Phase I, Randomized, Double-blinded, Placebo-Controlled Dose De-escalation Study to Evaluate the Safety and Immunogenicity of Alum Adjuvanted Zika Virus Purified Inactivated Vaccine (ZPIV) Administered by the Intramuscular Route in Adult Subjects who Reside in a Flavivirus Endemic Area

DMID Protocol Number: 16-0034

DMID Funding Mechanism: Vaccine and Treatment Evaluation Units (VTEU)

Pharmaceutical Support Provided by: Walter Reed Army Institute of Research (WRAIR)

IND Sponsor: Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

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Draft or Version Number: Version 7.0

19 SEPTEMBER 2022

STATEMENT OF COMPLIANCE

The study 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 Harmonization (ICH) E6; 62 Federal Register 25691 (1997);
- National Institutes of Health (NIH) Clinical Terms of Award.

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 study) 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.

Study Investigators:

Signed: Date:

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LIST OF ABBREVIATIONS

AFI	Acute Febrile Illness
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Aminotransferase
ARI	Acute Rash Illness
AST	Aspartate Aminotransferase
BMI	Body Mass Index
C	Centigrade
CAIMED	Centro Ambulatorio de Investigaciones Medicas
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHIKV	Chikungunya virus
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
DCC	Data Coordinating Center
DENV	Dengue Virus
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DRM	Data Review Meeting
DoD	Department of Defense
F	Fahrenheit
FDA	Food and Drug Administration
FWA	Federal Wide Assurance

GCP	Good Clinical Practice
GMT	Geometric Mean Titer
HCT	Hematocrit
HD	High Dose
HGB	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention to Treat
JAMA	Journal of the American Medical Association
JEV	Japanese Encephalitis Virus
LD	Low Dose
MedDRA ®	Medical Dictionary for Regulatory Activities
MD	Medium Dose
Mcg	Microgram
Mg	Milligram
mL	Milliliter
mmHg	Millimeters of Mercury
MOP	Manual of Procedure

N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PBMCs	Peripheral Blood Mononuclear Cells
PFU	Plaque Forming Units
PHI	Protected Health Information
PI	Principal Investigator
PP	Per Protocol
PREP	Public Readiness and Emergency Preparedness
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOC	System Organ Class
SOP	Standard Operating Procedure
TBEV	Tick-borne Encephalitis Virus
US	United States
VTEU	Vaccine and Treatment Evaluation Unit
WBC	White Blood Cells
WHO	World Health Organization

WNV	West Nile Virus
YFV	Yellow Fever Virus
ZIKV	Zika Virus
ZPIV	Zika Virus Purified Inactivated Vaccine

PROTOCOL SUMMARY

Title:	Phase I, Randomized, Double-blinded, Placebo-Controlled Dose De-escalation Study to Evaluate the Safety and Immunogenicity of Alum Adjuvanted Zika Virus Purified Inactivated Vaccine (ZPIV) Administered by the Intramuscular Route in Adult Subjects Who Reside in a Flavivirus Endemic Area
Phase:	1
Population:	This study will enroll 90 healthy male and non-pregnant female subjects between the ages of 21 and 49 inclusive. The study will be conducted at Ponce Medical School Foundation, Inc.-CAIMED in Ponce, Puerto Rico.
Number of Sites:	1 VTEU subcontract site
Study Duration:	This study is expected to take approximately 49 months to complete from initiation through availability of a final report on the primary and secondary outcomes of safety and humoral immune responses to ZPIV after a two-dose, homologous regimen.
Subject Participation Duration:	The entire duration for each individual subject's participation is approximately 26 months from recruitment through the last study visit.
Description of Agent or Intervention:	ZPIV is an aluminum hydroxide adjuvanted, purified inactivated vaccine using a 2015 Puerto Rico isolate (PRVABC59 strain). ZPIV will be administered in a homologous prime-boost regimen on Day 1 and Day 29 at two doses: 5 mcg or 2.5mcg. The placebo will be normal saline (0.9% Sodium Chloride, USP).

Objectives:

Primary

1. Assess the safety and reactogenicity of a homologous prime boost regimen of ZPIV given at two different dose levels in a dose de-escalation format in healthy adult subjects who live in Puerto Rico, a flavivirus endemic area.
2. Compare the safety and reactogenicity profile of ZPIV after each vaccination, between dosage groups, and by pre-vaccination flavivirus immune status.

Secondary

1. Assess the humoral immune response to a homologous prime-boost regimen of ZPIV after each dose of vaccine as determined by kinetics of the immune responses, seroconversion rates, and Geometric Mean Titers (GMT) overall, and compare results between dosage groups and by pre-vaccination flavivirus immune status.
2. Assess the durability of the humoral immune response to ZPIV after the second vaccine administration overall, and compare results between dosage groups and by pre-vaccination flavivirus immune status.

Exploratory

1. Estimate the incidence of ZIKV and DENV infections between Visit 00 and the subject's last visit and describe the clinical presentation including disease severity overall, by dosage group, product received, and pre-vaccination flavivirus immune status.

Outcome Measures

Primary Endpoints

<u>Safety and Reactogenicity:</u>

1. Frequency and severity of solicited injection site and systemic reactogenicity from time of study vaccine administration through Day 8 after each administration of study vaccine overall and by dosage group and pre-vaccination flavivirus immune status.
2. Frequency and severity of unsolicited vaccine-related adverse events (AE), including vaccine-related laboratory AE, from first administration of study vaccine until 28 days after the last

vaccination overall and by dosage group and pre-vaccination flavivirus immune status.

3. Frequency, type, and duration of serious adverse events (SAE) and adverse events of special interest (AESI) considered related to study vaccine from the first administration of study vaccine until the end of the study overall, and by dosage group and pre-vaccination flavivirus immune status.
4. Frequency of new onset chronic medical conditions reported at any time from the first administration of study vaccine until the end of the study overall, and by dosage group and pre-vaccination flavivirus immune status.
5. Comparison of the frequency, type, and duration of vaccine-related Grade 3 local, systemic, or laboratory AE, and Grade 2 or greater local or systemic reactogenicity through Day 8 after each study vaccine administration between dosage groups and by pre-vaccination flavivirus immune status.
6. Comparison of study withdrawals and discontinuation of study vaccination due to any reason between dosage groups and by pre-vaccination flavivirus immune status.

Secondary Endpoints

Immunogenicity:

1. Frequency of seroconversion to ZIKV measured by ZIKV ELISA and neutralization assay in comparison with baseline sample (collected on Visit 00) overall, and by dosage group and pre-vaccination flavivirus immune status.
2. Per visit GMT as measured by ZIKV ELISA and neutralization assay after each study vaccine administration overall and by dosage group and pre-vaccination flavivirus immune status.
3. Peak GMT as measured by ZIKV ELISA and neutralization assay after each vaccination and overall, and by dosage group and pre-vaccination flavivirus immune status.
4. Proportion of subjects with at least a 4-fold rise in ZIKV GMT as measured by ZIKV ELISA and neutralization assay at 4 weeks

after each vaccination compared with baseline overall, and by dosage group and pre-vaccination flavivirus immune status.

Exploratory Endpoints

1. Number and proportion of subjects who develop an acute febrile illness (AFI) or an acute rash illness (ARI) who have evidence of acute ZIKV or DENV infection within 14 days of symptom onset. This endpoint will be analyzed for all subjects overall, and by treatment group and pre-vaccination flavivirus immune status.
2. Number and severity of laboratory confirmed ZIKV and DENV infections during the study, overall, by treatment group, and pre-vaccination immune status, where severity is described by:
   - Duration of symptoms
   - Grade of symptoms (mild, moderate, severe)
   - Use of concomitant medications
   - Need for hospitalization or other medical care.

   Laboratory confirmation will be made by AFI or ARI with either: 1) evidence of acute ZIKV or DENV infection within 14 days of symptom onset or 2) a 4 fold rise in DENV or ZIKV neutralization titers between samples collected at the last visit prior to and at the first visit following the AFI/ARI illness visit, where available.

Description of Study Design:

This study is a single-center, double-blinded, placebo-controlled, Phase 1, dose de-escalation study to evaluate the safety, reactogenicity, and immunogenicity of ZPIV administered in a homologous prime-boost regimen to healthy male and non-pregnant female adult subjects. Two dose levels will be evaluated. Each subject will receive either placebo or 5 mcg (Group 1) or 2.5 mcg (Group 2) of ZPIV administered by intramuscular (IM) injection on Days 1 and 29. The study will consist of a screening period of up to 28 days, a vaccination period in which subjects will receive a prime dose of vaccine on Day 1 followed by a homologous boost on Day 29, and a follow-up period of 24 months post boost vaccination. Blood for evaluation of antibodies to ZIKV by ELISA and neutralizing antibody assays will be collected at Visit 00, prior to each vaccination, and at multiple timepoints afterward (See Section 8 and Table 2).

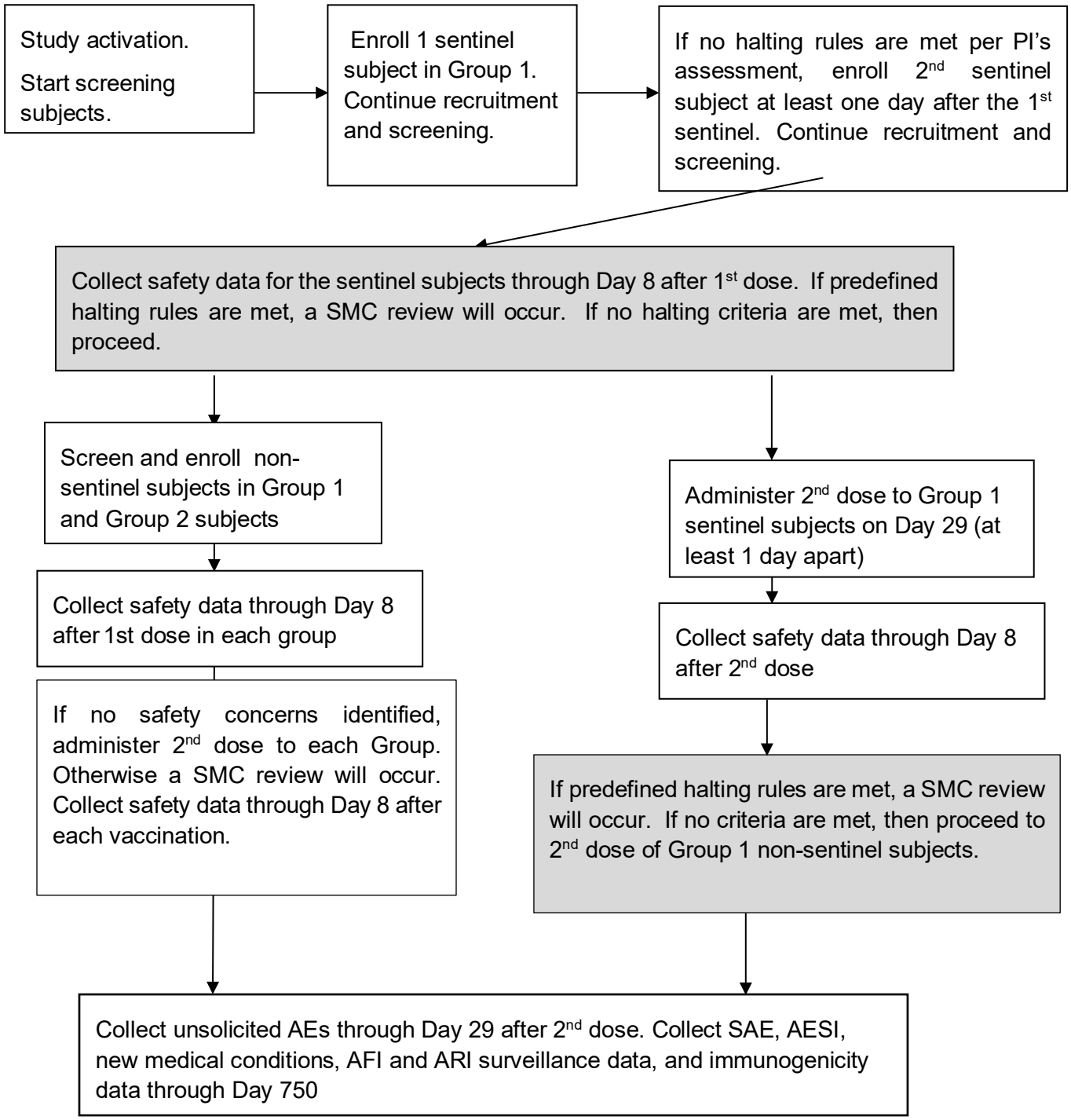
This study will also include enhanced surveillance of acute febrile illness (AFI) and acute rash illness (ARI) from the time Informed Consent is signed until the subject's last study visit. Data will be collected on the incidence and clinical presentation of flavivirus (i.e., ZIKV and DENV) infection and disease. Subjects who develop grade 1 or higher fever for at least 2 consecutive days accompanied by any of the following: rash, arthralgia, or nonpurulent conjunctivitis (AFI) or new rash not limited to the vaccination site (ARI) at any time after signing the ICF will be asked to contact the clinic within 3 days of symptom onset and come in within 14 days for evaluation and blood and urine collection to test for acute ZIKV and DENV infection. Subjects will also be asked about any history of these symptoms at each visit after Visit 00. Follow-up serology will be monitored for a 4-fold rise in neutralization titers to ZIKV and DENV in samples as closely flanking the AFI/ARI illness visit as possible, where available..

