on Subject Inclusion or Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed toward the DMID Medical Officer.

5.1 Subject Inclusion Criteria

Subjects eligible to participate in this study must meet all of the following inclusion criteria:

1. Provide written informed consent before initiation of any study procedures.
2. Are able to understand and comply with planned study procedures and be available for all study visits/phone calls.
3. Males or non-pregnant females ages 18-49, inclusive.
4. Are in good health *
   * *As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days which would affect the assessment of the safety of subjects or the immunogenicity of study vaccinations. Chronic medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, ER or urgent care for condition and no adverse symptoms that need medical intervention such as medication change/supplemental oxygen). This includes no change in chronic prescription medication, dose, or frequency as a result of deterioration of the chronic medical diagnosis or condition in the 60 days prior to enrollment. Any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as*

determined by the site principal investigator or appropriate sub-investigator, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition. Similarly, medication changes subsequent to enrollment and study vaccination are acceptable provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination. Note: Topical, nasal, and inhaled medications (apart from steroids as outlined in the Subject Exclusion Criteria), herbals, vitamins, and supplements are permitted.

5. Oral temperature is less than 100.0 °F (37.8ºC).
6. Pulse is 47 to 105 beats per minute (bpm), inclusive.
7. Systolic blood pressure (BP) is 85 to 150mm Hg, inclusive.
8. Diastolic blood pressure (BP) is 55 to 95 mm Hg, inclusive.
9. Have acceptable screening laboratories[*,**] within 28 days prior to enrollment.
   [*] *Refer to Appendix B for range of acceptable laboratory values.*
   [*] *Screening laboratory values that are outside acceptable range but are thought to be due to an acute condition or due to laboratory error may be repeated once. [see Manual of Procedures (MOP)]*
10. Urine protein screen is negative or trace.
11. Drug screen for opiates is negative.
12. HgbA1C <6.3% at screening.
13. HIV – 1/2 antibody negative.
14. HCV antibody negative.
15. HBsAg negative.
16. Women of childbearing potential[*], must be using an effective method of contraception[**] from 30 days prior to the first study vaccination until 90 days after the last study vaccination.
    [*] *Women of childbearing potential are defined as those <u>who have not been</u> sterilized via tubal ligation, 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), AND are still menstruating or < 1 year since the last menses if perimenoapausal.*

    [**] *For this study, we define an effective contraceptive method as one that results in a failure rate of less than 1% per year when it is used consistently and correctly. This includes, but is not limited to,*

non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with a vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables or oral contraceptives ("the pill").

17. Women 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.
    * *see definition of women of childbearing potential above.*
18. Sexually active male participants whose partner is a woman of childbearing potential* and has not had a vasectomy** must agree not to father a child until 90 days after the last vaccination***.
    * *see definition of women of childbearing potential above.*
    ** *performed > 1 year prior to screening*
    *** *must agree to use a barrier method of birth control e.g., either condom with spermicidal foam/gel/film/cream or partner reports usage of occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.*
19. Women agree to not donate eggs (ova, oocytes) and male subject agrees not to donate sperm from the start of screening onwards until at least 90 days after the last vaccination.
20. Agree not to participate in another clinical trial during the study period.
21. Agree not to donate blood to a blood bank for 3 months after receiving the last study vaccine.

5.2 Subject Exclusion Criteria

Subjects eligible to participate in this study must not meet any of the following exclusion criteria:

1. Women who are pregnant, planning to become pregnant or lactating*.
    * *Includes breastfeeding or planning to breastfeed at any given time from the receipt of study vaccination through the 12-month trial period.*
2. Known allergy or history of anaphylaxis, severe local or other serious adverse reactions to vaccines or vaccine products*, or history of severe allergic reactions.
    * *This includes a known allergy to an aminoglycoside (e.g., gentamicin, tobramycin, neomycin, streptomycin).*
3. Received an experimental agent* within 3 months prior to study vaccination, or expects to receive an experimental agent** during the 12-month trial-reporting period.
    * *Including vaccine, drug, biologic, device, blood product, or medication.*
    ** *Other than from participation in this study.*

4. Received any licensed live vaccine within 28 days prior to or after each study vaccination.
5. Received a licensed inactivated vaccine within 14 days prior to or after each study vaccination.*
   * *Allowable exception for inactivated seasonal influenza vaccine received more than 7 days prior to or after a study vaccination.*
6. Individuals in whom the ability to observe possible local reactions at the eligible injection sites (deltoid region) is, unacceptably obscured due to a physical condition or permanent body art.
7. Have an acute illness*, as determined by the site PI or appropriate sub-investigator, within 72 hours prior to study vaccination.
   * *An acute illness which is nearly resolved with only minor residual symptoms remaining is allowable if, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol. Subjects may re-screen after an acute illness is resolved*
8. Any confirmed or suspected immunosuppressive or immunodeficient condition* or use of anticancer chemotherapy or radiation therapy (cytotoxic) within 3 years prior to study vaccination.
   * *including HIV infection*
9. Administration of chronic (defined as more than 14 days) immunosuppressants or other immune modifying drugs within 6 months of receipt of study vaccine*
   *~ *For corticosteroids, this means prednisone, or equivalent, greater than or equal to 0.5 mg/kg/day. Intranasal and topical steroids ARE allowed; daily inhaled steroids for treatment of asthma NOT allowed.*
10. History of receipt of a Hantavirus vaccine, including vaccines for Hantaan virus, Puumala virus, or combination of both.
11. Exposed to ANDV* or plans to travel to an endemic area^^ from enrollment through 6 months post last vaccination.
    *$ *Residence in an ANDV endemic area in the last 3 years or >2 consecutive weeks of travel to an ANDV endemic area^^ in the last 3 years.*
    ** *ANDV endemic areas include Chile, Brazil and Argentina*[6,7]
12. Any chronic or active neurologic disorder, including seizures and epilepsy, excluding febrile seizures as a child.
13. History of receiving immunoglobulin or other blood product within the 3 months before enrollment in this study.

14. Current or past history of alcohol or drug abuse in the last 5 years.
15. Subjects with autoimmune disorders, chronic inflammatory disorders or neurological disorders with a potential autoimmune correlation.
16. Have any diagnosis, current or past, of schizophrenia, bipolar disease, or other psychiatric diagnosis that may interfere with subject compliance or safety evaluations.
17. Have been hospitalized for psychiatric illness, history of suicide attempt, or confinement for danger to self or others within 10 years prior to study vaccination.
18. Have received any antiviral within 3 days of study vaccination.
19. A diagnosis of Type I or II diabetes.
20. Current employee or staff paid entirely or partially by the contract for this trial, or staff who are supervised by the PI or Sub-Investigators.
21. Any condition that would, in the opinion of the Site Investigator or appropriate sub-investigator, is a contraindication to study participation. *

    * *Including acute or chronic (persisting for at least 90 days) clinically significant medical disease or condition, that would place the subject at an unacceptable risk of injury, render the subject unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the subject's successful completion of the study.*

5.3 Eligibility Criteria for Doses 2-4

Subjects may not receive subsequent vaccinations if any of the criteria in section 5.4.3 are met. Subsequent vaccinations may be deferred in subjects with transient acute illness as described in Section 5.4.3.

5.4 Treatment Assignment Procedures

5.4.1 Enrollment and Randomization Procedures

Per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the VTEU site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical and Data Coordinating Center's (SDCC) Advantage eClinical^{SM} (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subjects will be enrolled. The first 24 participants in Cohorts 1 and 2 will include two sentinel subjects since this is a first-in-human Phase 1 study

Table **1:** Treatment Arms. Sentinel subjects will be randomized 1:1 to dose regimen and will receive 2 mg of study vaccine open label. The 22 non-sentinel subjects in Cohorts 1 and 2 will be randomized in a 9:2:9:2 ratio (Treatment Arms 1b:1c:2b:2c) to receive either study vaccine or placebo at a dose of 2 mg in a 3 or 4 dose regimen, in double-blind fashion.

The 24 participants in Cohorts 3 and 4 will follow the same schedule as outlined for Cohorts 1 and 2 above. Sentinel subjects will receive 4 mg of study vaccine open label and be followed for safety through Day 8. The remaining 22 non-sentinel subjects in Cohort 3 and 4 will be randomized in a 9:2:9:2 (Treatment Arms 3b:3c:4b:4c) ratio to receive study vaccine or placebo at a dose of 4 mg in a 3 or 4 dose regimen, in double blind fashion.

Enrollment of subjects will be done online using the enrollment module of Advantage eClinical^{SM}. The randomization code will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. Advantage eClinical^{SM} will assign each subject to a treatment arm after the demographic and eligibility data have been entered into the system. A designated individual at the VTEU site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the Advantage eClinical^{SM} User's Guide. Manual back-up randomization procedures and instructions are provided for use in the event that the VTEU site temporarily loses access to the Internet or the online enrollment system is unavailable.

Subjects who sign the informed consent form (ICF) and are randomized but do not receive study vaccine or placebo may be replaced. Subjects who sign the ICF, and are randomized and vaccinated, and subsequently withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up will not be replaced.

5.4.2 Masking Procedures

Except for the sentinel subjects who will receive study vaccine open label, this is a double-blind clinical trial within each dose escalation group (i.e., double blinded within Cohorts 1 and 2 and double blinded within Cohorts 3 and 4).

Investigators, study personnel performing any study-related assessments following study vaccine administration, and laboratory personnel performing antibody assays will be blinded to treatment arm assignment (i.e., placebo vs. study vaccine, and dose regimen).

Sentinel subjects will be blinded to cohort, but not dosage group. Non-sentinel subjects will be blinded to treatment arm assignment and cohort.

The randomization scheme will be generated by the SDCC and provided to unblinded study personnel (i.e., research pharmacists performing study vaccination preparations and unblinded study vaccine administrators) at the VTEU site.

The unblinded study vaccine administrator is a study personnel member credentialed to administer vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

The SMC may receive data in aggregate and presented by treatment arm. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request the treatment arm be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. Refer to the protocol-specific Manual of Procedures (MOP) for unblinding procedures.