Please see Section 4 for a detailed study description.

The entire duration of each subject's participation is approximately 26 months including recruitment and collection of data on the safety and reactogenicity of the study vaccine and samples for the assessment of immunogenicity. This study is expected to take approximately 49 months to complete from initiation through availability of a final report on the primary outcomes of safety and the secondary outcomes of humoral immunity to ZIKV.

Estimated Time to Complete Enrollment: Enrollment is expected to take up to 15 months from study start.

Figure 1: Schematic of Study Design:



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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background Information

Zika virus (ZIKV) disease (also known as Zika) is an emerging mosquito-borne disease caused by an RNA virus from the family *Flaviviridae,* genus *Flavivirus*. As a Flavivirus, ZIKV is related to the four dengue viruses (DENV 1-4), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV), and tick-borne encephalitic virus (TBEV). ZIKV was first isolated in Uganda in 1947 from a sentinel rhesus monkey, and the virus has two known lineages, an Asian and African lineage. The majority of human ZIKV infections result in an asymptomatic infection or a benign, self-limited acute febrile illness. ZIKV infections are characterized primarily by the presence of rash, fever, conjunctivitis, and/or arthralgia, which have been found in the majority of cases seeking care or detected by public health surveillance (Duffy, 2016).

Until recently, there were few reported cases of ZIKV disease in the literature and no major Zika outbreaks were detected until 2007. In 2007, an outbreak of the Asian lineage of ZIKV was detected on the island of Yap, in Federated States of Micronesia. During that outbreak, approximately 75% of the population was infected with ZIKV within 3 years (Duffy, 2016). Subsequently, large outbreaks have occurred in French Polynesia in 2013 and Brazil in 2015. In February, 2016, the World Health Organization (WHO) declared the emerging ZIKV epidemic a “Public Health Emergency of International Concern.” Active mosquito-borne transmission of ZIKV has now been reported in more than 35 countries in South America, Central America, and the Caribbean including Puerto Rico (PAHO 2016).

A disturbing clinical picture has emerged from these recent outbreaks. Nine months after ZIKV transmission was first documented in Brazil, public health officials detected an increase in babies born with microcephaly in northeastern part of the country (WHO 2016). Epidemiologic studies have now found a significant association between ZIKV infections during pregnancy and the occurrence of serious birth defects including microcephaly, brain malformations and ocular defects (Rasmussen, 2016). An increase in Guillain-Barré syndrome (GBS) cases, which coincided temporally and geographically with the occurrence of ZIKV cases, has also been detected in people with primary ZIKV infections (Cao-Lormeau, 2016). As the recent outbreak has spread, cases of meningitis and encephalitis in patients with laboratory-confirmed ZIKV have been reported (Carteaux, 2016), (Broutet, 2016). Although causality has not been firmly established, emerging clinical and non-clinical data suggest ZIKV as a cause of neurologic disease both in the developing fetus and patients with ZIKV.

The intrinsic incubation period for ZIKV is believed to be similar to that of dengue with acute signs and symptoms typically occurring 3-10 days post infection. Following infection, ZIKV has been detected in blood, urine, semen, cerebral spinal fluid, saliva, amniotic fluid, and breast milk

(Calvetg, 2016, Atkinson, 2016, Dupont-Rouzeyrol, 2016). In most ZIKV infected individuals, the virus is detected in the blood from a few days to one week post onset of symptoms; however, recent reports suggest that the viremia may be present significantly longer in pregnant women (Driggers, 2016). Virus has also been found to persist longer in urine and semen, and sexual transmission of ZIKV has been recently documented (D'Ortenzio, 2016). ZIKV has recently been implicated in fatalities (Arzuza-Ortega, 2016).

2.1.1 ZPIV

ZPIV is purified inactivated vaccine using a 2015 isolate from Puerto Rico (PRVABC59 strain). The vaccine was produced in VERO cells and manufactured via a process that has been used successfully for several other flavivirus vaccines (e.g. Japanese encephalitis virus vaccine (JEV).

The inactivated vaccine induced neutralizing antibody responses when administered to mice and non-human primates. Mice challenged after one immunization delivered by the intramuscular route were protected against challenge by the homologous strain of virus as evidenced by no detection of viremia. Non-human primates (NHP) immunized with ZPIV 5mcg/dose with alum were similarly protected against viremia when challenged 28 days post second vaccination with this vaccine (Abbink 2016). The protection against viremia was observed whether the challenge was with the homologous strain (PRVABC59) or a heterologous strain from Brazil. In the NHP immunogenicity and challenge study, the animals were observed clinically over 8 weeks post immunization for changes in behavior, feeding patterns, local and systemic reactogenicity and body weight. Basic hematologic (white blood cell counts, hematocrits) and clinical chemistry parameters (electrolytes, renal function, hepatic function, etc) were also measured for up to 6 weeks post immunization. No significant changes were observed in any parameters measured (Abbink 2016).

While there is little human experience with the ZIKV vaccine, two other Vero cell-derived, purified inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent dengue virus (serotype 1) vaccine (manufactured by Walter Reed Army Institute of Research [WRAIR]) and a Japanese encephalitis virus vaccine (manufactured as IXIARO). Studies with both vaccines indicate that purified inactivated flavivirus vaccines are reasonably well tolerated (Martinez, 2015).

2.1.2 Public Readiness and Emergency Preparedness

This protocol, the ZPIV vaccine manufactured by WRAIR, are covered under the Public Readiness and Emergency Preparedness Act (PREP Act). Under the PREP Act, covered persons are immune from liability actions brought from the administration or use of a covered countermeasure that is the subject of a declaration. The PREP Act provides immunity for covered persons (such as manufacturers, distributers, program planners and other qualified persons who prescribe, administer or dispense the study vaccine) from tort liability, unless the injury was caused by willful misconduct.

The PREP Act also authorized a "Covered Countermeasures Process Fund" to provide compensation to eligible individuals who suffer specified injuries from administration or use of a countermeasure pursuant to the declaration. Any requests for compensation must be filed within one year of administration or use of the countermeasure. Requests would go to the HRSA Preparedness Countermeasures Injury Compensation Program (http://www.hrsa.gov/cicp/). Compensation may then be available for medical benefits, lost wages and death benefits to eligible individuals for specified injuries in accordance with regulations published by the Secretary. Eligibility for compensation and the injuries for which compensation may be available are further defined by regulation.

An individual who suffers a serious physical injury or death from administration and use of the study vaccine must first seek compensation from the Covered Countermeasures Process Fund. A serious physical injury means an injury that is life threatening, results in, or requires medical or surgical intervention to prevent, permanent impairment of a body function or permanent damage to body structure. Any compensation will be reduced by public or private insurance or worker's compensation available to the injured individual.

If no funds have been appropriated to the compensation program, the Secretary does not make a final determination on the individual's request within 240 days, or if the individual decides not to accept the compensation, the injured individual or his representative may pursue a tort claim in the United States District Court for the District of Columbia, but only if the claim involves willful misconduct, is pled with particularity required under the PREP Act, verified, and accompanied by an affidavit by a physician who did not treat the individual and certified medical records. Any award is reduced by any public or private insurance or worker's compensation available to the injured individual. Awards for non-economic damages, such as pain, suffering, physical impairment, mental anguish, and loss of consortium are also limited. If the individual accepts compensation, or if there is no willful misconduct, then the individual does not have a tort claim that can be filed in a United States Federal or a State court.

2.2 Rationale

There is no specific treatment or vaccine currently available to treat or prevent ZIKV infections, other than mosquito control measures. Development of a preventive vaccine against ZIKV infections is a high global public health priority. The ZIKV vaccine to be used in this trial is a purified inactivated virus vaccine that was produced by WRAIR. The strategy for the initial development of this vaccine is to conduct a series of phase I studies to assess the safety and immunogenicity in flavivirus-naïve and flavivirus-experienced subjects. Because we do not know how previous exposure to flaviviruses will affect safety and immunogenicity profile of this vaccine, this strategy will address a critical gap necessary to complete an early and rapid assessment of the viability of this ZIKV vaccine candidate. All trials will initiate with a 5 mcg dose. The first two clinical trials (conducted by DMID/NIAID and WRAIR), will explore the safety and immune response to the vaccine in flavivirus naïve subjects (DMID/NIAID), and in subjects that have been previously vaccinated with JEV or YFV vaccines (WRAIR). The third trial (conducted by DMID/NIAID) will be conducted in Puerto Rico, whose population has been exposed to DENV 1-4, ZIKV, and potentially YFV and/or WNV. A fourth clinical trial will be conducted by Beth Israel Deaconess Medical Center to evaluate the safety and immunogenicity of an accelerated vaccination schedule with the Zika vaccine candidate in healthy adults. The initial doses and regimens that will be utilized in these initial trials was selected based on past experience with other inactivated flavivirus vaccines, and recent data from mouse and NHP studies. Experience with licensed flavivirus vaccines suggests a 5 mcg dose should be safe and immunogenic; and the 5 mcg dose was safe and protective in a NHP model (Abbink 2016). Therefore for these trials, subjects will be enrolled initially in the 5 mcg group. To determine if lower doses of ZPIV are equally or comparably immunogenic to the 5 mcg dose, subjects will then be enrolled in the 2.5 mcg group (Saint Louis University and CAIMED) and 1.25 mcg group (Saint Louis University).

ZPIV is intended to be used in flavivirus endemic areas and among US military members and travelers who may have already received another flavivirus vaccine. Therefore, evaluating the safety and immune response in both flavivirus-naïve and flavivirus-immune subjects is important. The Puerto Rico study gives us the opportunity to evaluate ZPIV among a largely flavivirus-immune population who resides in an area with active ZIKV and DENV transmission. Subjects will be followed closely for potential immune-mediated complications and subsequent natural flavivirus infections will be assessed for 24 months after second dose of ZPIV is administered. Based on experience from other inactivated flavivirus vaccines and early animal testing, two 5 mcg doses of alum adjuvanted ZPIV is expected to be well-tolerated and generate moderate neutralizing antibody titers against ZIKV.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

This is a phase I study of ZPIV. The potential risks of this study are those related to the investigational vaccine, having blood drawn, intramuscular injection, other risks, and breach of confidentiality. There may be other unknown risks, discomforts, or side effects.