5.4.3 Discontinuation of Study Product

There are several reasons why a study subject may be prevented from receiving the second, third or fourth vaccination. If a subject's study vaccine must be discontinued before the end of the vaccination schedule, this will not result in automatic withdrawal of the subject from the study.

Subjects who discontinue study vaccine will be encouraged to remain in this trial for the following follow-up assessments:

- Safety assessments: Venous blood sample at approximately 7 days after the last study vaccination. Subjects will be asked to complete the remaining study assessments per Appendix A, Schedule of Events which may be conducted by phone call/electronic communication (e.g., email, text message) rather than in person continuing through approximately 6 months after the last study vaccination.
- Immunogenicity assessments: Venous blood sample at Day 29, 85 and approximately 6 months after the last study vaccination See the protocol-specific Manual of Procedures (MOP) for alternate follow-up requirements.

Follow-up study vaccinations (2nd, 3rd and/or 4^{th} dose) will <u>not</u> be administered to a subject if any of the following criteria are met:

- Pregnancy
- Receipt of disallowed licensed vaccine, experimental product or medication (see Section 5.2)
- New onset of illness or condition that meets the Exclusion Criteria (see Section 5.2)
- 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. Note: Medication changes subsequent to the first study vaccination are not exclusionary for receipt of the follow-up study vaccination provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination.
- Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity.
- Any laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- Any generalized urticaria after administration of study product that is considered related to study product.
- Serious adverse event related to the study vaccination.
- Grade 3 solicited or unsolicited (clinical sign or symptom) adverse event that occurs in the 7 days following study vaccination, lasts for 24 hours or more without decreasing to a Grade 1 or Grade 2, and does not have an alternative etiology. If the subject has a Grade 3 adverse event as per Reactogenicity Tables 3, 4, 5 or 6 in the seven days following study vaccination and it lasts < 24 hours before decreasing to a Grade 2 or Grade 1 adverse event, see discontinuation criteria for Grade 2 below.
- Grade 3 solicited or unsolicited (clinical sign or symptom) adverse event that occurs >7 days following study vaccination and is ongoing at the time of the subsequent study vaccination (i.e., Day 29, Day 57 or Day 169). Conversely, if this Grade 3 adverse event was resolved or reduced to a Grade 1 or Grade 2 adverse event at the time of the subsequent study vaccination, IP may only be given, 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 clinical safety laboratory value (according to the toxicity table, Section 9.2.2) that does not decrease to Grade 1 or less prior to the follow-up study vaccination (i.e., Day 29,

Day 57 or Day 169). Any clinical safety laboratory parameter may be re-evaluated only once at the local clinical laboratory to assess eligibility prior to the follow-up study vaccination. If the clinical safety laboratory value decreases to Grade 1 or less, the subject may receive the follow-up study vaccination. The study vaccination should be scheduled to occur within the acceptable protocol-specified window for that visit.

- Subjects who experienced any Grade 2 adverse event that is an unresolved Grade 2 or a Grade 1 at the time of the next vaccination, IP may only be given 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.
- 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.

Subjects with transient injection site or systemic signs or symptoms, or with an acute illness, including an oral temperature greater than or equal to 100.0°F, follow-up study vaccination/s should be postponed/deferred until signs, symptoms, or acute illness have resolved, or are improving as further specified below. Study vaccine given outside the protocol-specified window must be approved by the DMID Medical Officer.

- Note for afebrile, acute illness only: If a subject is afebrile, his/her acute illness is nearly resolved with only minor residual symptoms remaining, this occurs within the acceptable protocol-specified window for that visit, and, in the opinion of the site principal investigator or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol, the subject may receive the follow-up study vaccination without DMID Medical Officer approval.

5.4.4 Study Withdrawal

Subjects may voluntarily withdraw their consent for trial participation at any time and for any reason, without penalty. The site principal investigator or appropriate sub-investigator may also choose to remove a subject from the study. A subject may withdraw or be withdrawn from this trial for any of the following reasons:

- Medical disease or condition, or any new clinical finding for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of this trial, or would interfere with the evaluation of adverse events or immunologic response.
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Subject dies.
- Termination of this trial.
- New information becomes available that makes further participation unsafe.

5.4.5 Handling of Withdrawals

The primary reason for withdrawal from this trial will be recorded on the Study Status data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.8.

In the case of subjects who are lost to follow-up, extensive effort (i.e., three documented contact attempts via phone calls, emails, text messages, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject's study records. Every attempt will be made to follow all adverse events, including solicited local site and systemic AEs, unsolicited non-serious adverse events and SAEs, ongoing at the time of early withdrawal through resolution as per applicable collection times defined for the specific type of adverse event.

Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the ICF, randomization, and receipt of study vaccine will not be replaced. Subjects who withdraw, or are withdrawn or terminated from this trial, or are lost to follow-up after signing the ICF and randomization but before receipt of study vaccine may be replaced.

5.4.6 Termination of Study

Although the sponsor has every intention of completing this trial, it reserves the right to terminate this trial at any time for clinical or administrative reasons. Reasons for termination

include, but are not limited to, study closure due to the recommendation after a SMC review and at the discretion of DMID.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

ANDV DNA vaccine is a deoxyribonucleic acid (DNA) vaccine. The ANDV DNA vaccine plasmid is constructed on a well-characterized plasmid backbone, pWRG7077, and elements of the ANDV genome. The plasmid used for the ANDV DNA vaccine, pWRG/AND-M (opt2), is optimized for homo sapien codon usage and mRNA stability by Genewiz. The vaccine was developed by the Department of Molecular Virology, US Army Medical Research Institute of Infectious Diseases (USAMRIID; Fort Detrick, Maryland), and the final vaccine was manufactured by Aldevron (Fargo, ND).

The ANDV DNA vaccine drug substance, plasmid pWRG/AND- M(opt2), Lot # 87763, date of manufacture 01 Dec 2017, was diluted to a concentration of 4.0 +/- 0.2 mg/mL in phosphate-buffered saline (PBS) at a pH of 7.2, sterile-filtered, and stored as bulk after aliquots were removed for stability, sterility, and quality control (QC) testing.

The ANDV DNA plasmid that forms the active component for the vaccine is suspended in PBS. Each injection will contain 1 or 2 mg of DNA in 0.5 mL of PBS depending on randomization assignment; it is clear, colorless and free of visible particulate.

6.1.1 Acquisition

The ANDV DNA vaccine will be provided by Aldevron (Fargo, ND). Placebo and Diluent, 0.9% Sodium Chloride Injection, USP will be provided by the DMID Clinical Materials Services (CMS), Fisher BioServices, and will be shipped to the investigational site upon request and approval by DMID.

Upon request by DMID, ANDV DNA Vaccine will be shipped to the following address:
DMID Clinical Materials Services
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876

ANDV DNA vaccine, placebo and diluent will be provided through the DMID Clinical Materials Services (CMS) to the VTEU site prior to the start of this trial upon request and with prior approval from DMID. Should the site principal investigator require additional ANDV vaccine during this trial, further instructions are provided in the protocol-specific MOP.

6.1.2 Formulation, Packaging, and Labeling

ANDV DNA Vaccine

The ANDV DNA vaccine comes in single-use vials. Vials for the Phase 1 clinical trial will be fill-finished at 1.3 mL per vial. This will allow two 0.5 mL PharmaJet Stratis® Needle-Free Injection System syringes to be filled from each vial.

The vaccine formulates will be administered using the PharmaJet Stratis® Needle-Free Injection System. This device delivers a 0.5 mL jet of liquid at high pressure that penetrates through the skin into the muscle. The device is FDA 510K cleared for use with approved medicines.

The study product will be labeled according to manufacturer specifications and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use".

Placebo/Diluent

Placebo and diluent will be supplied as 0.9% Sodium Chloride Injection, USP which is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection (WFI). Each mL contains sodium chloride 9 mg without any preservative. It contains no bacteriostatic, antimicrobial agent, or added buffer and is supplied only in single-dose containers. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3 [4.5 to 7.0]).

The placebo will be administered using the PharmaJet Stratis® Needle-Free Injection System. This device delivers a 0.5 mL jet of liquid at high pressure that penetrates through the skin into the muscle. The device is FDA 510K cleared for use with approved medicines.[17]

6.1.3 Product Storage and Stability

ANDV DNA Vaccine

The vials containing study product must be stored at ≤ - 65º C. Vials are removed from the product freezer and thawed at room temperature for at least 30 minutes and administered within 1 hour after thawing.

Placebo/Diluent

Placebo and diluent must be stored at 20ºC to 25ºC (68°F to 77ºF) [See USP Controlled Room Temperature; excursions between 15°C and 30°C (59°F and 86°F) are permitted]. See protocol-specific MOP for further instructions.

The temperature of the storage unit must be recorded daily (excluding non-business days and holidays as applicable), continuously monitored and recorded during the duration of this trial per the VTEU site's standard operating procedures (SOP), and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). The research pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, the affected study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team (see MOP for contact information) for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine are provided in the protocol-specific MOP.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

See the protocol-specific MOP for detailed information on the preparation, labeling, storage, and administration of study product for each cohort. Study product preparation will be performed by the VTEU site's research pharmacist on the same day of study vaccine administration.

Visually inspect the ANDV DNA vaccine upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter or if there are any concerns regarding its integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined at ≤ - 65 ºC and labeled as 'Do Not Use' (until further notice). The site principal investigator or responsible person should immediately contact the DMID Product Support Team (see MOP for contact information) and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the

affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. If the ANDV vaccine is unusable, study personnel will use another vial from the study supply. Replacement vials may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

The study product will be prepared for administration by thawing at room temperature for at least 30 minutes. Vials should be gently inverted at least 15 times once thawed and administered within 1 hour after thawing. The study product should have a clear appearance after thawing.

Dilution is not required for subjects allocated to the 4 mg dosage cohorts, the dose will be divided into 2 mg per 0.5 mL administration into the left and right deltoid muscles. Dilution with 0.9% Sodium Chloride Injection, USP is required for subjects allocated to the 2 mg dosage cohorts, the dosage will be divided into 1 mg per 0.5 mL administration into the left and right deltoid muscles. See protocol-specific MOP for further instructions on dilution.