ZPIV

Potential risks related to ZPIV administration may be similar to those of other inactivated flavivirus vaccines. Two other cell-derived formalin-inactivated purified inactivated flavivirus vaccines have been evaluated in clinical trials in humans: a monovalent dengue virus (serotype 1) vaccine developed by WRAIR and a JEV vaccine (IXIARO) that is licensed and widely used. Studies with both vaccines indicate that purified inactivated flavivirus vaccines are reasonably well tolerated.

In a Phase 1 clinical trial of two doses (2.5 mcg and 5 mcg per 0.5 mL) of an inactivated DENV-1 vaccine administered intramuscularly in a 2-dose regimen at 1 and 29 days, few AEs were detected among the 20 adult subjects (Martinez, 2015). Local reactogenicity consisted of pain and tenderness with one report of induration. The most common solicited systemic AEs included fatigue, headache, myalgia, nausea/vomiting and fever with the last three AEs occurring after the second administration of the 5 mcg dose. Unsolicited AEs detected among subjects in the 2.5 mcg group were mild lymphadenopathy, mild elevation of alanine transaminase (ALT), and moderate elevation of aspartate aminotransaminase (AST), in one subject each. In the high dose group, one subject reported mild chills and another subject reported mild arthralgia (Martinez, 2015).

A formaldehyde-inactivated JEV vaccine (IXIARO) has been licensed for use in the United States since 2009. The package insert indicates that the most common adverse reactions reported were headache, myalgia, influenza-like illness, and fatigue. (Valnera Austria GmBH, 2015) No effect was seen on the safety profile of IXIARO compared to placebo (aluminum hydroxide) when examined by age, sex, or ethnic origin. In a Phase 3 clinical trial involving 2675 subjects who received either two doses of IXIARO (6 mcg) or placebo (phosphate buffered saline (PBS) plus aluminum hydroxide), there was with little difference between the active and placebo groups (Valnera Austria, 2015). The overall percentage of subjects who experienced at least one AE was 59% in the active group versus 57% in the placebo. Injection site reactions were mild to moderate in severity, and consisted of (in descending order of occurrence) pain, tenderness, erythema, induration, edema and pruritus. The most common systemic AEs post first dose and second dose were headache, myalgia, fatigue, influenza-like illness, nausea, nasopharygnitis, and fever. Sixteen SAEs were reported in 10 subjects who received vaccine

and 6 subjects who received placebo. The SAEs that occurred among subjects in the IXIARO group were dermatomyositis, appendicitis, rectal hemorrhage, limb abscess involving contralateral arm, chest pain, ovarian torsion, ruptured corpus luteal cyst, and three orthopedic injuries. No deaths occurred in the trial.

IXIARO is also licensed at one half the dose (3 mcg) for infants and children aged 2 months to <3 years; the full strength is recommended for use in older children. Fever was the most commonly observed AEs up to 12 years of age (IXIARO package insert).

The association of ZIKV infections with the rare autoimmune disease GBS and acute disseminated encephalomyelitis (ADEM) is a concern for ZIKV vaccines in general. It is not yet clear whether the increased incidence of GBS is the result of an autoimmune reaction or is the result of neurotropism of the virus. Meningoencephalitis from ZIKV infection has also recently been reported (Carteaux, 2016).

In summary, inactivated flavivirus vaccines appear to be reasonably well tolerated. However, typical safety considerations with inactivated flavivirus vaccines include local reactions of pain, tenderness, erythema, induration, edema and pruritus, and mild to moderate systemic reactions including headache, myalgia, fatigue, influenza-like illness, nausea, and fever. The potential for a vaccine or ZPIV to induce GBS is not known. The safety and efficacy of ZPIV in an area of endemic flavivirus transmission is also not known. As ZPIV is intended for use in ZIKV-endemic areas where transmission of other arboviruses/flaviviruses occurs and in flavivirus-exposed populations, this trial will evaluate the safety and immunogenicity of ZPIV in a flavivirus endemic area/exposed population.

Aluminum hydroxide (Alum) Adjuvant

The use of alum as an adjuvant is well established and generally well tolerated. Specific limitations of the use of alum as an adjuvant include increased production of IgE antibodies and occurrence of allergic reactions, neurotoxicity, and granulomas among individuals given vaccine adjuvanted with alum. The occurrence of granulomas has been associated with subcutaneous or intradermal administration. Alum is typically excreted in the urine; however, it may accumulate in persons with renal insufficiency. In cases of accumulation, high levels of aluminum in the body may affect the brain and bone tissues. While there is a theoretical concern that immunostimulatory adjuvants may precipitate autoimmune disorders, there is not thought to be an issue when aluminum hydroxide is used as an adjuvant.

Allergic Reaction

Acute and potentially life-threatening allergic reactions are also possible. Very rarely, occurring in about 1 in 4 million people given a vaccination, there can be a serious allergic reaction to a vaccine. These reactions can manifest as skin rash (hives), swelling around the mouth, throat or eyes (angioedema), difficulty breathing (bronchospasm), a fast pulse (tachycardia), or loss of

blood pressure (hypotension). If these reactions occur, they can usually be stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a fatal reaction (death), although researchers do not expect this to occur.

Blood Draw

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down with the feet raised. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure to the draw site for several minutes after the blood draw.

IM injection

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.

Pregnancy

It is unknown if ZPIV poses any risks to an unborn child. As such, women of childbearing potential, defined as women who are not post-menopausal (i.e., have been amenorrhea for ≥1 year without other medical cause), or who have not been surgically sterilized (i.e., had a tubal ligation, bilateral oophorectomy, hysterectomy, bilateral salpingectomy, or successful Essure placement), must agree to use an effective method of birth control for the duration of the study.

Human Leukocyte Antigen (HLA) Testing

Blood will be collected for HLA testing to explore the impact of host genetics on immune responses to ZPIV. The study will not do genetic tests to determine hereditary diseases. The HLA results will be part of research records and will not become part of a subject's medical record and will not be shared with the subject's doctor. It is possible that if others found out information from these tests (such as information about HLA type) it could cause problems with the subject's family (e.g., family member learns about risk for a disease that may be passed on or learns who may be the true parent of a child). The risk of this is extremely low because the results will not be part of the health records and will only be part of research records.

Breach of Confidentiality

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 records will be kept in a locked file cabinet or maintained in a locked room at the participating VTEU and subcontract sites. Electronic files

will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected. Any publications from this study will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating VTEU sites for quality assurance and data analysis include groups, such as NIAID or its designee and the Food and Drug Administration (FDA) and Department of Defense.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This web site will not include information that can identify subjects. At most, this web site will include a summary of the results.

2.3.2 Known Potential Benefits

As the efficacy of experimental vaccines that protect against ZIKV infection and/or disease have not been proven, there are no known benefits to the human subjects participating in this clinical trial who will receive ZPIV. Society will benefit if ZPIV is able to protect people from ZIKV infection and disease as a result of this and other vaccine trials.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary

1. Assess the safety and reactogenicity of a homologous prime boost regimen of ZPIV given at two different dose levels in a dose de-escalation format in healthy adult subjects who live in Puerto Rico, a flavivirus endemic area.
2. Compare the safety and reactogenicity profile of ZPIV after each vaccination, between dosage groups, and by pre-vaccination flavivirus immune status.

3.1.2 Secondary

1. Assess the humoral immune response to a homologous prime-boost regimen of ZPIV after each dose of vaccine as determined by kinetics of the immune responses, seroconversion rates, and Geometric Mean Titers (GMT) overall, and compare results between dosage groups and by pre-vaccination flavivirus immune status.
2. Assess the durability of the humoral immune response to ZPIV after the second vaccine administration overall, and compare results between dosage groups and by pre-vaccination flavivirus immune status.

3.1.3 Exploratory

1. Estimate the incidence of ZIKV and DENV infections between Visit 00 and the subject's last visit and describe the clinical presentation including disease severity overall, by dosage group, product received, and pre-vaccination flavivirus immune status.

3.2 Study Outcome Measures

3.2.1 Primary Endpoints

Safety and Reactogenicity:

1. Frequency and severity of solicited injection site and systemic reactogenicity from time of study vaccine administration through Day 8 after each administration of study vaccine overall and by dosage group and pre-vaccination flavivirus immune status.

2. Frequency and severity of unsolicited vaccine-related adverse events (AE), including vaccine-related laboratory AE, from first administration of study vaccine until 28 days after the last vaccination overall and by dosage group and pre-vaccination flavivirus immune status.

3. Frequency, type, and duration of serious adverse events (SAE) and adverse events of special interest (AESI) considered related to study vaccine from the first administration of study vaccine until the end of the study overall, and by dosage group and pre-vaccination flavivirus immune status.

4. Frequency of new onset chronic medical conditions reported at any time from the first administration of study vaccine until the end of the study overall, and by dosage group and pre-vaccination flavivirus immune status.

5. Comparison of the frequency, type, and duration of vaccine-related Grade 3 local, systemic, or laboratory AE, and Grade 2 or greater local or systemic reactogenicity through Day 8 after each study vaccine administration between dosage groups and by pre-vaccination flavivirus immune status.

6. Comparison of study withdrawals and discontinuation of study vaccination due to any reason between dosage groups and by pre-vaccination flavivirus immune status.

3.2.2 Secondary Endpoints

Immunogenicity:

1. Frequency of seroconversion to ZIKV measured by ZIKV ELISA and neutralization assay in comparison with baseline sample (collected on Visit 00) overall, and by dosage group and pre-vaccination flavivirus immune status.

2. Per Visit GMT as measured by ZIKV ELISA and neutralization assay after each study vaccine administration and overall, and by dosage group and pre-vaccination flavivirus immune status.

3. Peak GMT as measured by ZIKV ELISA and neutralization assay after each vaccination and overall, and by dosage group and pre-vaccination flavivirus immune status.

4. Proportion of subjects with at least a 4-fold rise in ZIKV GMT as measured by ZIKV ELISA and neutralization assay at 4 weeks after each vaccination compared with baseline overall, and by dosage group and pre-vaccination flavivirus immune status.

3.2.3 Exploratory Endpoints

1. Number and proportion of subjects who develop an acute febrile illness (AFI) or an acute rash illness (ARI) who have evidence of acute ZIKV or DENV infection detected within 14 days of symptom onset. This endpoint will be analyzed for all subjects overall, and by dosage group, product received, and pre-vaccination flavivirus immune status.
2. Number and severity of laboratory confirmed ZIKV and DENV infections during the study, overall, by treatment group, and pre-vaccination immune status, where severity is described by:
    - Duration of symptoms
    - Grade of symptoms (mild, moderate, severe)
    - Use of concomitant medications
    - Need for hospitalization or other medical care

    Laboratory confirmation will be made by AFI or ARI with either: 1) evidence of acute ZIKV and DENV infection within 14 days of symptom onset or 2) a 4 fold rise in DENV or ZIKV neutralization titers between samples collected at the last visit prior to and at the first visit following the AFI/ARI, where available.

4 STUDY DESIGN

This study is a single-center, double-blinded, placebo-controlled, Phase 1, dose de-escalation study to evaluate the safety, reactogenicity, and immunogenicity of a purified inactivated, alum-adjuvanted ZIKV vaccine (ZPIV) administered in a homologous prime-boost regimen to healthy male and non-pregnant female adult subjects living in a flavivirus-endemic area. Two dose levels will be evaluated. Each subject will receive either placebo or 5 mcg (Group 1), or 2.5 mcg (Group 2) of ZPIV administered by IM injection. The study will consist of a screening period of up to 28 days, a vaccination period in which subjects will receive a prime dose of vaccine on Day 1 followed by a boost on Day 29, and a follow-up period of 24 months post boost vaccination. As this is a phase I study, the study will begin with enrollment of 2 sentinel subjects in Group 1 who will receive 5 mcg ZPIV open label. One sentinel subject will be vaccinated, followed for one day for safety and reactogenicity, and if no halting rules (Section 9.5.1) are met per determination of the PI and co-PI, then the second sentinel subject will receive 5 mcg ZPIV open-label. Both sentinels will be followed for safety through Day 8 and if no predefined halting rules are met (9.5.1) and no safety concerns are identified, then enrollment of the Group 1 non-sentinel subjects will proceed in double-blind fashion. The same procedure will be used for administration of the boost vaccination to the Group 1 sentinels: 1 sentinel (can be either) will receive 5 mcg ZPIV open-label, be followed for 1 day for safety and reactogenicity, and if no halting rules are met (9.5.1), then the 2nd sentinel will receive the boost vaccine. Both sentinels will be followed until Day 8 after 2nd vaccination (Study Day 36) for safety and reactogenicity and if no halting rules are met (9.5.1) then boost vaccination of the non-sentinel Group 1 subjects can proceed. If predefined halting rules are met or safety concerns are identified during the trial, a Safety Monitoring Committee (SMC) meeting will be held to conduct an electronic review (E-Review) of clinical and laboratory safety and reactogenicity data. Subjects and investigators will be blinded as to the product administered for all subjects except the sentinel subjects but they will not be blinded to the Group. Solicited local and systemic reactogenicity data will be collected from all subjects through Day 8 after each vaccination. Unsolicited adverse events will be collected from all subjects through Day 57 (28 days after 2nd vaccination).

Enrollment of the 2.5 mcg ZPIV group (Group 2) can begin after or at the same time non-sentinel subjects in Group 1 receive the 1st dose of vaccine. As of December 18, 2017, the majority of non-sentinel subjects in Group 1 have already received the 1st dose and have been followed until Day 8 for safety and reactogenicity, and no halting rules have been met or safety concerns identified. As this is a dose de-escalation study, concurrent enrollment of some remaining non-sentinel subjects in Groups 1 is permitted after enrollment of Group 2 subjects has begun; also, no sentinel subjects will be used in Group 2. All subjects in Group 2 will receive study product or placebo in double-blind fashion.

The original study design planned to enroll 40 ZPIV recipients and 5 placebo recipients in each Group. However, due a natural disaster (hurricane Maria), 11 subjects enrolled in Group 1 had loss of samples at key timepoints. To rebalance the number of evaluable subjects between Groups, Group 1 enrollment will be increased by 5 subjects and Group 2 enrollment will be decreased by 5 subjects, for a total enrollment of 50 subjects in Group 1 and 40 in Group 2. Treatment assignments for both groups will be assigned according to the originally planned 8:1 ratio of ZPIV:placebo. Without compromising the blind of the study, we can anticipate approximately 35 evaluable ZPIV recipients and approximately 5 evaluable placebo recipients in each group.

All subjects will receive a homologous boost of ZPIV or placebo 28 days post-prime if no halting rules precluding second vaccination are met (Section 9). All subjects will be monitored for occurrence of unsolicited AEs until 28 days after the second vaccination.

Table 1: Schematic of Study Design

Dosage Group	Approximate # of Subjects (8:1 ratio)	Treatment at Days 1 and 29
Group 1 Sentinels	2	5 mcg ZPIV
Group 1 Non-Sentinels	43	5 mcg ZPIV
	5	Placebo
Group 2	35	2.5 mcg ZPIV
	5	Placebo

The safety and reactogenicity of the study vaccine will be assessed as described in Section 7 and outlined in the Schedule of Procedures and Evaluations. Solicited local and systemic reactogenicity will be recorded through Day 8 after the prime and boost administration of study vaccine by the subject in a memory aid, and will be reviewed with the clinic staff during clinic visits on Days 2 and 8, and during a telephone visit on Day 4. Safety laboratories will be collected on Days 8 and 15 after administration of the prime and boost dose. Unsolicited AEs will be recorded and evaluated by medical history and targeted physical examinations as needed until 28 days after the second vaccination. SAEs, AESIs, and history of new medical conditions with onset after the first vaccination will be collected for the duration of the study.

Blood will be drawn to evaluate humoral immunity to ZIKV and DENV pre- and at multiple visits post- each vaccination, including baseline serology testing on Visit 00 to determine pre-vaccination flavivirus immune status. CPT tubes will be collected and peripheral blood mononuclear (PBMCs) cells will be harvested and stored for future assessment of immunity

including cellular and humoral immunity and systems biology. Refer to section 7.3.1 for collection timepoints for these assays.

Subjects who develop an AFI (grade 1 or higher fever on at least 2 consecutive days accompanied by any of the following: new rash not limited to vaccination site, arthralgia, or nonpurulent conjunctivitis) or ARI (new rash not limited to vaccination site) at any time after signing the ICF until the end of study will be asked to contact the clinic within 3 days of symptom onset and return as soon as possible and at least within 14 days for blood and urine collection to evaluate for acute ZIKV and DENV infection. Subjects will also be asked about any history of AFI and ARI at each clinic visit and phone call after Visit 00. Subjects presenting for evaluation of AFI/ARI will have a medical history including travel history collected, all symptoms recorded, physical exam if indicated, and concomitant medications and AEs collected, regardless of when the visit occurs.

The entire duration of each subject's participation is approximately 26 months including recruitment and collection of data on the safety and reactogenicity of the study vaccine and collection of samples for the assessment of immunogenicity. This study is expected to take approximately 49 months to complete from initiation through availability of a final report on the primary outcomes of safety and the secondary outcomes of humoral immunity to ZIKV.

4.1 Substudies (if applicable)

No sub studies are planned.

5 STUDY ENROLLMENT AND WITHDRAWAL

Approximately ninety males and non-pregnant, non-breastfeeding females, 21-49 years of age, inclusive, who are in good health (on the basis of physical examination, medical history and clinical judgment) and meet all eligibility criteria, will be enrolled. The study population's racial and ethnic make-up should reflect the demographics of the larger community at the VTEU subcontract site. Estimated time to complete enrollment of all subjects in this study is approximately 15 months. Screening visits for eligible subjects will be performed within 28 days prior to administration of the study vaccine on Day 1. Subjects who do not receive the second vaccination or who withdraw consent for future use of samples after receiving the first vaccination may be replaced following written approval of the sponsor.

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/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed to 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. Must be a male or non-pregnant, non-breastfeeding female between the age of 21 and 49 years, inclusive at the time of screening and enrollment.
2. Must be willing and able to read, sign and date the informed consent document before study related procedures are performed.
3. Must be willing and able to comply with study requirements and available for follow-up visits for the entire study.
4. Must have a means to be contacted by telephone.
5. Must have a body mass index (BMI) ≥18.1 and <35.0 kg/m^2.
6. Must have acceptable* screening laboratory findings within 28 days before enrollment.

**Acceptable clinical laboratory parameters include:*
-Hemoglobin: women: ≥11.5 g/dL; men ≥13.5 g/dL
-Hemotocrit: women: ≥ 34.5%; men ≥ 40.5%
-White blood cell count: ≥3.500 cells/mm^3 but ≤10,800 cells/mm^3
-Platelets: ≥150,000 but ≤450,000 per mm^3
-Urine dipstick (clean urine sample): protein <1+, glucose negative
-Serum creatinine ≤1 x institutional upper limit of normal (ULN)
-Blood urea nitrogen (BUN) <25
-Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) <1.25 x institutional ULN
-Total bilirubin <1.25 x institutional ULN

**Note: 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.*

7. Must be in good health based on the investigator's clinical judgment when considering findings from past medical history, medication use, vital signs, and an abbreviated physical examination.

   Note 1: Good health is defined by the absence of any medical condition described in the exclusion criteria in a subject with a normal abbreviated physical exam and vital signs. If the subject has a preexisting condition not listed in exclusion criteria, it cannot meet any of the following criteria: 1.) first diagnosed in last 3 months; 2.) worsening in terms of clinical outcome in last 6 months; or 3.) involves need for medication that may pose a risk to subject's safety or impede assessment of adverse events or immunogenicity if they participate in study.

   Note 2: An abbreviated physical exam differs from a complete exam in that it does not include a genitourinary and rectal exam.

   Note 3: Vital signs must be normal by protocol toxicity grading scale or determined to be normal-variant by investigator. In the event of an abnormal heart rate or blood pressure due to physiological variation or activity, the subject may rest for 10 minutes in a quiet room, and then blood pressure and/or heart rate may be re-measured. Repeated vital signs may be used to determine eligibility.

8. Women of childbearing potential* must have a negative serum pregnancy test at screening and a negative urine pregnancy test immediately prior to each vaccination.

   Note: All female subjects are considered of childbearing potential unless postmenopausal or surgically sterilized and ≥3 months have passed since sterilization procedure. Postmenopausal is defined as amenorrhea for ≥12 months without an alternative medical cause. Permanent female sterilization procedures include tubal ligation, bilateral salpingectomy, hysterectomy, bilateral oophorectomy, or successful Essure placement.

9. Women of childbearing potential must use an acceptable method of contraception* from one month (30 days) prior to the first vaccination until the end of the study.

   **Acceptable methods of contraception include the following:*
   -Use highly effective contraceptive methods, defined by <1% failure rate per year independent of user adherence, including long-acting reversible contraception (LARC): progestin-releasing subdermal implants and intrauterine devices (IUD), OR
   -Use effective contraceptive methods, defined by 5-9% failure rate with typical use and <1% failure rate with consistent and correct use, including: prescription oral contraceptives, contraceptive injections, combined pill, progestin-only pill, hormone-releasing transdermal patch or vaginal ring, and depot medroxyprogesterone acetate injection (Depo-Provera), OR
   -Male sex partners must have had a vasectomy ≥3 months prior to first vaccination, OR
   -Practice abstinence defined as refraining from heterosexual intercourse from 30 days before first vaccination until the end of the study.

10. Female subjects must agree to not donate eggs (ova, oocytes) from the start of screening period until the end of the study.

11. Subjects must provide concurrent consent at the time of enrollment and 1st vaccination to future use of stored blood samples to measure immunity to ZIKV.

5.2 Subject Exclusion Criteria

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

1. Has plans to become pregnant during the course of the study, or is currently pregnant or breastfeeding.
2. Plans to receive a licensed flavivirus vaccine or participate in another flavivirus vaccine trial during the study.
3. Has positive serology for HIV 1/2, Hepatitis C virus, or Hepatitis B surface antigen.
4. Has known or suspected congenital or acquired immunodeficiency, or recent history or current use of immunosuppressive therapy*

   **Anti-cancer chemotherapy or radiation therapy within the preceding 6 months, or long-term (at least 2 weeks within the previous 3 months) systemic corticosteroids therapy (at a dose of at least 0.5 mg/kg/day). Intranasal or topical prednisone (or equivalent) is allowed.*
5. Had organ and/or stem cell transplantation whether or not on chronic immunosuppressive therapy.
6. Has history of malignancy other than squamous cell or basal cell skin cancer, unless there has been surgical excision that is considered to have achieved cure*.

   **Subjects with a history of skin cancer must not be vaccinated at the previous tumor site.*
7. Has history of chronic or acute severe neurologic condition*.

   **Including history of Guillain-Barre syndrome, seizure disorder or epilepsy, Bell's palsy, meningitis, or disease with any focal neurologic deficits.*
8. Has diabetes mellitus type 1 or type 2, including cases controlled with diet alone.

   **Note: history of isolated gestational diabetes is not an exclusion criterion.*
9. Has history of thyroidectomy, or thyroid disease requiring medication during the last 12 months.
10. Has major psychiatric illness during last 12 months that in the investigator's opinion would preclude participation.
11. Has history of other chronic disease or condition*.

    **Includes the conditions and diagnoses defined as AESI in section 9, as well as autoimmune disease, hypercholesterolemia, chronic hepatitis or cirrhosis, chronic pulmonary disease, chronic renal disease, and chronic cardiac disease including hypertension even if medically controlled*
    – Vital signs must be normal by protocol toxicity grading scale or determined to be normal-variant by investigator. In the event of an abnormal heart rate or blood pressure due to physiological variation or activity, the subject may rest for 10 minutes in a quiet room, and then blood pressure and/or heart rate may be re-measured. Repeated vital signs may be used to determine eligibility.
12. Has current or past history of substance abuse that in the investigator's opinion would preclude participation.