The volume administered to Cohort 1 and 2 subjects will be a total of 1.0 ml; 0.5 ml of ANDV DNA vaccine (diluted to 1.0 mg/0.5 mL) administered into both the left and right deltoid muscles.

The volume administered to Cohort 3 and 4 subjects will be a total of 1.0 ml; 0.5 ml ANDV DNA vaccine (2.0 mg/0.5 ml) administered into both the left and right deltoid muscles.

Study product administration will be performed by an unblinded study personnel member who is credentialed to administer vaccines and may also participate in dose preparation, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

6.3 Modification of Study Intervention/Investigational Product for a Participant

Subjects who do not receive follow-up vaccinations (2, 3 or 4) within the protocol defined window will be followed for safety and immunogenicity.

Outside of protocol defined windows, there will be no dose modifications.

If a subject's follow-up vaccinations are deferred, attempts will be made to reschedule the vaccination to occur within the acceptable protocol-specified window for that visit.

Subjects who do not receive the follow-up study vaccination will continue with follow-up safety and immunogenicity assessments continuing through approximately 6 months after their last study vaccination. See Sections 5.4.3, 5.4.4 and 5.4.5 for reasons for and handling of withdrawals and discontinuation of treatment. See the protocol-specific MOP for alternate follow-up requirements.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

After receipt of the ANDV vaccine, the site principal investigator is responsible for study product distribution and disposition and has ultimate responsibility for study product accountability. As this is a blinded study, the site PI will delegate this responsibility to the unblinded site pharmacist. Study vaccine records must be maintained and document logs of receipt, accountability, and storage temperature conditions. The study product accountability records and dispensing logs will also capture vial numbers, including final vial number, date of study vaccine preparation/ administration, time of study vaccine preparation, time study vaccine is drawn into the syringe, and amount of study vaccine withdrawn for administration. Time of study vaccine administration to the subject will be captured on the appropriate data collection form. All study product(s), whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. The sponsor's monitoring staff will verify the VTEU site's study product accountability records and dispensing logs per the site monitoring plan.

Used and unused vials of vaccine, will be retained until monitored and released for disposition as applicable. The injectors are re-usable, syringes are disposable. Used syringes post dosing may be disposed of upon completion of administration. Filled, un-used syringes will be retained until monitored and released for disposition as applicable. Final disposition of the unused vaccine will be determined by DMID and communicated to the enrolling site.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

Study products will be administered to subjects via intramuscular injection according to subject treatment assignment and as described in Section 6.2. Thus, subject compliance is not anticipated to be an issue. Deviations from the dose schedule may only occur as described in Section 6.3.

6.6 Concomitant Medications/Treatments

Administration of any medications, therapies, or non-study vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications and medications taken within 60 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. 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 5.2). In addition, the site principal investigator or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity. Use of medications (anti-inflammatories or analgesics) as prophylaxis prior to study vaccination is prohibited. There are no known drug-vaccine interactions with the study vaccine and subjects are not being asked to discontinue current medications not listed in the exclusion criteria.

In the event medical conditions dictate use of medications, subjects are encouraged to obtain adequate care, comply with the course of therapy as prescribed by their physician and inform the site as soon as practicable. Details of all medications taken during the medication reporting period for this study (date, indication, brand or generic name) will be recorded. Use of new medication should prompt evaluation for the occurrence of an AE or worsening of a pre-existing medical condition.

7 STUDY SCHEDULE

Complete study schedule details listed by type of visit are described below. Refer also to Sections 4 and 8, and Appendix A: Schedule of Events .

7.1 Screening
Day -28 to -1, Visit 00

Potential subjects will be screened for eligibility within 28 days prior to the administration of study vaccination. The following activities will be performed at screening and may be done all at one visit or split into separate visits for all Treatment Arms:

- Subjects will be provided with a description of this trial (purpose and study procedures) and asked to read and sign the ICF. The ICF will be signed prior to performing any study procedures, including any screening procedures.
- Demographic information will be obtained by interview of subjects.
- Eligibility criteria will be reviewed with subjects. The following screening procedures will be completed to ensure that the potential subject is eligible for the study:
- Complete medical history will be obtained by interview of subjects.
- History of all concomitant medications taken within 60 days prior to signing the ICF will be reviewed with subjects. Medications reported in the eCRF are limited to those taken within 30 days prior to study vaccination. Assessment of study eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Section 5.1, 5.2, 5.3).
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- Height and weight will be collected.
- A physical examination will be performed 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 by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- A serum pregnancy test will be performed on all females of childbearing potential and must be negative.
- Venous blood will be collected for screening laboratory tests to be run at the local clinical laboratory. These include: WBC, Hgb, platelet count, ANC, sodium, potassium, and creatinine, HgbA1C, total bilirubin, and ALT. BUN will be obtained if creatinine is above the normal range. In addition, participants will be screened for HIV – 1/2 antibody, HCV antibody, and HBsAg. Testing results must be confirmed to meet the eligibility criteria as outlined in the Inclusion Criteria (see Section 5.1 and Appendix B) prior to randomization.
- Urine will be collected for screening urinalysis (dipstick) for urine protein and urine toxicology screen for opiates. Values must meet the eligibility criteria (see Section 5) prior to randomization.

7.2 Enrollment/Baseline Day 1, Visit 1

Forty-eight subjects who meet all inclusion and no exclusion criteria will be administered study product. The following procedures will occur for all Treatment Arms:

- Subject's willingness to participate will be reconfirmed prior to performing any further study procedures, including administration of the study vaccination.
- Eligibility criteria, including results of all clinical screening laboratory evaluations, will be reviewed with subjects prior to study vaccination to ensure continued eligibility.
- Complete medical history and any updates obtained by interview of subjects since the screening visit will be reviewed with subjects prior to study vaccination to ensure continued eligibility.
- All concomitant medications will be reviewed with subjects prior to study vaccination for accuracy and completeness. Any new concomitant medications taken since the screening visit will be reviewed with subjects and assessed for continued eligibility prior to study vaccination. Medications reported in the eCRF are limited to those taken within 30 days prior to study vaccination.
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained to ensure eligibility. Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

- A targeted physical examination may be performed prior to study vaccination, if indicated based on review of complete medical history and any updates obtained by interview of subjects since the screening visit, by a study clinician listed on the Form FDA 1572.
- A urine pregnancy test will be performed within 24 hours prior to study vaccination on all females of childbearing potential. Results must be negative and known prior to randomization and study vaccination.
- Subjects will be enrolled in Advantage eClinicalSM and assigned randomly to a treatment arm prior to study vaccination.
- Venous blood will be collected immediately prior to the study vaccination for baseline ANDV PRNT and PsVNA antibody assays.
- Venous blood will be collected immediately prior to the study vaccination for baseline safety labs.
- Venous blood will be collected immediately prior to the study vaccination for baseline ICS and LPA cellular assays.
- Pre-administration systemic reactogenicity assessments will be performed prior to study vaccination to establish a baseline. Subjects will then receive study vaccination via intramuscular injection into the left and right deltoid one after another or simultaneously. The time of administration will be recorded on the appropriate data collection form. Subjects will be observed for at least 30 minutes after the last study vaccination was given. Post-administration local and systemic reactogenicity assessments will be performed. Any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.
- Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited site and systemic AEs, unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their oral temperature around the same time each day. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Subjects will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the subject on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

7.3 Follow-up, Vaccination Visits Day 29 (+2), 57 (+2), 169 (+5)

Follow-up visits are scheduled in reference to study vaccination date as indicated for each visit window, see Appendix A, SOE. All Treatment Arms will return for vaccination on D29 (V3), D57 (V5) and D169 (V9).

- Eligibility criteria will be reviewed with subjects prior to study vaccination to ensure continued eligibility.
- Interim medical history will be obtained by interview of subjects to ensure continued eligibility prior to study vaccination. Any changes since the previous clinic visit or contact will be noted at each clinic visit.
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form at each clinic visit. Any new concomitant medications taken since the screening visit will be reviewed with subjects and assessed for continued eligibility prior to study vaccination.
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained to ensure eligibility. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed prior to study vaccination, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician listed on the Form FDA 1572.
- A urine pregnancy test will be performed within 24 hours prior to study vaccination on all females of childbearing potential. Results must be negative and known prior to administration of study vaccination.
- Venous blood will be collected immediately prior to the study vaccination (D29) for ANDV PsVNA antibody assays.
- Venous blood will be collected at Days 57 and 169 immediately prior to the study vaccination for ANDV PsVNA and PRNT antibody assays.
- Venous blood will be collected immediately prior to the study vaccination for safety labs on Day 169 only.
- Venous blood will be collected immediately prior to the study vaccination for ICS and LPA cellular assays.

- Pre-administration systemic reactogenicity assessments will be performed prior to study vaccination to establish a baseline. Subjects will then receive the dose of study vaccine via intramuscular injection into the left and right deltoid one after another or simultaneously. The time of administration will be recorded on the appropriate data collection form. Subjects will be observed for at least 30 minutes after the last study vaccination was given. Post-administration local and systemic reactogenicity assessments will be performed. Any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic.
- Subjects will be provided with a memory aid and other study-related materials to record daily oral temperature, solicited site and systemic AEs, unsolicited AEs, and concomitant medications. Subjects will be encouraged to take their oral temperature around the same time each day. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Subjects will be instructed on how to use their memory aid and how to measure and record AEs prior to discharge from the clinic. Subjects will be instructed to notify the study center if they develop any severe reactions after study vaccination. If the site principal investigator or appropriate sub-investigator deems the reaction severe enough, further instructions will be given to the subject on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.
- All AEs and SAEs will be collected and recorded on the appropriate data collection form at each clinic visit.

7.4 Follow-up, Day 8 post each vaccination Day 8 (+2), 36 (+2), 64 (+2), 176 (+2)

Follow-up visits are scheduled in reference to study vaccination date as indicated for each visit window, see Appendix A, SOE. All Treatment Arms will return on D8 (V2), D36 (V4), D64 (V6) and D176 (V10).

- Interim medical history will be obtained by interview of subjects to ensure continued eligibility. Any changes since the previous clinic visit or contact will be noted at each clinic visit.
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form at each clinic visit. Any new concomitant medications taken since the last clinic visit or phone contact will be reviewed with subjects and assessed for continued eligibility.

- A targeted physical examination may be performed, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician listed on the Form FDA.
- Venous blood will be collected for safety labs.
- All AEs and SAEs will be collected and recorded on the appropriate data collection form at each clinic visit.
- Memory aid information will be reviewed with subjects at each clinic visit.

7.5 Follow-up, Day 29 post each vaccination Day 85, 197

Follow-up visits are scheduled in reference to study vaccination date as indicated for each visit window, see Appendix A, SOE. All Treatment Arms will return 28 days post vaccination on D85 (V7) and D197 (V11).

- Interim medical history will be obtained by interview of subjects to ensure continued eligibility. Any changes since the previous clinic visit or contact will be noted at each clinic visit.
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form at Day 85 and Day 197 visit. Any new concomitant medications taken since the previous clinic visit or contact will be reviewed with subjects and assessed for continued eligibility.
- A targeted physical examination may be performed, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician listed on the Form FDA 1572.
- Venous blood will be collected for ANDV PRNT and PsVNA antibody assays.
- Venous blood will be collected for future exploratory assays
- Venous blood will be collected for ICS and LPA cellular assays.
- All AEs and SAEs will be collected and recorded on the appropriate data collection form at each clinic visit.

7.6 Follow-up Visits Day 141 & 253

Follow-up visits are scheduled in reference to study vaccination date as indicated for each visit window, see Appendix A, SOE. All Treatment Arms will return on D141 (V8) and D253 (V12).

- Interim medical history will be obtained by interview of subjects to ensure continued eligibility. Any changes since the previous clinic visit or contact will be noted at each clinic visit.
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form at the Day 141 clinic visit. Any new concomitant medications taken since the previous clinic visit or contact will be reviewed with subjects and assessed for continued eligibility.
- A targeted physical examination may be performed, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician listed on the Form FDA 1572.
- Venous blood will be collected for ANDV PsVNA antibody assays at Day 253.
- All AEs will be collected and recorded on the appropriate data collection form at day 141.
- SAEs will be collected and recorded on the appropriate data collection form at each clinic visit.

7.7 Final Study Visit Day 337

Final visits are scheduled in reference to study vaccination date as indicated for each visit window, see Appendix A, SOE. All treatment Arms will return on D337 (V13).

- Interim medical history will be obtained by interview of subjects. Any changes since the previous clinic visit or contact will be noted.
- A targeted physical examination may be performed, if indicated based on review of complete medical history and any updates obtained by interview of subjects, by a study clinician listed on the Form FDA 1572.
- Venous blood will be collected for future exploratory assays.
- Venous blood will be collected for ANDV PsVNA antibody assays.

- All SAEs will be collected and recorded on the appropriate data collection form.

7.8 Early Termination Visit

The following activities will be performed on all Treatment Arms at the early termination visit on subjects who withdraw, or are withdrawn or terminated from this trial:

- Interim medical history will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted (if indicated).
- Memory aid information will be reviewed with subjects (if within 7 days post study vaccination).
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form (if prior to 28 days post last study vaccination).
- All AE/SAEs will be recorded on the appropriate data collection form. AEs will be recorded if prior to 28 days post last study vaccination. AEs will be limited to SAEs, if after 28 days post last study vaccination.
- Vital signs, including oral temperature, pulse, and blood pressure, may be obtained if indicated. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician licensed listed on the Form FDA 1572.
- Post-administration reactogenicity assessments will be performed (if within 7 days post study vaccination).
- Venous blood will be collected for safety laboratory evaluations and performed by the local (clinical) laboratory (if within 7 days post last study vaccination).
- Venous blood will be collected for cellular (ICS, LPA) assays (if prior to 28 days post last study vaccination).
- Venous blood will be collected for PsVNA antibody assays.

7.9 Unscheduled Visit

Unscheduled visits may occur at any time during this trial. Any of the following activities may be performed:

- Interim medical history will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted (if indicated).
- Memory aid information will be reviewed with subjects (if within 7 days post study vaccination).
- All concomitant medications (including solicitation for receipt of any non-study vaccines) will be recorded on the appropriate data collection form (if prior to 28 days post last study vaccination).
- All AE/SAEs will be recorded on the appropriate data collection form. AEs will be recorded if prior to 28 days post last study vaccination. AEs will be limited to SAEs, if after 28 days post last study vaccination.
- Vital signs, including oral temperature, pulse, and blood pressure, may be obtained if indicated. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination may be performed, if indicated based on review of interim medical history, by a study clinician listed on the Form FDA 1572.
- Post-administration reactogenicity assessments will be performed (if within 7 days post study vaccination).
- Venous blood will be collected for safety laboratory evaluations and performed by the local (clinical) laboratory (if within 7 days post last study vaccination).
- Venous blood will be collected for cellular (ICS, LPA) assays (if prior to 28 days post last study vaccination).
- Venous blood will be collected for PsVNA antibody assays.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Complete medical history will be obtained by interview of subjects at the screening visit and will be reviewed and/or updated on Day 1 prior to study vaccination. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits after study vaccination, an interim medical history will be obtained by interview of subjects noting any changes since the previous clinic visit or contact. The interim medical history should include an assessment for new medical conditions.

Medication history (concomitant medications) will include a review of all current medications and medications taken within 60 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 reported in the eCRF are limited to those taken within 30 days prior to the first study vaccination through approximately 28 days after the last study vaccination. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. Use of new medication should prompt evaluation for the occurrence of any AE. Assessment of eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Sections 5.1 and 5.2). In addition, the site principal investigator or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity. History of non-study vaccines received from 30 days prior to the first study vaccination through approximately 28 days after the last study vaccination will be solicited and recorded on the eCRF.

A full physical examination will be performed on all subjects at the screening visit. This exam will include the following organs and organ systems: general appearance, skin, head and neck, lungs, heart, liver, spleen, extremities, musculoskeletal, lymph nodes and nervous system, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

Vital signs (oral temperature, pulse, and blood pressure) will be collected at the screening visit. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Height and weight will be collected at the screening visit prior to study vaccination.

On Day 1 prior to the study vaccination and at follow-up visits after the study vaccination, a targeted physical examination may be performed, if indicated based on the subject's interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

On Day 1 and all vaccination visits, vital signs (oral temperature, pulse, and blood pressure) will be collected prior to study vaccination. A urine pregnancy test will be performed within 24 hours prior to study vaccination on all females of childbearing potential. Pre-administration reactogenicity assessments will be performed prior to each study vaccination to establish baseline, then the study vaccination will be given. Subjects will be observed in the clinic for at least 30 minutes after each study vaccination. The study vaccination site will be examined, post-administration reactogenicity assessments will be performed and any AE/SAEs will be recorded on the appropriate data collection form prior to discharge from the clinic. All subjects will receive and complete a subject memory aid from the time of study vaccination through 7 days after study vaccination. Subject memory aids will be reviewed with the subjects for adverse events, (solicited local and systemic AEs and unsolicited AEs) approximately 7 days after each vaccination.

Reactogenicity assessments will include an assessment of solicited adverse events occurring from the time of study vaccination through 7 days after study vaccination. These include local reactions such as pain, tenderness, erythema (redness), induration (hardness/swelling), bruising and skin discoloration as well as systemic AEs such as fever, feverishness (chills, shivering, sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), nausea, dizziness and headache.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Serum pregnancy tests will be done at the screening visit and processed locally by the site laboratory at the screening visit and a urine pregnancy test will be performed within 24 hours prior to study vaccination on all females of childbearing potential. Urine will be collected in a

sterile urine cup for processing by research staff using a CLIA waived urine pregnancy test kit. Results must be negative and known prior to randomization on Day 1 and prior to administration of study vaccination on Day 29, Day 57 and Day 169.

Clinical screening laboratory tests will be performed at the screening visit and processed by the local (clinical) laboratory. Parameters to be evaluated to confirm trial eligibility as per Section 5.1 and Appendix B, include the following:

- Hematology: WBC, Hgb, platelet count, ANC, and HgbA1c.
- Biochemistry: Total bilirubin, ALT, creatinine, sodium, and potassium. BUN will be done if creatinine is above the normal range.
- Serum serology testing for HIV – 1/2 antibody, HCV antibody, and HBsAg.
- Serum βHCG pregnancy test (all females of childbearing potential) at screening and urine pregnancy test as described above.
- Urinalysis will be done to test for urine protein via dipstick in the clinic. Clean-catch, mid-stream, urine specimen will be collected in a sterile urine cup.
- Urine test for opiates: A clean-catch, mid-stream urine specimen will be collected in a sterile urine cup and transported to the clinical laboratory for processing and examination.

Clinical safety labs will be performed at Day 1 and 169, and approximately 7 days after each study vaccination. If they are abnormal, tests will be repeated at the next scheduled visit (or sooner if medically indicated) and followed to normal or stabilization. Clinical safety laboratory parameters to be evaluated will include WBC, Hgb, platelet count, ANC, sodium, potassium, Creatinine, total bilirubin and ALT. These evaluations will be performed by the local (clinical) laboratory and will not be used for screening purposes prior to dosing.

The volume of venous blood to be collected for clinical screening, immunologic assessments and safety laboratory evaluations is presented in the tables below:

Table 2: Blood Volume (mL) – All Treatment Arms

Study Visit (V)	00	01	02	03	04	05	06	07	08	09	10	11	12	13	U/S or ET
Study Day and Window	Screen	D1	D8	D29	D36	D57	D64	D85	D141	D169	D176	D197	D253	D337	
Screening Labs	20														
Clinical Safety Evaluations		6.5	6.5		6.5		6.5			6.5	6.5				6.5
PBMCs (ICS, LPA)		40		40		40		40		40		40			40
Immunogenicity (ANDV PRNT)		10				10		10		10		10			
Immunogenicity (ANDV PsVNA)		10		10		10		10		10		10	10	10	10
Future Exploratory Assays								40				40		40	
Total volume of blood collected/visit	20	66.5	6.5	50	6.5	60	6.5	100	0	66.5	6.5	100	10	50	56.5
Cumulative volume of blood	20	86.5	93	143	149.5	209.5	216	316	316	382.5	389	489	499	549	N/A

8.2.2 Acceptable Laboratory Values for Eligibility and Defining Normal Values

If laboratory screening tests are out of acceptable range, repeat of screening tests is permitted once, provided there is an alternative explanation for the out of range value.