13. Has tattoos, scars, or other marks on both deltoid areas that would, in the opinion of the investigator, interfere with assessment of the vaccination site.
14. Has a history of chronic urticaria (recurrent hives).
15. Has known allergy or history of anaphylaxis or other serious reaction to a vaccine or vaccine component*.

    **Including aluminum hydroxide (alum) or aminoglycosides (e.g., neomycin and streptomycin).*
16. Had major surgery (per the investigator's judgment) in the month prior to screening or plans to have major surgery during the study.
17. Received blood products or immunoglobulin in the 3 months prior to screening or planned use during the course of the study.
18. Donated a unit of blood within 8 weeks before Day 1 or plans to donate blood during the course of the study.
19. Received live attenuated vaccine from 30 days before Day 1 or plans to receive a live attenuated vaccine from Day 1 until 30 days after the last vaccination.
20. Received killed or inactivated vaccine from 14 days before Day 1 or plans to receive a killed or inactivated vaccine from Day 1 until 14 days after the last vaccination.
21. Received experimental therapeutic agents within 3 months prior to the first study vaccination or plans to receive any experimental therapeutic agents during the course of the study.
22. Is currently participating or plans to participate in another clinical study involving an investigational product, blood drawing, or an invasive procedure listed below.

    **An invasive procedure requiring administration of anesthetics or intravenous dyes or removal of tissue would be excluded. This includes endoscopy, bronchoscopy, or administration of IV contrast.*
23. Has an acute illness or temperature ≥38.0ºC on Day 1 or Day 29* or within 2 days prior to vaccination.

    **Subjects with fever or an acute illness on the day of vaccination or in the 2 days prior to vaccination may be re-assessed and enrolled if healthy or only minor residual symptoms remain within 2 days of Day 1 or Day 29.*
24. Is a study site employee* or staff paid entirely or partially by the OCRR contract or subcontract for the trial, or staff who are supervised by the PI or Sub-Investigators.

    **Including the Principal Investigator, sub-Investigators listed in Form FDA 1572 or Investigator of Record Form*

25. In the investigator's opinion, the subject cannot communicate reliably, is unlikely to adhere to the study requirements, or has a condition that would limit their ability to complete the study.

5.3 Study Withdrawal and Discontinuation of Study Vaccination

5.3.1 Study Withdrawal

Subjects have the right to withdraw their consent for study participation at any time and for any reason, without penalty, before completing the study. The investigator will make at least three documented attempts followed by a certified letter to contact any subject who does not return for scheduled follow-up. Although the subject is not obliged to give reasons for withdrawing early, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the subject's rights.

An investigator may also withdraw a subject from the study for any reason. If a subject withdraws early, an early withdrawal assessment should be obtained via a clinic visit or via telephone contact if this is the only available means (see Section 7).

A subject may withdraw or be withdrawn from the study for any of the following reasons:

- Subject withdraws their consent.
- Subject is lost to follow-up.
- Subject dies.
- Decision by the site principal investigator or appropriate sub-investigator to withdraw for noncompliance with protocol requirements or other reasons.
- Decision by the sponsor to stop or cancel the study
- Decision by local regulatory authorities and the VTEU site or subcontract site IRB/IEC to stop or cancel the study
- New medical disease or condition, worsening of any medical disease or condition, or any new clinical findings 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 study, or would interfere with the evaluation of responses.

5.3.2 Discontinuation or Withdrawal of Study Vaccination

Subjects who have received the first vaccination may choose to discontinue receipt of study vaccine for any reason but may choose to remain in the study. In addition, a subject may be discontinued from receipt of the second (boost) vaccination as is described in Section 9.5.2.

However, a discontinuation from receipt of the second vaccination will not result in automatic withdrawal from the study. Subjects meeting criteria for discontinuation of study vaccination must not receive any further study vaccine, but should continue to be monitored for safety and immunogenicity and collection of samples for future immunity and systems biology assays if this does not result in a safety risk for the subject and the subject agrees to continue with safety and immunogenicity monitoring.

5.3.3 Handling of Withdrawals and Discontinuation of Study Vaccination

The primary reason for study withdrawal or discontinuation of study vaccination will be recorded on the Study Status and/or Discontinuation of Treatment case report forms.

Those subjects who received one dose of study vaccine and have chosen not to receive a second dose of study vaccine or are not qualified to receive the second dose, will be asked to remain in this trial for follow-up safety and immunogenicity assessments including collection of samples for future studies unless subject safety precludes this. Safety assessments may be done by phone call, rather than in person, if this is the only means available of obtaining safety information.

Those subjects who withdraw or are withdrawn from the study will be asked to complete an early termination visit. The subject has the right to refuse to attend this visit. In the case of subjects who fail to appear for a safety follow-up assessment or an early termination visit, extensive effort (i.e., three documented contact attempts via phone and e-mails will be 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 records. See the protocol-specific MOP for description of follow-up post study withdrawal cases.

Every attempt will be made to follow all adverse events, including solicited injection site and systemic reactions, unsolicited non-serious adverse events, serious adverse events, AESIs, and new-onset chronic medical conditions, 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 from this study after signing the informed consent form (ICF), randomization, and receipt of the first dose of study vaccine may be replaced following written approval from sponsor. Subjects who withdraw or are withdrawn from this study after signing the ICF and randomization but before receipt of the first dose of study vaccine will be replaced.

5.3.4 Withdrawal from the Future Use of Research Samples

Subjects must give concurrent consent for collection and storage of blood samples for measurement of immunity to ZIKV and other arboviruses in protocol-specified and future immunity and systems biology assays. Future use samples (Section 14.8) will be collected and stored at the time of enrollment, first vaccination, and thereafter for use in future studies of immunity and systems biology.

Subjects may withdraw their consent for future use of stored samples at any time after receipt of the first study vaccination. Withdrawal of consent for future use of samples must be in writing. Withdrawal of consent for future use of stored samples will not result in automatic withdrawal from the study. Subjects will continue to be monitored for safety, and samples for protocol-specified safety and immunogenicity assays will continue to be obtained if this does not result in a safety risk for the subject and the subject agrees to continue with sample collection. Samples from subjects who withdraw consent for future use will be destroyed after all protocol-specified assays, including humoral immunity assays and evaluations of AFI and ARI, are completed.

Subjects who withdraw consent for future use of samples after receiving the first vaccination may be replaced following written approval of the sponsor.

5.3.5 Termination of Study

Although the study Sponsor has every intention of completing this study, it reserves the right to terminate this study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to SMC recommendation and at the discretion of DMID.

5.4 Treatment Assignment Procedures

5.4.1 Randomization Procedures

Per International Conference on Harmonization (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the VTEU subcontract 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) AdvantageEDC^{SM} (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subject will be enrolled. Two sentinel subjects in Group 1 will receive 5 mcg ZPIV open-

label. All other subjects will be dosed in double-blind randomized fashion. Subjects will receive the same study product and dose at their first and second study vaccinations. Subjects and investigators will be blinded as to study product received except for the sentinel subjects who will be dosed open label. Subjects and investigators will not be blinded to study group (1 or 2).

Enrollment of subjects will be done online using the enrollment module of AdvantageEDC. The list of randomized treatment assignments will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. AdvantageEDC will assign each subject a treatment code after the demographic and eligibility data have been entered into the system. A designated individual at each site will be provided with a treatment key, which links the treatment code to the actual treatment assignment, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the AdvantageEDC User's Guide. Manual back-up randomization procedures are provided in the MOP for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

5.4.2 Masking Procedures

This is a double-blinded study with the exception of the two sentinel subjects in Group 1. Non-sentinel subjects in Group 1 and all subjects in Group 2, investigators, study personnel performing any study-related assessments following study vaccine administration, and laboratory personnel performing assays will be blinded to study treatment within a group. The two sentinel subjects will receive open-label 5 mcg ZPIV (i.e., sentinels, investigators and study personnel will not be blinded to sentinel study treatment). Laboratory testing personnel will remain blinded to the identity and timepoint of individual samples.

The unblinded study vaccine administrator(s) is a study clinician credentialed to administer vaccines, but will not be involved in study-related assessments or have subject contact for data collection following study vaccine administration.

6 STUDY INVESTIGATIONAL VACCINE

6.1 Study Vaccine Description

6.1.1 Alum Adjuvanted ZPIV

The clinical material used to develop the ZIKV purified inactivated virus vaccine (ZPIV) was manufactured in Vero cells cultured in medium containing heat inactivated fetal bovine serum, Neomycin and Streptomycin. Following infection with ZIKV (Puerto Rico PRVABC59 strain), culture supernatants were collected, and clarified by centrifugation and filtration (0.45 µm followed by 0.22 µm). The clarified viral fluids were treated with Benzonase to remove cellular DNA, and then concentrated by ultrafiltration followed filtration (0.45 µm). The concentrated virus was purified by column chromatography using a Capto™Core 700 column. The virus-containing fractions were pooled. The purified pool was filtered (0.22µm). Sucrose was added and the virus was inactivated for 7 days at 22 °C with 0.05% formalin (1:2000 dilution of 37% formaldehyde). On day two of the inactivation, the virus was filtered (0.22 µm). Following inactivation, the formalin-treated virus pool was filtered (0.22 µm), concentrated to 50ug/mL, and diafiltered to remove residual formaldehyde. Sucrose in PBS was added to a final concentration of 3% sucrose. The bulk was sterile filtered with a 0.22 sterile in-line filter. Inactivation was confirmed by inoculation of Vero cells. The purified inactivated virus (ZPIV) was then adsorbed to aluminum hydroxide (Alhydrogel).

6.1.2 Aluminum Hydroxide Adjuvant (Alhydrogel)

Aluminum Hydroxide (or Alum) Adjuvant (Alhydrogel®) will be used to adjuvant the ZPIV vaccine and to further dilute the adjuvanted vaccine for the 2.5 mcg dose. Use of alum as an adjuvant is well established and generally well tolerated.

6.1.3 Placebo (Normal Saline, 0.9% Sodium Chloride, USP)

The placebo will consist of an injection of normal saline (0.9% sodium chloride solution, United States Pharmacopeia [USP]).

6.2 Acquisition

ZPIV and the alum adjuvant is manufactured and will be provided by WRAIR. Upon DMID authorization, the vaccine and adjuvant will be transferred to the following address:

DMID-Clinical Agents Repository (CAR), Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CAR@ThermoFisher.com

Normal saline be provided by the DMID CAR, Fisher BioServices.

All study vaccine (i.e., ZPIV, alum adjuvant, and normal saline) will be shipped to the participating VTEU subcontract site prior to the start of this study upon request and with prior approval from DMID. Should the site principal investigator require additional study vaccine or study products during this trial, further instructions are provided in the protocol-specific MOP.

6.3 Formulation, Storage, Packaging, and Labeling

6.3.1 Alum Adjuvanted ZPIV

The ZIKV purified inactivated vaccine with aluminum hydroxide (ZPIV) is supplied as a white to slightly yellowish suspension with white particulates, in a phosphate buffered saline formulation. The vaccine is formulated to contain 5mcg of antigen adjuvanted with 500mcg of aluminum hydroxide adjuvant per 0.5 mL (or 10 mcg/mL PIV protein and 1000 mcg/mL Alhydrogel). Each 2-mL sterile, single-dose vial contains a fill volume of 0.7mL and must be stored at 2°C to 8°C. During storage, a clear liquid with a white to slightly yellow precipitate will be observed; this is the alum adjuvant and this appearance is to be expected following refrigerated storage.

6.3.2 Alhydrogel® (Aluminum Hydroxide [Alum])

Alhydrogel® (aluminum hydroxide adjuvant) is supplied as a 1mg/mL (or 1000mcg/mL), liquid in a PBS formulation. The adjuvant appears as a white to slightly yellowish suspension with white particulates. Each 2-mL sterile, single-dose glass vial contains a fill volume of 1 mL and must be stored at 2°C to 8°C. During storage, a clear liquid with a white precipitate will be observed; this is the alum adjuvant and this appearance is to be expected following refrigerated storage. The adjuvant will be used to further dilute the ZPIV vaccine to attain the 2.5mcg dose.

6.3.3 Placebo (Normal Saline, 0.9% Sodium Chloride, USP)

Normal saline (0.9% sodium chloride injection, USP) is a clear to colorless, sterile, nonpyrogenic, isotonic solution. Each mL contains sodium chloride 9 mg and contains no preservatives, bacteriostatic, antimicrobial agent, or added buffer. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment. The product will be used as the placebo and will be supplied as single-use vials. The vials 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].

6.4 Dosage, Preparation and Administration of Study Vaccine

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

Visually inspect the study vaccine (ZPIV, alum adjuvant, and placebo [normal saline]) upon receipt and prior to preparation and use. If the study vaccine appear(s) to have been damaged, contaminated or discolored, or if there are any concerns regarding its integrity, do NOT use the affected study vaccine. The affected study vaccine must be quarantined and labeled as "Do Not Use (until further notice)." The Site Principal Investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov 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 vaccine can be used. If it cannot be used, the site will receive specific final disposition instructions from DMID. If the study vaccine is unusable, the participating VTEU subcontract sites pharmacist will prepare another dose. Replacement vials may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Depending on randomization, each subject will either receive either placebo, 5 mcg, or 2.5 mcg of ZPIV vaccine as a 0.5mL injection administered intramuscularly on Days 1 and 29. Each 0.5mL dose of the inactivated, adjuvanted ZPIV vaccine will contain 500 mcg of aluminum hydroxide.

Study vaccine administration will be performed by an unblinded study vaccine administrator(s) 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. On the assigned days, each dose of study vaccine or placebo will be administered to subjects via single injection in the preferred arm via IM injection.

6.5 Accountability Procedures for the Study Investigational Vaccine

The site Principal Investigator is responsible for the distribution and disposition of study vaccine, and has ultimate responsibility for accountability. The site Principal Investigator may delegate this responsibility to the site Research Pharmacist. If delegated, the site Research Pharmacist will be responsible for maintaining complete records and documentation of study vaccine and study product receipt, accountability, dispensation, temperature monitoring, storage conditions, and final disposition of the study vaccine and study products. All study vaccine, whether administered or not, must be documented on the appropriate study vaccine accountability record or dispensing log. Used and unused study vaccine and placebo will be retained until accountability by the study monitor is completed and as per DMID requirements.

Upon completion of the study and after the final monitoring visit, any remaining used and unused vials of study vaccine and placebo will either be returned or destroyed appropriately at the clinical site as per sponsor requirements and instructions, or in accordance with disposition plans determined at study conclusion or termination.

6.6 Concomitant Medications and Treatments

Assessment of study eligibility will include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Section 5). Prescription and over-the-counter drugs and vaccines will be recorded, as well as herbals, vitamins, and supplements. Pre-study and concomitant use of any medications, therapies, or vaccines will be on the appropriate case report form. Medications will include all current medications and non-study vaccinations taken within 30 days prior to signing the ICF through approximately Day 29 after the last study vaccination. Subjects who do not receive all study vaccinations will have concomitant medications collected through approximately Day 29 after the last study vaccination, or early termination, whichever occurs first.

Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Subjects presenting with a history of AFI or ARI will have all concomitant medications taken since start of illness recorded.

Medications that might interfere with the evaluation of the investigational vaccine should not be used during the study period unless absolutely necessary. Subjects will be evaluated for continuation in the protocol or withdrawn and followed for safety and reactogenicity if there is a medical need to take medications that may interfere with the evaluation of the study vaccine. Medications in this category include the prohibited medications per the Subject Exclusion

Criteria. 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 as prophylaxis prior to study vaccination is prohibited.

To the Sponsor's knowledge, there are no drug-vaccine interactions with the study vaccines 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 Investigator as soon as practicable. Details of all medications taken during the medication reporting period for this study (date, dose, frequency, brand or generic name) must be recorded.

7 STUDY PROCEDURES AND EVALUATIONS

7.1 Clinical Evaluations

7.1.1 Medical History

A complete medical history will be obtained by interviewing the subjects at the first study visit. 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. A focused vaccination history will be obtained. A history of pre-study and concomitant medication use will be collected as described in Section 6, however the focus will be on history of receipt of medications which modify the host immune response (e.g., cancer chemotherapeutic agents, parenteral corticosteroids). In addition, subjects will be asked if they received any experimental therapeutic agent, blood product or immunoglobulin within 3 months of the screening visit. A history of current use of contraception and recent menstrual history (in females) will be solicited.

At follow-up visits after the first study visit, an interim medical history will be obtained by interview of the subjects noting any changes since the previous clinic visit or phone call. The interim medical history should include an assessment for AFI, ARI, and new medical conditions and symptoms as well as an interim medication history.

Subjects reporting AFI/ARI within 14 days will be evaluated as per section 8.7 or asked to come in for evaluation within 14 days and preferably as soon as possible if the report is made during a telephone visit.

Subjects reporting AFI/ARI more than 14 days previously will have history, including symptoms (grade, duration), use of concomitant medications, healthcare seeking behavior and hospitalizations, and any prior travel collected.

7.1.2 Physical Examination

At the screening visit (Visit 00), an abbreviated physical examination (excluding pelvic and rectal examinations) will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. For all visits following the screening visit, a targeted physical examination may be performed by a study

clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on subject's interim medical history.

Vital signs (oral temperature, pulse, and blood pressure) will be collected if indicated at the time of physical examination. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Vital signs must be normal by protocol toxicity grading scale or determined to be normal-variant by investigator. In the event of an abnormal heart rate or blood pressure due to physiological variation or activity, the subject may rest for 10 minutes in a quiet room, and then blood pressure and/or heart rate may be re-measured. The repeated measurement may be used to determine eligibility per the judgment of the investigator.

Height and weight will be collected on the screening visit to determine Body Mass Index (BMI).

Pre-administration reactogenicity assessments will be performed prior to each study vaccination, then the study vaccination will be given. A subject with mild pre-administration reactogenicity that is transient, resolving, or clinically insignificant may be enrolled at the investigator's discretion.

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 or SAEs will be assessed and recorded on the appropriate case report form prior to discharge from the clinic.

Reactogenicity assessments will include an assessment of solicited adverse events occurring from the time of each study vaccination through Day 8 after each study vaccination, which includes an assessment of injection site reactions including pruritus (itching), erythema (redness), ecchymosis (bruising), induration (hardness)/swelling, pain, and tenderness as well as systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, rash, nausea, vomiting, , diarrhea, and abdominal pain. All subjects will complete a subject memory aid from Day 1 through Day 8 after each study vaccination to record solicited injection site and systemic reactogenicity. Subject memory aids will be reviewed with the subjects by phone visit on Day 4 and at scheduled clinical visits on Days 2 and 8 after each study vaccination.

7.1.3 Febrile or Rash Illness Assessment

For this study, AFI will be defined as grade 1 or higher fever lasting at least two consecutive days and accompanied by any one of:

- 1) new rash not limited to vaccination site,
- 2) arthralgia, or

- 3) nonpurulent conjunctivitis.

ARI will be defined as new rash not limited to vaccination site.

Subjects will be informed during the screening period about the long term surveillance and follow-up of all AFI and ARI during the study. They will be told to report all AFI and ARI to the study site within 3 days of symptom onset and come in for an assessment within 14 days (as soon as possible).

Subjects will also be asked at every follow-up visit after signing the ICF about AFI or ARI since the last visit.

The following assessment will be completed if a report of AFI or ARI is made during a regular or unscheduled visit. If it occurs during a regular visit, all procedures outlined in Section 8 appropriate for that day should be performed as well. The febrile/rash illness visit procedures include:

- Obtain interim medical history with focus on collecting signs and symptoms consistent with arboviral disease including duration and severity, and history of medical care received.
- Obtain interim travel history for any trips outside of Puerto Rico since the previous clinic visit or phone call, and record information including travel destination and duration of time at destination on the appropriate form.
- Record concomitant medications taken from time of onset of illness on the appropriate case report form.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Perform a targeted physical examination.
- Collect blood (whole blood and serum) and urine to evaluate for possible ZIKV, DENV, and chikungunya virus (CHIKV) infection with qRT-PCR and for other evidence of acute ZIKV and DENV infection.
- Assess for unsolicited AEs, SAEs and AESIs and new onset chronic medical conditions and record on the appropriate case report form.

If the subject reports history of AFI/ARI which began more than 14 days ago, the subject will be asked questions about their illness including onset date, signs and symptoms, severity and

duration of symptoms, medications used, healthcare seeking behavior, and travel history and this information will be recorded.

7.2 Clinical Laboratory Evaluations

7.2.1 Screening Laboratory Tests

In order to be eligible for participation in the study and receipt of the first dose of study vaccine, the subject's clinical screening laboratory evaluations must be confirmed to meet the eligibility criteria as outlined in the Inclusion and Exclusion Criteria (see Section 5).

- WBC, hemoglobin, hematocrit, and platelets; ALT, AST, total bilirubin, BUN, creatinine, and urine dipstick for glucose and protein will be performed.
- Subjects will be screened for HIV 1/2, hepatitis B surface antigen, antibody to hepatitis C virus. If a positive result occurs, the subject will be referred for appropriate follow-up and results will be reported as required by state/territory law. These screening tests must be negative for the subject to be eligible to participate as described in the Inclusion and Exclusion Criteria.
- Serum pregnancy tests will be performed at the screening visit. Urine pregnancy tests will be performed by the site laboratory within 24 hours prior to each study vaccination on Day 1 and approximately Day 29 on all female subjects of childbearing potential. Results must be known and negative prior to randomization on Day 1 and prior to receipt of the second vaccination on Day 29. A final urine pregnancy test will be performed on Day 29 post boost vaccination, including in subjects who do not receive the boost.
- If the initial screening laboratory tests are more than 28 days old, these tests will need to be repeated. However, repeat testing for HIV, HCV, and HBV are not needed.

7.2.2 Safety Laboratory Tests

Clinical safety laboratory tests will be evaluated on Day 8 and 15 after receipt of the prime and boost dose of study vaccine or placebo. Tests will include WBC, hemoglobin, hematocrit, and platelets; ALT, AST, total bilirubin, BUN, creatinine, and urine dipstick for protein and glucose.

7.2.3 Baseline Flavivirus serology

Subjects enrolled in the study will have blood drawn on Visit 00 after signing the ICF to determine baseline flavivirus immune status. Serum will be tested for antibodies to ZIKV and DENV. This can be done at any time during the study as the results will not be used to determine study eligibility.

7.3 Special Assays or Procedures

7.3.1 Immunogenicity Endpoints and Future Studies Sample Collection

Refer to SOE (Appendix A), Section 8, and Table 2 for blood collection timepoints.

Subjects will have blood collected pre-vaccination for determination of baseline flavivirus immunity as described in 7.2.3 and this sample will serve as a baseline comparison for protocol-specified and future humoral immunity studies. Relatively little is currently known regarding the human immune response to ZIKV, and the role of humoral, cellular, and other immune responses in protection from either infection or disease, as well as clearance of the virus. Multiple ongoing natural history clinical studies as well as animal studies are trying to identify answers to these questions. To allow this trial to be as informative as possible, blood samples for future studies of humoral, and cellular immunity to ZIKV and systems biology will be collected. Blood for serum for protocol-specified and future humoral immunity studies will be collected. CPT tubes for PBMCs for cellular and humoral immunity and systems biology will be collected. Plasma fractions from the CPT tubes will be saved for future systems biology studies. Paxgene tubes for systems biology will be collected. Samples on Days 1 and 29 are collected prior to vaccination. Subjects who do not receive 2nd vaccination will continue to have blood for humoral immunity and CPT tubes for cellular and humoral immunity and systems biology collected for protocol-specified and future studies as safety and subject's consent allows but will not have samples collected in Paxgene tubes. As knowledge is gained regarding appropriate immune responses to be assessed, these samples will be tested accordingly.