Refer to Appendix B for acceptable values for study eligibility and clinical laboratory reference ranges.

- Absolute neutrophil counts (ANC) below the laboratory normal reference range will be allowable if they are in keeping with the levels seen in a condition that is prevalent in our population known as “benign ethnic neutropenia.” For subjects who are of African American or Middle Eastern decent, ANC must be greater than or equal to 0.8 K/mcL for inclusion.
- Creatinine, BUN, and ALT values that are below the lower limit of normal as presented in Appendix B are not exclusionary as these values are considered to be not clinically significant (NCS).

- If a creatinine level is abnormal (elevated above the clinical laboratory normal reference range) at screening, a BUN will be collected to accompany the repeat creatinine. If the BUN is within normal range (or less than the lower limits of normal), then the out of range screening and/or repeat creatinine value will be considered NCS and subject will be acceptable for study inclusion (see Appendix B for acceptable values for study eligibility).

8.2.3 Special Assays or Procedures

Cellular Immunology Assays

Functional T cell immune responses in PBMCs collected from the study participants at different time points during the course of study will be studied by flow-based ICS and LPA. These assays will be performed at CCHMC. Venous blood samples will be collected from each subject at day 1, 29, 57, 85, 169 and 197. PBMCs will be isolated and cryopreserved for future use. Batched PBMCs from each subject will be thawed and stimulated with ANDV GP peptide pools and the frequencies of CD4 and CD8 T cell secreting IFNg, IL-2 and TNF will be measured by ICS. In separate experiments thawed PBMCs will be stained with CFSE dye and stimulated with ANDV GP peptide pools. Five days later degree of proliferation of CD4 and CD8 T cells will be determined by measuring the dilution of CFSE on these cells by flow cytometry.

ANDV, PsVNA and PRNT Assays

Serum from ANDV DNA vaccinated individuals will be evaluated for immunogenicity by a neutralizing antibody response to ANDV measured by PsVNA and PRNT. These assays will be performed at USAMRIID. Venous blood samples will be collected from each subject prior to study vaccination (Day 1) and on Day 29, 57, 85, 169, 197, 253, and 337 (all Treatment Arms). Serum from all time points will be analyzed by ANDV PsVNA. Serum from Day 1, 57, 85, 169 and 197 will be analyzed by ANDV PRNT. Those time points represent prior to the initial vaccination (Day 1), 1 month after the initial series of vaccinations (Day 57 and 85), prior to the six-month boost (Day 169) and 1 month after the six-month boost (Day 197).

8.2.4 Specimen Preparation, Handling, and Shipping

8.2.4.1 Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP as appropriate.

8.2.4.2 Specimen Shipment

Specimen shipment will occur when all specimens are available following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the local (clinical) laboratory manual and protocol-specific MOP as appropriate.

All specimens for clinical screening and safety laboratory evaluations will be transported from the VTEU site to the local (clinical) laboratory.

Specimens for cellular assays (ICS, LPA) will be transported from the VTEU site to the local (research) laboratory for processing.

Specimens for antibody assays (ANDV, PsVNA and PRNT) will be shipped from the VTEU site to the Clinical Materials Services (CMS).

Further instructions for specimen shipment are included in the local (clinical) laboratory manual and protocol-specific MOP, as appropriate.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the occurrence of:

1. Serious adverse events occurring from the time of study vaccination through approximately 6 months after the last study vaccination.

2. Solicited Adverse Events – reactogenicity events occurring from the time of study vaccination through 7 days after each study vaccination:
   a) Local AEs including pain, tenderness, erythema (redness), induration (hardness/swelling), bruising and skin discoloration.
   b) Systemic AEs including fever, feverishness (chills, shivering, sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain), nausea, dizziness and headache.

3. Clinical safety laboratory adverse events occurring from the time of study vaccination through approximately 7 days after each study vaccination. Parameters to be evaluated include WBC, Hgb, platelet count, ANC, sodium, potassium, Creatinine, total bilirubin and ALT.

4. Unsolicited Adverse Events –non-serious adverse events occurring from the time of study vaccination through approximately 28 days after the last study vaccination.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event (AE): ICH E6 defines an 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 a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited local (application site) and systemic (subjective and quantitative) reactions, not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data collection form and eCRF.

Information to be collected for unsolicited non-serious AEs includes event description, date of onset, licensed study clinician's assessment of severity and relationship to study product and alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator), date of resolution of the event, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases from the time of study vaccination through 28 days post study vaccination, it will be recorded as an AE.

AEs must be graded for severity and assessed for relationship to study product (see definitions below). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: AEs will be assessed by a licensed study clinician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator using a protocol-defined grading system (see Section 9.2.2). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): Events interrupt the subject's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Relationship to Study Product: The licensed study clinician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2 Reactogenicity

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited local (application site) and systemic (subjective and quantitative) AEs:

Table 3: Local (Injection Site) Reactogenicity Grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site (spontaneous discomfort)	Subject is aware of pain, but it does not interfere with daily activity, **and** no pain medication is taken	Subject is aware of pain; there is interference with daily activity **or** it requires repeated use of a non-narcotic pain reliever for >24 hours	Subject is aware of pain, **and** it prevents daily activity or requires any use of a prescription medication
Tenderness – hurts only when injection site is touched or the arm is moved	The area immediately surrounding the injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it prevents daily activity
Erythema (Redness)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Induration (Hardness/Swelling)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Skin Discoloration	Barely perceptible	Clearly discernable difference compared to	Unsightly difference

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
	difference compared to surrounding skin	surrounding skin	when compared to surrounding skin
Ecchymosis (Bruising)*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

Ecchymosis (bruising), erythema (redness) and induration (hardness)/swelling as analyzed by measurement will be graded as follows:

Table 4: Local (Injection Site) Reactogenicity Measurements

Local (Injection Site) Reaction	Small	Medium	Large
Ecchymosis (Bruising)*	<20 mm	20 mm – 50 mm	>50 mm
Erythema (Redness)*	<20 mm	20 mm – 50 mm	>50 mm
Induration (Hardness)/Swelling*	<20 mm	20 mm – 50 mm	>50 mm

* In addition to grading the measured local reaction at the greatest single diameter, they should be evaluated and graded using the functional scale as well as the actual measurement. Size will not be used as halting criteria by itself.

Table 5: Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness (chills/shivering/sweating)	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference in that it prevents daily activity
Malaise (General Unwell Feeling)	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Fatigue (Tiredness)	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Myalgia (Body Aches/Muscular Pain)*	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Headache	noticeable but does not interfere with daily activity	Any use of pain reliever or interferes with daily activity	Prevents daily activity or requires use of a prescription medication
Nausea	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity
Dizziness	noticeable but does not interfere with daily activity	interferes with daily activity	Significant interference, prevents daily activity

* Not at injection site.

Oral temperature[#] will be graded as follows:

Table 6: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral[†]	37.8°C – 38.4°C 100.00°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

[#] Oral temperature assessed on Day 1 prior to study vaccination will be considered as baseline.
* A fever can be considered not related to the study product if an alternative etiology can be documented.
† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

Pulse and blood pressure[#] will be graded as follows:

Table 7: Blood Pressure and Pulse Grading

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia - beats per minute	45-46	40 – 44	<40
Tachycardia - beats per minute	106 – 130	131 – 155	>155
Hypotension (systolic) mmHg	80 – 84	75 – 79	<75
Hypotension (diastolic) mmHg	50 – 54	45 – 49	<45
Hypertension (systolic) mmHg	151 – 155	156 – 160	>160

Hypertension (diastolic) mmHg	96 – 100	101 – 105	>105

[#] Pulse and blood pressure assessed on Day 1 prior to study vaccination will be considered as baseline.

Clinical safety laboratory results[#] will be graded as follows:

Table 8: Clinical Safety Laboratory Adverse Event Grading (Hematology)

Hematology	Clinical Laboratory Reference Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC K/mcL (Decrease, 18 to < 21 years)	4.5 – 13.0	2.5 – 4.4	1.5 – 2.4	<1.5
WBC K/mcL (Decrease, ≥ 21 years)	4.5 – 11.0	2.5 – 4.4	1.5 – 2.4	<1.5
WBC K/mcL (Increase 18 to < 21 years)	4.5 – 13.0	13.1 – 15.0	15.1 – 20.0	>20.0
WBC K/mcL (Increase ≥ 21 years)	4.5 – 11.0	11.1 – 15.0	15.1 – 20.0	>20.0
Hgb g/dL (Decrease) (Female)	11.7 – 15.7	10.1 – 11.6	8.5 – 10	<8.5
Hgb g/dL (Decrease) (Male)	13.3 – 17.7	11.0 – 13.2	9.5 – 10.9	<9.5
Platelet count K/mcL (Decrease)	135 - 466	125 – 134	100 – 124	<100
Platelet count K/mcL (Increase)	135 - 466	467 - 517	518 – 750	>750
Absolute Neutrophil Count, K/mcL* (18 to < 21 years)	1.80 – 8.00	1.5-<1.8	1.0-<1.5	<1.0
Absolute Neutrophil Count, K/mcL* (≥ 21 years)	1.80 – 7.70	1.5-<1.8	1.0-<1.5	<1.0
Absolute Neutrophil Count, K/mcL - Benign Ethnic Neutropenia*	≥ 0.8	0.6 – 0.7	0.4 – 0.5	< 0.4

* ANC for subjects that are of African American and Middle Eastern descent may have values as low as 0.8 K/mcL. Subjects of this descent must have an ANC ≥ 0.8 K/mcL to be eligible to participate in the study if all other study criteria are met.