To determine the study outcome measures of humoral immune responses induced by vaccination with ZPIV, barcoded specimens will be sent from the repository to a central laboratory for testing. Humoral immunity will be assessed at WRAIR Pilot Bioproduction Facility under the direction of Rafael De La Barrera, M.S. LTC Natalie D. Collins, Ph.D., MPH, MLS (ASCP) , will serve at the WRAIR POC. The specimens will be labeled without personal identifiers. WRAIR investigators will not interact with study participants or have access to identifiable data. The analysis will include some de-identified data on the individuals who contributed the specimens. The barcoded data and specimens are not of a sensitive nature and

only contain basic sociodemographic and clinical information that cannot be traced back to individual volunteers by WRAIR personnel.

7.3.2 HLA Typing

HLA testing is planned as part of the evaluation of T-cell responses at baseline (Day 1). The human leukocyte antigen (HLA) system is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. These cell-surface proteins are responsible for the regulation of the immune system in humans. For those subjects who consent to have human leukocyte antigen (HLA) typing/genetic testing on their blood samples, genotyping/sequencing of genes related to immune responses such as HLA will be performed using well-established methods in the field to explore the impact of host genetics on immune responses to ZPIV.

7.4 Specimen Preparation, Handling, and Shipping

7.4.1 Instruction for Specimen Preparation, Handling, and Storage

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

7.4.2 Specimen Shipment

Specimen shipment will occur at intervals during the course of this study following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in the protocol-specific MOP.

8 STUDY SCHEDULE

Complete study schedule details listed by study visit/day are described below. Refer also to Sections 4 and 7 and Appendix A.

8.1 Screening/Baseline Visit

8.1.1 Visit 00, Day -28 to -1, Screening Visit

Potential subjects will be screened for eligibility within 28 days prior to the administration of the first study vaccination during Visit 00. The following activities will be performed during that visit:

- Provide description of this study (purpose and study procedures) and ask subjects to read and sign the ICF. The ICF will be signed prior to performing any screening procedures. Review eligibility criteria.
- Interview subjects to collect medical history, vaccination history, and travel history in last 30 days. Ask about recent history of blood donation, and receipt of blood products, immunoglobulin or experimental agents (as per exclusion criteria). Interviewing subjects is sufficient to obtain medical history. Solicitation of medical records from the subject's primary care provider is not required.
- Collect and review history of medications taken within 30 days prior to signing the ICF.
- Obtain vital signs, including oral temperature, pulse, and blood pressure to assure eligibility.
- Collect height and weight to calculate BMI.
- Perform an abbreviated physical examination (does not include pelvic or rectal exam). All physical exams will be done by a clinician licensed to make medical diagnoses and listed on Form FDA 1572 (see Section 7).
- Collect blood samples for baseline ZIKV and DENV serology.
- Collect venous blood for screening laboratory tests including: WBC, hemoglobin, hematocrit, platelets, ALT, AST, total bilirubin, BUN, creatinine, HIV-1/2 antibody, Hepatitis B surface antigen, and Hepatitis C antibody.

- Collect urine for urine dipstick for glucose and protein as part of screening laboratory tests.
- Collect serum for pregnancy test for all female subjects of childbearing potential as part of screening laboratory tests.
- Discuss with subject surveillance for AFI/ARI and need to contact clinic within 3 days if symptoms compatible with AFI/ARI develop. Provide subject with thermometer for measuring temperature and instruct in use.

The overall eligibility of the subject to participate in the study will be assessed once all the results are available from the screening tests and findings from the subject interview are available. Study subjects who qualify for inclusion will be contacted and scheduled for enrollment and prime vaccination within 28 days.

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.

8.2 Study Enrollment and First Vaccination Visit

8.2.1 Visit 01, Study Day 1, Enrollment and First Vaccination

- Reconfirm subject's willingness to participate prior to performing any study procedures, including consent for future use of samples prior to administration of the first study vaccination.
- Perform a urine pregnancy test within 24 hours prior to study vaccine administration on all female subjects of childbearing potential. Results must be known and negative prior to enrollment and receipt of study vaccine.
- Review eligibility criteria, including results of all screening laboratory tests, prior to the first study vaccination to assure continued eligibility.
  - If the initial screening laboratory tests are more than 28 days old, these tests will need to be repeated (see Section 7.2.1). However, repeat testing for HIV, HCV, and HBV are not needed.
- Review medical history and any updates obtained by interview of subjects since the screening visit to assure continued eligibility.

- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness assessment and sample collection as per Section 8.7.

- Review all concomitant medications and recent vaccinations (within 30 days of signing the ICF) with subjects prior to the first vaccination for accuracy and completeness. Any new medications taken since the screening visit will be recorded on the appropriate case report form and assessed for continued eligibility.

- Vital signs including oral temperature, blood pressure, and pulse, will be obtained prior to the first study vaccination.

- Perform a targeted physical examination prior to the first study vaccination, if indicated based on review of complete medical history and updates obtained by interview of subjects since the screening visit.

- Collect blood samples for, humoral immunity, HLA typing, CPT tubes and a Paxgene tube prior to vaccination.

- Enroll and randomize subjects in Advantage EDC prior to the first study vaccination.

- Perform pre-administration reactogenicity assessment prior to the first study vaccination to establish baseline. Subjects will then receive a single dose of study vaccine via IM injection into the deltoid muscle of the preferred arm. The site of injection (right or left arm) and time of administration will be recorded on the case report form.

- Observe subject in the clinic for at least 30 minutes after the vaccination to monitor for any acute reactions and evaluate the vaccination site and assess for reactogenicity.

- Assess for unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

- Instruct subject about importance of reactogenicity monitoring and remind about the Day 2 follow-up visit.

- Provide subject with a memory aid, thermometer, and ruler for recording daily maximum oral temperature and systemic and local AEs beginning with the day of vaccination and continuing through Day 8. Subjects will be encouraged to take their temperature around the same time each day and also if they feel feverish, beginning with the day of vaccination. Subjects will be instructed on how to use the memory aid and how to rate

any adverse events. Subjects will be asked to bring their paper Memory Aid with them to their next study visit.

8.2.2. Visit 02, Study Day 2, Day 2 Clinic Visit after First Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Review the Memory Aid with the subject.
- Examine vaccination site for local reactions.
- Collect blood samples for a CPT tube and a Paxgene tube
- Assess for unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.2.3 Visit 03, Study Day 4+1; Day 4 Phone Call Visit after First Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interviewing subject by phone. Record any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come in for a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.

- Ask subject about information that they have collected since Day 1 on their paper Memory Aid. If subject has an AE that they are concerned about, encourage them to come into the study clinic to be evaluated.

- Ask about unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.2.4 Visit 04, Study Day 8±1, Day 8 Clinic Visit after First Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.

- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.

- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.

- Perform a targeted physical examination, if indicated based on review of complete medical history.

- Obtain vital signs including oral temperature, blood pressure, and pulse.

- Review the Memory Aid with the subject.

- Examine vaccination site for local reactions.

- Collect blood samples for CPT tubes and a Paxgene tube.

- Collect venous blood for safety laboratory tests including WBC, hemoglobin, hematocrit, platelets, ALT, AST, total bilirubin, BUN, creatinine.

- Collect urine for urine dipstick for glucose and protein as part of the safety laboratory tests.

- Assess for unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.2.5 Visit 05, Study Day 15±1, Day 15 Clinic Visit after First Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Examine vaccination site for local reactions.
- Collect blood samples for humoral immunity, CPT tubes and a Paxgene tube.
- Collect venous blood for safety laboratory tests including WBC, hemoglobin, hematocrit, platelets, ALT, AST, total bilirubin, BUN, creatinine.
- Collect urine for urine dipstick for glucose and protein as part of the safety laboratory tests.
- Assess for unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3 Second Vaccination Visit

8.3.1 Visit 06, Study Day 29±3, Second Vaccination Clinic Visit

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Review eligibility criteria*

  *Except for: Inclusion Criteria 1, 2, 5, 6; and Exclusion Criteria 3.
- Review section 9.5.2 to be sure subject has not met a Halting Criteria for 2nd vaccination.

- Collect urine for a urine pregnancy test, which must be performed within 24 hours prior to study vaccine administration on all female subjects of childbearing potential. Results must be known and negative prior to receipt of study vaccine.
- Review medical history to assure continued eligibility.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Collect blood samples for humoral immunity, CPT tubes and a Paxgene tube.
- Do a pre-administration reactogenicity assessment prior to the second study vaccination to establish baseline. Subjects will receive a single dose of study vaccine via IM injection into the deltoid muscle of the preferred arm.
- Observe in the clinic for at least 30 minutes after the vaccination to monitor for any acute reactions and evaluate the vaccination site and assess reactogenicity.
- Assess for unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.
- Instruct subject about importance of reactogenicity monitoring and remind about the Day 2 clinic follow-up visit.
- Provide subject with a memory aid, thermometer, and ruler for recording daily maximum oral temperature and systemic and local AEs beginning with the day of vaccination and continuing for the next 7 days. Subjects will be encouraged to take their temperature around the same time each day and also if they feel feverish, beginning with the day of vaccination. Subjects will be instructed on how to use the memory aid and how to rate any adverse events. Subjects will be asked to bring their Memory Aid with them at their next study visit.

8.3.2 Visit 07, Study Day 30, Day 2 Clinic Visit after Second Vaccination.

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Review the Memory Aid with the subject.
- Examine vaccination site for local reactions.
- Collect blood samples for a CPT tube and a Paxgene tube.
- Assess for unsolicited AE, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.3 Visit 08, Study Day 32 +1; Day 4 Phone Visit after Second Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interviewing subject by phone. Record any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come in for a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.
- Ask subject about information that they have collected since Day 1 on their paper Memory Aid. If subject has an AE that they are concerned about, encourage them to come into the study clinic to be evaluated.

- Ask about unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.4 Visit 09, Study Day 36±1, Day 8 Clinic Visit after Second Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Review the Memory Aid with the subject.
- Examine vaccination site for local reactions.
- Collect blood samples for CPT tubes and a Paxgene tube.
- Collect venous blood for safety laboratory tests including WBC, hemoglobin, hematocrit, platelets, ALT, AST, total bilirubin, BUN, creatinine.
- Collect urine for urine dipstick for glucose and protein.
- Assess for unsolicited AE, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.5 Visit 10, Study Day 43±2, Day 15 Clinic Visit after Second Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.

- Collect information on all concomitant medications taken since last visit and record on the appropriate case report form.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Examine vaccination site for local reactions.
- Collect venous blood for safety laboratory tests including WBC, hemoglobin, hematocrit, platelets, ALT, AST, total bilirubin, BUN, creatinine.
- Collect urine for urine dipstick for glucose and protein as part of the safety laboratory tests.
- Collect blood samples for humoral immunity, CPT tubes and a Paxgene tube.
- Assess for unsolicited AE, SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.6 Visit 11, Study Day 57±3, Day 29 Clinic Visit after Second Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- All concomitant medications will be recorded on the appropriate case report form.
- A targeted physical examination will be performed, if indicated based on review of complete medical history.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Examine vaccination site for local reactions.
- A urine pregnancy test must be performed on all female subjects of childbearing potential.

- Collect blood samples for humoral immunity and CPT tubes.
- Any unsolicited AEs, SAEs, AESIs, and new onset chronic medical conditions will be assessed and recorded on the appropriate case report form.

8.3.7 Visit 12, Study Day 107±14, Day 79 (2.5 Month) Phone Visit after Second Vaccination

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.
- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come in for a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Any unsolicited SAEs, AESIs, and new onset chronic medical conditions will be assessed and recorded on the appropriate case report form.