Table 9: Clinical Safety Laboratory Adverse Event Grading (Chemistry)

Chemistry	Clinical Laboratory Reference Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
ALT unit/L (Increase)	≤49	50-123	124-245	> 245

Total Bilirubin mg/dL (Increase) – when accompanied by any increase in ALT	0.10 – 1.2	1.3 – 1.5	1.6 – 1.8	>1.8
Total Bilirubin mg/dL (Increase) – when ALT is normal	0.10 – 1.2	1.3 – 1.8	1.9 – 2.4	>2.4
Creatinine mg/dL (Increase) (Female)	0.50 – 0.80	0.81 – 1.70	1.71 – 2.00	>2.00
Creatinine mg/dL (Increase) (Male)	0.60 – 1.10	1.11 – 1.70	1.71 – 2.00	>2.00
Sodium, low, mmol/L	136 – 145	130 - 135	123-129	<123
Sodium, high, mmol/L	136 - 145	146 - 150	151-157	>157
Potassium, high, mmol/L	3.5 – 5.1	5.2 - 6.0	6.1-6.5	>6.5
Potassium, low, mmol/L	3.5 – 5.1	3.0 - 3.4	2.5-2.9	<2.5
Blood Urea Nitrogen (BUN) mg/dL	9.00 – 23.00	24 – 26	27 - 31	>31

[#] Clinical laboratory evaluations assessed at the Day 1 visit will be considered as baseline.

9.2.3 Serious Adverse Events

Serious Adverse Event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations 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.

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

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 principal investigator or sub-investigator.
- Recorded on the appropriate SAE form and eCRF.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Reviewed and evaluated by an Independent Safety Monitor (ISM) (as deemed necessary), the SMC (periodic review unless related), DMID, and the IRB.

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site principal investigator or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this trial, regardless of the relationship to study product. AE/SAEs, abnormal laboratory test values, or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately.

For baseline laboratory results that are abnormal according to the local laboratory reference range and fall within Grade 1 toxicity table range, these will not be considered laboratory adverse event (AE) and will thus not be graded. However, if baseline clinical labs fall within Grade 1 range, then a laboratory AE is reported only if the value changes such that it falls into Grade 2 or higher when subsequent safety laboratory testing is done.

9.3 Reporting Procedures

Solicited local and systemic AEs will be documented and reported from the time of study vaccination through 7 days after each study vaccination.

Clinical safety laboratory adverse events will be documented and reported at Day 1, 169 and 7 days after each study vaccination. Additional safety labs will be reported if the subject returns to the clinic for re-evaluation of abnormal laboratory values through 7 days after the last study vaccination.

Unsolicited non-serious AEs will be documented and reported from the time of study vaccination through approximately 28 days after the last study vaccination.

SAEs (including lab values that meet SAE criteria) will be documented and reported from the time of study vaccination through approximately 6 months after the last study vaccination.

At any time after completion of this trial, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.3.1 Serious Adverse Events

Any AE that meets a protocol-defined serious criteria must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into Advantage eClinical[SM]. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM (as deemed necessary) when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group.

The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site principal investigator or appropriate sub-investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and the site principal investigators (i.e., all principal investigators to whom the sponsor is providing drug under its IND(s) or under any principal investigator's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via Advantage eClinical^{SM} on the Pregnancy Report form. With the subject's permission, all protocol-required venous blood samples will be obtained and the subject will continue to be followed for safety for the duration of this trial. Efforts will be made to follow all pregnancies reported during the course of this trial to pregnancy outcome pending the subject's permission.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be collected, assessed, and followed through resolution from the time of study vaccination through approximately 28 days after the last study vaccination.

SAEs will be collected, assessed, and followed from the time of study vaccination through resolution even if this extends beyond the trial-reporting period (approximately 6 months after the last study vaccination).

Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

If the site principal investigator or appropriate sub-investigator becomes aware of an acute febrile illness and the site principal investigator or appropriate sub-investigator decides to bring the subject in for an evaluation to determine etiology, then the site principal investigator or appropriate sub-investigator, at their own discretion, can determine if specific viral testing should be done by PCR to determine if the infectious agent was RSV.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5 Halting Rules

9.5.1 Sentinel Subject Halting Rules

If any of these conditions are met, further vaccinations will be halted pending SMC electronic review.

9.5.1.1 If none of the following conditions are met, may proceed to the second sentinel subject:

- Any SAE regardless of the relationship to the study product
- Any laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- One Grade 3 unsolicited adverse event that is considered related to study product.

9.5.1.2 If none of the following conditions are met, may proceed to the remainder of the cohort:

- Any SAE regardless of the relationship to the study product
- Any laryngospasm, bronchospasm, or anaphylaxis within 1 day after administration of study product that is considered related to study product.
- Any generalized urticaria after administration of study product that is considered related to study product.

- One Grade 3 unsolicited adverse event that is considered related to study product and not resolved or improved to lower grade within 2 days.
- Same Grade 2 or higher solicited local adverse event in both sentinel subjects that is considered related to study product and not resolved or improved to lower grade within 2 days.
- Same Grade 2 or higher solicited systemic adverse event in both sentinel subjects that is considered related to study product and not resolved or improved to lower grade within 2 days.
- Same Grade 2 or higher laboratory adverse event in both sentinel subjects that is considered related to study product.

9.5.2 Study Halting Rules

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 two body parts) after administration of study product that is considered related to study product.
- Two or more subjects experience a grade 3 unsolicited AE (in the same MedDRA High Level Term) after administration of study product that is considered related to study product and not resolved or improved to lower grade within 2 days.

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 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 9.2.2

Grading scales for clinical safety laboratory adverse events are included in Section 9.2.2.

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 to proceed.

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

9.6 Safety Oversight (ISM plus SMC)

9.6.1 Independent Safety Monitor (ISM)

An ISM is a physician with relevant expertise whose primary responsibility is to provide to DMID an independent safety assessment in a timely fashion. Participation is for the duration of the DMID study and is a voluntary position that does not receive payment. The ISM must meet the requirements of the NIAID conflict of interest policy.

The ISM:

- Is in close proximity to the study site and has the authority and ability to readily access study participant records in real time.
- May be a member of the participating institution's staff but preferably be from a different organizational group within the institution.
- Should not be in a direct supervisory relationship with the investigator.
- Should have no direct involvement in the conduct of the study.

The ISM will:

- Sign a Conflict of Interest (COI) certification at the time they are asked to participate and provide updates to this information as needed.
- Receive reports of Serious Adverse Events (SAEs) from the site investigator and will be notified by email when DMID is notified of the SAE.

- Evaluate the SAE and report their clinical assessment to DMID, through DMID-CROMS SOCS in a timely manner using the attached report form and email the report to DMID-CROMS SOCS.
- Communicate with the investigator at the site as needed.
- Review additional safety related events at the request of DMID.
- Provide additional information to DMID and/or the SMC by teleconference as requested.

9.6.2 Safety Monitoring Committee (SMC)

This clinical study will utilize an SMC, which is an independent group of experts that advises DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to the DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in a SMC charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study.

The SMC will review the safety data at the following milestones:

- Organizational meeting (prior to start of the study)
- Data Review meeting (DRM) will be held if any sentinel halting rules are met for Cohort 1 and 2 sentinel subjects. The SMC will conduct an electronic review of available safety data for the first 2 sentinel subjects in Cohorts 1 and 2 (2 mg dose group) that have completed 7 days of the safety assessment after receiving study product. No new subjects may be enrolled during this follow-up and review period, but screening may continue.
- DRM: The SMC will conduct a review of available safety data for the first 24 subjects in Cohort 1 and 2 (2 mg dose group) that have completed 7 days of the safety assessment after receiving the second dose of study product. No new subjects may be enrolled during this follow-up and review period, but screening for Cohort 3 and 4 and vaccination of the remaining doses in Cohorts 1 and 2 may continue.
- DRM will be held if any sentinel halting rules are met for the Cohort 3 and 4 sentinel subjects. The SMC will conduct an electronic review of available safety data for the first 2 sentinel subjects in Cohort 3 and 4 (4 mg dose group) that have completed 7 days of the safety assessment after receiving study product. No new subjects may be enrolled during this follow-up and review period, but screening may continue.

- An 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.
- Final review meeting: approximately 6 to 8 months after final clinical database lock to review the cumulative safety and immunogenicity data for this trial. The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID.

The SMC will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the SMC. At this time, each data element that the SMC needs to assess will be clearly defined. Procedures for SMC reviews/meetings will be defined in the charter. The SMC will review applicable data to include, but not limited to, study progress and participant clinical, safety, and reactogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, and solicited and unsolicited AE/SAEs. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC may receive data in aggregate and presented by cohort. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request the treatment assignment be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this trial as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

DMID or the SMC chair may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of this trial. The DMID Medical Monitor is empowered to stop enrollment and study vaccinations if adverse events that meet the halting criteria are reported. The DMID Medical Monitor and the ISM (as deemed necessary) will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during this trial.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor SOPs. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the VTEU site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

This is a phase I, randomized, placebo controlled, double-blind, dose escalation trial and is not designed to test a specific hypothesis. Rather, it is intended to obtain preliminary estimates in healthy adults of the safety, reactogenicity, and immunogenicity of the ANDV vaccine in healthy adults.

11.2 Sample Size Considerations

Rare adverse events are not demonstrable in a clinical study of this size; however, the probabilities of observing one or more AEs given various true event rates are presented in Table 10.

Table 10: Probability of Observing an Adverse Event for Various Event Rates

N	"True" Event Rate	Probability of Observation (%)	N	"True" Event Rate	Probability of Observation (%)
10	0.1%	1.0	20	0.1%	2.0
	0.5%	4.9		0.5%	9.5
	1.0%	9.6		1.0%	18.2
	2.0%	18.3		2.0%	33.2
	3.0%	26.3		3.0%	45.6
	4.0%	33.5		4.0%	55.8
	5.0%	40.1		5.0%	64.2
	10.0%	65.1		10.0%	87.8
	20.0%	89.3		20.0%	98.8

11.3 Planned Interim Analyses (if applicable)

11.3.1 Interim Safety Review

After the completion of enrolling 24 subjects in Cohorts 1 and 2 (2 mg dose group), the SMC will review the data for Cohorts 1 and 2 through 7 days post the second vaccination.
These reviews, however, may not involve any hypothesis testing and will not be considered in estimating the precision of any estimates made at the conclusion of the study.

11.3.2 Interim Analysis of Safety and Immunogenicity Data

An interim analysis of safety, reactogenicity, and immunologic response data (excluding exploratory outcomes) is planned once all subjects (Cohort 1-4) have completed the Day 197 visit and the data are entered in the database, validated and monitored according to the clinical monitoring plan. The study statistician will provide the analysis of aggregate data unblinded at the group level to the investigators and sponsor staff for the purpose of manuscript, abstract preparation or presentation. The information from the primary immunogenicity and safety review may be published or otherwise presented, pursuant upon executed agreements between NIAID and VTEU investigators. The laboratory staff that run the assays for the Day 253 and 337 visits will remain blinded, and the assessment of relationship to study product of any SAEs that may be reported at the Day 253 and 337 visits will be delegated to blinded sub-investigators at the clinical site. While the results will not be used to make any decisions concerning the conduct of this trial, they may be used to make decisions on activities external to this trial such as the design of future trials of this vaccine. Since this early analysis of the data is not intended to impact the conduct of the trial, it has no impact on Type I error and adjustments are not planned.

11.4 Final Analysis Plan

The final analysis will be performed and clinical study report completed when all primary safety endpoint data and all secondary immunogenicity endpoint data are available. The interim analysis based on the primary clinical data base will be included in the final clinical study report (CSR). The CSR will be completed after the final data lock (through approximately Day 337 follow-up) and when all primary endpoint data are received by SDCC. Any available data from the exploratory immunogenicity endpoints may also be included or if agreed upon, in an addendum to the CSR. A formal statistical analysis plan will be developed by SDCC and finalized prior to the primary data lock.

11.4.1 Analysis Populations

The Safety Analysis population includes all subjects who received one dose of study vaccine.

The intent-to-treat (ITT) population includes all subjects who received one dose of study vaccine and contributed both pre- and at least one post-study vaccination venous blood samples for immunogenicity testing for which valid results were reported. For analyses using the ITT population, subjects will be grouped based on randomized treatment arm.

In the final analysis, if there are major protocol deviations, a per-protocol (PP) analysis may be performed. The per protocol (PP) population includes all subjects in the ITT subset with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline.
- Data from all visits subsequent to major protocol deviations, such as:
  - Receipt of non-study licensed live vaccine within 28 days before or after study vaccination,
  - Receipt of non-study licensed inactivated vaccine within 14 days before or after study vaccination,*
    **Allowable exception for inactivated seasonal influenza vaccine received more than 7 days prior to or after a study vaccination.*
  - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days before or after study vaccination.
- Data from any visit that occurs substantially out of window.

For analyses using the PP population, subjects will be grouped based on study vaccinations received.

11.4.2 Safety Data

Summaries and analysis of safety data will be presented for the Safety Analysis Population.

Solicited AEs will be summarized by severity for each day after vaccination (Days 1-7 post study vaccination) and as the maximum severity over all 7 days. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals (CI), to summarize the proportion of subjects reporting each symptom, any application site symptom, and any systemic symptom.

Unsolicited non-serious AEs will be collected from the time of first study vaccination through approximately 28 days post last vaccination**.** Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA®) for preferred term and system organ class (SOC). SAEs will be collected from the time of first study vaccination through approximately 6 months post last vaccination**.** The numbers of SAEs are likely to be small in this trial and will be reported by detailed listings showing the event description, MedDRA®preferred term and SOC, relevant dates (study vaccinations and AEs), severity, relatedness, and outcome for each event.

Non-serious unsolicited AEs will be summarized as number and percentage of subjects reporting at least one event in each MedDRA®preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the proportion of subjects and exact 95% confidence intervals of AEs in aggregate and by MedDRA®categories will be computed.

Clinical laboratory data will be summarized by severity for each visit, and as the maximum over all post-study vaccination visits. Graphical presentations may include box plots and shift plots.

11.4.3 Immunogenicity Data

11.4.3.1 Cellular Assays (ICS, LPA)

Summaries and analysis of cellular assay data will be presented for the ITT population. If there are major protocol deviations, a per-protocol (PP) analysis may also be performed.

Immune responses in terms of incidence of a cellular immune response to ANDV peptide pool >3 standard deviations (SD) above that on Day 1 will be summarized. Cellular responses will be measured by mean intracellular cytokine-secreting CD4+ and CD8+ T Cell responses and mean CD4+ and CD8+ T Cell lymphoproliferation. Analyses will include response (>3 SD) for the different groups at the different time points.

11.4.3.2 Immunogenicity (PsVNA, PRNT)

Summaries and analysis of immunogenicity data will be presented for the ITT population. If there are major protocol deviations, a per-protocol (PP) analysis may also be performed.

Immune responses in terms of a titer $\geq$ 20 in antibody post vaccination, measured by PsVNA and PRNT will be summarized. Analyses will include incidence of seroconversion at different time points and geometric mean titers for the different groups at the different time points will be determined and compared. The correlation between PsVNA and PRNT titers will be evaluated.

11.4.4 Missing Values and Outliers

All attempts will be made to collect all data per protocol. As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The study site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. The study site will permit authorized representatives of DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical trial records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

Interview of subjects is sufficient for obtaining medical history. Solicitation of medical records from the subject's primary care provider is not required unless deemed needed by the Investigator. Data collection forms for use as source documents will be derived from the CRFs and be provided by the SDCC. Supplemental site-specific source documents may be included as appropriate.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. Each site principal investigator will provide direct access to the site's study-related source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. Each site principal investigator will ensure all study personnel are appropriately trained and current applicable documentations are maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site principal investigator will ensure that this trial is conducted in full conformity with 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 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The site principal investigator's institution will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

Prior to enrollment of subjects into this trial, the protocol and ICF will be reviewed and approved by the appropriate IRB listed on its FWA.

The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this trial and a copy will be provided to DMID. The IRB FWA number will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the principal investigator and provided to the site IRB. Any amendments to the protocol or consent materials will be approved by the IRB before they are implemented.

14.3 Informed Consent Process

The site principal investigator will choose subjects in accordance with the eligibility criteria detailed in Section 5. Before any study procedures are performed, subjects must sign an ICF that complies with the requirements of ICH E6, 21 CFR Part 50 and 45 CFR 46 and the local IRB. Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site clinical staff may pre-screen via chart review and refer potential subjects to the research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, including pre-screening of subjects for eligibility, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products. This will include the nature, risks and possible benefits of this trial, alternate therapies, any known AEs, the investigational status of the study interventions/products, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum samples. Subjects will be allowed sufficient time to consider participation in this research trial, after having the nature, risks and possible benefits of this trial explained to them, and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

ICFs describing in detail the study interventions/products, study procedures, risks and possible benefits will be given to subjects. The ICF must not include any exculpatory statements. ICFs will be IRB-approved and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain this research trial to subjects and answer any questions that may arise. Subjects must sign the ICF, and written documentation of the informed consent process is required prior to starting any study procedures being done specifically for this trial, including determining eligibility and administering study product.

By signing the ICF, subjects agree to complete all study procedures required by this trial, unless the subject withdraws voluntarily, or is withdrawn or terminated from this trial for any reason. The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

DMID will provide the site principal investigator, in writing, any new information that significantly impacts the subject's risk of receiving the investigational products. This new information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all ICFs that they sign.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all adults who meet the Subject Inclusion Criteria (see Section 5.1) and do not meet the Subject Exclusion Criteria (see Section 5.2), regardless of religion, sex, or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated including those in other populations. Children will not be included in this trial as they are not the target population at this time.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the site principal investigators, other study personnel, the sponsor, and their agents. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects. Subjects will have code numbers and will not be identified by name.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning this trial or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All information provided by the sponsor and all data and information generated by the VTEU site as part of this trial (other than a subject's medical records) will be kept confidential by the site principal investigators and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigators or other study personnel for any purpose other than conducting this trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigators or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of this trial (3) information which is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published as described in Section 16.

In addition, 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.

The study monitor, applicable regulatory authorities, such as the FDA, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the site principal investigators. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. The VTEU site will permit access to such records.

14.6 Study Discontinuation

If this trial is discontinued, subjects, who have signed the ICF and are randomized and vaccinated, will continue to be followed for safety for the duration of this trial. No further study vaccinations will be administered.

14.7 Future Use of Stored Specimens and Data

Residual samples are those that are left over after the study has been completed and are stored for possible use in future research studies. Future use samples are extra tube/s of blood collected and stored for possible use in future research studies. Retention of residual samples for future use and the collection and storage of samples for use in future research studies is a condition of study participation. Subjects who sign the informed consent form for the study are consenting to allow the collection, storage and use of both residual and future use samples.

Residual samples (serum or cells) derived from venous blood samples will be maintained for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. These samples may be shared for purposes other than per protocol analysis with investigators at the participating VTEU site and with other investigators at other institutions once the clinical study report has been finalized.

Future use samples (whole blood, serum or cells) will be collected at Day 85, 197 and 337. It is anticipated that approximately two to five aliquots of serum (4 ml/each) from venous blood samples will be available specifically for the purpose of future research at Day 85, 197 and 337. PBMCs collected at day 337 will also be processed for future use. Future use research studies may include but are not limited to non-traditional immune assay development, assessing innate immune factors, cytokines and other virologic evaluations and Human Leukocyte Antigen (HLA) testing to explore the impact of host genetics on immune responses to ANDV DNA vaccine. If HLA testing is performed, results 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.

Residual and future use research samples will be stored indefinitely at a DMID designated central clinical storage facility. These samples will not be sold or used directly for production of any commercial product. Genetic tests may be performed on samples. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality. There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will NOT be kept in their health records.

Residual samples will be available upon the completion of the study; however, future use samples may be requested from DMID and shipped from the DMID CMS at any time.

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.

15 DATA HANDLING AND RECORD KEEPING

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRF and provided by the SDCC to record and maintain data for each subject enrolled in this trial. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF derived from the data collection forms should be consistent with the data collection forms or the discrepancies should be explained.

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events must be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the site principal investigator or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at the VTEU site under the supervision of the respective site principal investigator. During this trial, the site principal investigator must maintain complete and accurate documentation for this trial.

The SDCC for this trial will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), reactogenicity, and immunogenicity data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic

range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical and reactogenicity data will be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, and immunogenicity data).

15.4 Timing/Reports

See Section 9.3 for further reporting details.

Interim statistical reports may be generated as outlined in the protocol or as deemed necessary and appropriate by DMID. Upon request and DMID approval, the SDCC may provide the study team with interim summary results for purposes of designing future trials or for publication/presentation of the data. Safety and immunogenicity summary reports may be generated for the SMC.

After full analysis and final reporting is complete, and upon request and DMID approval, the SDCC will provide the VTEU site with a summary of results by treatment arm and/or subject treatment assignments. In this regard, the VTEU site requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, and study drug disposition records shall be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the drug, until 2 years after the investigation is discontinued and the FDA has been notified. ICFs for future use will be maintained as long as the sample exists.

The VTEU site must contact DMID for authorization prior to the destruction of any study records.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the study protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or other study personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the site principal investigator and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the SDCC's Advantage eClinical^{SM}.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File as well as in the subject's chart. Protocol deviations must be sent to the local IRB per its guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

NIH Public Access Policy, http://publicaccess.nih.gov/

NIH OER Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

Public Law 110-85, Section 801, Clinical Trial Databases

42CFR11

NIH NOT-OD-16-149

Results of any exploratory immunogenicity analysis will not be published prior to publication of the primary immunogenicity results for this trial.

*Journal Citation: De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004; 351:1250-1.

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18 SUPPLEMENTS/APPENDICES

Appendix A: Schedule of Events

All Treatment Arms																
Study Visit (V)	**00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**U/S[#]**	**ET[#]**
Study Day from Vaccine 1	**Day ≤ -28 Screen**	**Day 1 Vaccine 1**	**Day 8 +2**	**Day 29 +2**	**Day 36**	**Day 57**	**Day 64**	**Day 85**	**Day 141**	**Day 169**	**Day 176**	**Day 197**	**Day 253**	**Day 337**	-	-
Weeks from Vaccine 1		**0**	**1**	**4**	**5**	**8**	**9**	**12**	**20**	**24**	**25**	**28**	**36**	**48**	-	-
Study Day from Vaccine 2				**Day 1 Vaccine 2**	**Day 8 +2**	**Day 29 +2**	**Day 36**	**Day 57**	**Day 113**	**Day 141**	**Day 148**	**Day 169**	**Day 225**	**Day 309**	-	-
Study Day from Vaccine 3						**Day 1 Vaccine 3**	**Day 8 +2**	**Day 29 +2**	**Day 85 +/-5**	**Day 113+5**	**Day 120**	**Day 141**	**Day 197**	**Day 281**	-	-
Study Day from Vaccine 4										**Day 1 Vaccine 4**	**Day 8 +2**	**Day 29 +2**	**Day 85 +/-5**	**Day 169 +/-7**	-	-
Visit Type	**Screen**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**	**Clinic**
Obtain Informed Consent~∞	X															
Collect Demographic Information	X															
Height & Weight	X															
Physical Examination - Full	X															
Urine Dipstick, Opioid testing	X															
Screening Labs~	20[= & 2]															
Enrollment/Randomization		X														
Review Eligibility Criteria	X	X[†¬1]		X[†¬]		X[†¬]				X[†¬]						
Medical History@	X	X[†¬1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications [4]	X[√]	X[†¬]	X	X	X	X	X	X	X	X	X	X			X	X
Vital Signs (oral temp, pulse, BP)[%]	X	X[†$]		X[†]		X[†]				X[†]					X	X
Physical Examination – Targeted[0]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test^	X	X[†]		X[†]		X[†]				X[†]						
Clinical Safety Evaluations*		6.5	6.5		6.5		6.5			6.5	6.5				6.5	6.5

Study Visit (V)	00	01	02	03	04	05	06	07	08	09	10	11	12	13	U/S#	ET#
Study Day from Vaccine 1	Day ≤ -28 Screen	Day 1 Vaccine 1	Day 8 +2	Day 29 +2	Day 36	Day 57	Day 64	Day 85	Day 141	Day 169	Day 176	Day 197	Day 253	Day 337	-	-
Weeks from Vaccine 1		0	1	4	5	8	9	12	20	24	25	28	36	48	-	-
Study Day from Vaccine 2				Day 1 Vaccine 2	Day 8 +2	Day 29 +2	Day 36	Day 57	Day 113	Day 141	Day 148	Day 169	Day 225	Day 309	-	-
Study Day from Vaccine 3						Day 1 Vaccine 3	Day 8 +2	Day 29 +2	Day 85 +/-5	Day 113+5	Day 120	Day 141	Day 197	Day 281	-	-
Study Day from Vaccine 4										Day 1 Vaccine 4	Day 8 +2	Day 29 +2	Day 85 +/-5	Day 169	-	-
Visit Type	Screen	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic
PBMCs (ICS, LPA)		40		40		40		40		40		40			40	40
Immunogenicity ANDV PRNT		10				10		10		10		10				
Immunogenicity ANDV PsVNA		10		10		10		10		10		10	10	10	10	10
Future Exploratory Assays								40				40		40		
Pre-administration reactogenicity assessment		X		X		X				X						
Study Vaccination[3]		X		X		X				X						
30-minute evaluation post vax>		X		X		X				X						
Distribute Memory Aid/Materials		X		X		X				X						
Review Memory Aid/Site assessment			X		X		X				X				X	X
Assessment of Adverse Events		X	X	X	X	X	X	X	X	X	X	X			X	X
Assessment of SAEs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

∞ Prior to study procedures.
† Prior to study vaccination.
¬ Review/confirm information or activity in subjects previously consented and screened.
[1] Review results of clinical screening laboratory evaluations.
@ Complete medical history will be obtained by interview of subjects at the screening visit and will be updated on Day 1 prior to the first study vaccination and interim medical history will be obtained by interview of subjects at follow-up visits after the first study vaccination.
% Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
$ Vital signs assessed on Day 1 prior to the first study vaccination will be considered as baseline.
√ All current medications and medications taken within 60 days prior to signing the ICF. Concomitant medications taken within 30 days of enrollment through Day 28 post last vaccination are collected in Advantage eClinical[SM].
> Observation period will begin after the study vaccination has been given.
^ Serum pregnancy test will be performed on all females of childbearing potential at screening. A urine pregnancy test will be performed within 24 hours prior to study vaccination and results must be negative and known prior to each study vaccination.

~ Screening laboratories include: WBC, Hgb, platelet count, ANC, sodium, potassium, Creatinine (Blood Urea Nitrogen [BUN] will be obtained only if creatinine is above the normal range), HgbA1C, total bilirubin, Alanine aminotransferase (ALT), hepatitis B surface antigen, hepatitis C antibody, HIV types 1 and 2

= All clinical screening laboratory evaluations are to be performed at screening and the values are to be reviewed prior to the first study vaccination on Day 1; no additional blood draws for this are required on Day 1.

& Retesting of values that lead to exclusion is allowed once using an unscheduled visit during the screening period, provided there is an alternative explanation for the out of range value.

2 If the initial laboratory screening occurred more than 28 days before baseline (Day 1) but the subject was unable to be vaccinated within the 28-day window (e.g., due to meeting Exclusion Criteria or for other reasons), the subject must have laboratories repeated.

* Safety laboratories include: WBC, Hgb, platelet count, ANC, sodium, potassium, Creatinine, total bilirubin, Alanine aminotransferase (ALT).

Refer to protocol for specific procedures and time points for ET or U/S visit.

3 The 2mg dosage cohort will receive 1mg/0.5ml administered into the left and right deltoid muscles, one after another or simultaneously. The 4mg dosage cohort will receive 2mg/0.5ml administered into the left and right deltoid muscles, one after another or simultaneously.

() A targeted physical examination may be performed, if indicated based on review of medical history and any updates obtained by interview of subjects.

4 I**ncluding solicitation for receipt of any non-study vaccines**

Appendix B: Acceptable Ranges of Screening Laboratory Measurements

Lab Test Name	Clinical Laboratory Reference Range	Study Eligibility Acceptable Lower Limit	Study Eligibility Acceptable Upper Limit	Lab Unit
Hemoglobin, male	13.30 - 17.70	13.30	17.70	g/dL
Hemoglobin, female	11.70 - 15.70	11.70	15.70	g/dL
HgbA1C	na	na	<6.3	%
White blood cell count (WBC) (18 to <21 years)^	4.5 – 13.0	3.6	13.0	K/mcL
White blood cell count(WBC) (≥21 years)^	4.5 – 11.0	3.6	11.0	K/mcL
Absolute Neutrophil count*	1.80 – 7.70	1.80	7.70	K/mcL
Platelet count	135 - 466	135	466	K/mcL
Sodium	136.0 – 145.0	136.0	145.0	mmol/L
Potassium	3.5 – 5.1	3.5	5.1	mmol/L
Blood urea nitrogen (BUN)†	9.0 – 23.00	<9.0	23.00	mg/dL
Serum creatinine†^, male	0.60 – 1.10	<0.60	1.3	mg/dL
Serum creatinine†^, female	0.50 – 0.80	<0.50	1.2	mg/dL
Total bilirubin	0.10 – 1.2	0.10	1.2	mg/dL
Alanine transferase† (ALT)	na	na	≤49	unit/L
Hepatitis B surface antigen	Negative	Negative	Negative	n/a
Hepatitis C antibodies	Negative	Negative	Negative	n/a
HIV - 1/2 Ab	Negative	Negative	Negative	n/a
Serum hCG (females only)	Negative	Negative	Negative	n/a

* ANC for subjects that are of African American and Middle Eastern descent may have values as low as 0.8 K/mcL. Subjects of this descent must have an ANC ≥ 0.8 K/mcL to be eligible to participate in the study if all other study criteria are met.

† Values of serum creatinine and blood urea nitrogen (BUN) below the lower limit of normal (LLN) are acceptable for study enrollment.