8.3.8 Visit 13, Study Day 157±14, Day 129 (4.5 months) Phone Visit after Second Vaccination

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.
- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, as the subject to come in for a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Ask about unsolicited SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.9 Visit 14, Study Day 209±14, Day 181 (6 Month) Clinic Visit after Second Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- A targeted physical examination will be performed, if indicated based on review of complete medical history.
- Collect blood samples for humoral immunity and CPT tubes.
- Any unsolicited SAEs, AESIs, and new onset chronic medical conditions will be assessed and recorded on the appropriate case report form.

8.3.10 Visit 15, Study Day 268 ±14, Day 240 (8 months) Phone visit after 2nd vaccination.

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.
- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come in for a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Ask about unsolicited SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.11 Visit 16, Study Day 328±14, Day 300 (10 Months) Phone Call after Second Vaccination

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, as the subject to come in for a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Ask about unsolicited SAEs and AESIs and new onset chronic medical conditions and record on the appropriate case report form.

8.3.12 Visit 17, Study Day 388±14, Day 360 (12 months) Clinic Visit Following Second Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- A targeted physical examination will be performed, if indicated based on review of complete medical history.
- Collect blood samples for humoral immunity and CPT tubes.
- Any unsolicited SAEs, AESIs, and new onset chronic medical conditions will be assessed and recorded on the appropriate case report form.

8.3.13 Visit 18, Study Day 448±14, Day 420 (14 months) Phone Call following Second Vaccination.

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.
- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come if for a febrile/rash illness Assessment and sample collection as per Section 8.7.

- Ask about unsolicited SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.14 Visit 19, Study Day 508±14, Day 480 (16 months) Phone Call following Second Vaccination.

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.
- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come in for a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Ask about unsolicited SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.15 Visit 20, Study Day 569±14, Day 541 (18 months) Clinic Visit following Second Vaccination.

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness assessment and sample collection as per Section 8.7.
- A targeted physical examination will be performed, if indicated based on review of complete medical history.
- Collect blood samples for humoral immunity and CPT tubes.
- Any unsolicited SAEs, AESIs, and new onset chronic medical conditions will be assessed and recorded on the appropriate case report form.

8.3.16 Visit 21, Study Day 629±14, Day 601 (20 months) Phone Visit following Second Vaccination.

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.
- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come in for a febrile/rash illness assessment and sample collection as per Section 8.7.
- Ask about unsolicited SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.3.17 Visit 22, Study Day 689±14, Day 661 (22 months) Phone Visit following Second Vaccination.

- Contact subjects by phone to ask for any updates on contact information including email address and phone number.
- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.
- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, ask the subject to come in for a febrile/rash illness assessment and sample collection as per Section 8.7.
- Ask about unsolicited SAEs, AESIs, and new onset chronic medical conditions and record on the appropriate case report form.

8.4 Final Study Visit

8.4.1 Visit 23, Study Day 750±14, Day 722 (24 months) Clinic Visit Following Second Vaccination

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic or phone visit.

- Ask about febrile/rash illness. If the subject reports history of AFI/ARI within the past 14 days, conduct a febrile/rash illness assessment and sample collection as per Section 8.7.
- A targeted physical examination will be performed, if indicated based on review of complete medical history.
- Collect blood samples for humoral immunity and CPT tubes.
- Any unsolicited SAEs, AESIs, and new onset chronic medical conditions will be assessed and recorded on the appropriate case report form.

Table 2: Blood Volume Table

Study Visit	V00	V01	V02	V04	V05	V06	V07	V09	V10	V11	V14	V17	V20	V23
Study Day and Window	Screen D -28 to D -1	D1	D2	D8 ±1	D15 ±1	D29 ±3	D30	D36 ±1	D43 ±2	D57 ±3	D209 ±14	D388 ± 14	D569±14	D750±14
Study Day Post Second Study Vaccination		Vac # 1				Vac # 2	D2	D8±1	D15± 2	D29± 3	D181±14	D360 ±14	D541±14	D722±14
Blood Volume (in mL)														
Baseline Flavivirus serology	9													
Screening and Safety Clinical Laboratory Tests	22			10	10			10	10					
Serum for Humoral Immunity	0	27	0	0	27	27	0	0	27	27	27	27	27	27
CPT tubes for Immunity and systems biology	0	40	8	40	40	40	8	40	40	40	40	40	40	40
Paxgene tubes for Systems Biology	0	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5					
Daily Total Blood Volume	31	69.5	10.5	52.5	79.5	69.5	10.5	52.5	79.5	67	67	67	67	67
Cumulative Total Blood Volume	31	100.5	111	163.5	243	312.5	323	375.5	455	522	589	656	723	790

8.5 Early Termination Visit (if needed)

The following activities will be performed at the early termination visit for subjects who withdraw, or are withdrawn or terminated from this study:

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic visit or phone call.
- Ask about febrile/rash illness. If the subject reports history of febrile illness and either non-purulent conjunctivitis or arthraligia within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Record concomitant medications on the appropriate case report form if ≤ Day 29 after last study vaccination.
- Obtain vital signs including oral temperature, blood pressure, and pulse if visit occurs on or before Day 57.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Examine vaccination site for local reactions if visit occurs ≤28 days after first or 2nd vaccination.
- Review memory aid if visit occurs ≤8 days after 1st or 2nd vaccination.
- Obtain labs (safety, immunity (cellular and humoral) and systems biology) appropriate to that visit if visit occurs within window of a regular study visit.
- Collect urine for urine dipstick for glucose and protein if indicated as part of the safety laboratory tests.
- Assess for AEs if occurs on or prior to Day 57.
- Assess for unsolicited SAEs and AESIs and new onset chronic medical conditions and record on the appropriate case report form.
- Obtain urine for pregnancy if subject is a female of child bearing age and visit occurs on or before Day 57.

8.6 Unscheduled Visit (if needed)

Unscheduled visits may occur at any time during this study. Labs may be drawn at PI discretion. Any of the following activities may be performed:

- Obtain interim medical history, including an assessment for new medical conditions by interview of subjects and note any changes since the previous clinic visit or phone call.
- Ask about febrile/rash illness. If the subject reports history of febrile illness and either non-purulent conjunctivitis or arthralgia within the past 14 days, conduct a febrile/rash illness Assessment and sample collection as per Section 8.7.
- Record concomitant medications on the appropriate case report form if ≤ Day 29 after last study vaccination.
- Obtain vital signs including oral temperature, blood pressure, and pulse if visit occurs on or before Day 57.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Examine vaccination site for local reactions if visit occurs ≤28 days after first or 2nd vaccination.
- Review memory aid if visit occurs ≤8 days after 1st or 2nd vaccination.
- Obtain labs (safety, immunity (cellular and humoral) and systems biology) appropriate to that visit if visit occurs within window of a regular study visit.
- Collect urine for urine dipstick for glucose and protein if indicated as part of the safety laboratory tests.
- Assess for AEs if occurs on or prior to Day 57.
- Assess for unsolicited SAEs and AESIs and new onset chronic medical conditions and record on the appropriate case report form.
- May obtain urine for pregnancy if subject is a female of child bearing age and visit occurs on or before Day 57.

8.7 Febrile/Rash Illness Visit (if needed)

Febrile/Rash illness visits may occur at any time after the ICF is signed. Subjects will be encouraged to contact the clinic within 3 days of symptom onset and come in as soon as possible. This is a subtype of unscheduled visit where the subject reports a history of AFI or ARI within the prior 14 days and has come in for an evaluation, or reports AFI or ARI within the prior 14 days during a regular visit. If the report is made during a regular visit or within the window for a regular visit and the subject's safety allows, all the regular visit procedures should be performed as well.

- Obtain interim medical history, including querying for signs and symptoms of arboviral illness, any history of travel outside Puerto Rico, and record information including travel destination and duration of time at destination in the record, history of health care seeking for the illness, and an assessment for new medical conditions by interview of subjects. Note any changes since the previous clinic visit or phone call. Record all symptoms on the appropriate case report form.
- Record concomitant medications taken since the start of illness on the appropriate case report form.
- Obtain vital signs including oral temperature, blood pressure, and pulse.
- Perform a targeted physical examination, if indicated based on review of complete medical history.
- Collect blood (whole blood and serum) and urine for arbovirus qRT-PCR and to evaluate for other evidence of acute ZIKV and DENV infection.
- Assess for unsolicited AEs, SAEs and AESIs and new onset chronic medical conditions and record on the appropriate case report form.
- Obtain optional consent for photography if subject has a new rash.

9. ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Study vaccine-related serious adverse events occurring from the time of the first study vaccination through the end of the study period

2. Solicited Adverse Events – reactogenicity events occurring from Day 1 through Day 8 after the first and second administration of study vaccine:

    a) Injection site reactions: pruritus (itching), ecchymosis (bruising), erythema (redness), induration (hardness)/swelling, pain, and tenderness.

    b) Systemic reactions including fever, feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, rash, nausea, , vomiting, diarrhea, and abdominal pain.

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

4. New-onset chronic medical conditions, SAEs, and AESIs occurring from the time of first study vaccination through end of study follow up period.

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

9.2.1 Adverse Events

Adverse Event (AE): International Conference on Harmonisation (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 related to the use of a medicinal (investigational) product. FDA defines an AE as any untoward medical occurrence following the use of a study intervention/product in humans, whether or not considered related to study intervention.

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 (injection site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate case report form. Information to be collected for unsolicited AEs includes event description, date of onset, licensed study physician's assessment of severity and relationship to study vaccine or study product and alternate etiology (if not related to study vaccine) (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 study 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, it will be recorded as an AE.

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 case report form.

Severity of Event: AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator using a protocol-defined grading system. For events not included in the protocol-defined grading system (Section 9.2.2), 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 usual daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

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

- Related – There is a reasonable possibility that the study vaccine caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study vaccine and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study vaccine 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 as derived from the Division of Microbiology and Infectious Disease Revised 2013 Toxicity Table and will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Local (Injection Site) Reactogenicity Grading and Measurement

Reactogenicity	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Local reactions			
Pain	Does not interfere with activity **and** no pain medication is taken	Repeated use of non-narcotic pain reliever >24 hours **or** interferes with activity	Any use of narcotic pain reliever **or** prevents daily activity
Tenderness	Discomfort only to touch	Discomfort with movement **and** it interferes with daily activity	Significant discomfort at rest **and** it prevents daily activity
Pruritis (itching)	Does not interfere with daily activity	Intereferes with daily activity	Prevents daily activity
Ecchymosis (bruising)[a]	25-50 mm	51-100 mm	>100 mm
Erythema (redness)[a]	25-50 mm	51-100 mm	>100 mm
Induration (hardness)/swelling [b]	25-50 mm **and** does not interfere with activity	51-100 mm **or** interferes with activity	>100 mm **or** prevents daily activity

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable. The size of erythema, ecchymosis, and induration/swelling by itself will not be used as halting criteria.

[b] Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Feverishness (Chills/Shivering/Sweating)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue (Tiredness)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise (General Unwell Feeling)	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia (Body Aches/Muscular Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (Joint Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity or it requires > 24 hours of use of non-narcotic pain medication	Significant interference, prevents daily activity or it requires any use of narcotic pain medication
Nausea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Vomiting	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Diarrhea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Abdominal Pain	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Rash*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

* Not at injection site.

Oral temperature[#] will be graded as follows:

Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral†	38.0°C – 38.4°C 100.4°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 the first study vaccination will be considered as baseline.

* A fever can be considered not related to the study vaccine 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.

9.2.3 Additional Adverse Event Severity Grading

Pulse and blood pressure# will be graded as follows:

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Bradycardia - beats per minute	50 – 54 (or 45 – 49 if baseline < 60)	45 – 49 (or 40 – 44 if baseline < 60)	<45 (or <40 if baseline < 60)
Tachycardia - beats per minute	101 – 115	116 – 130	>130 (or ventricular dysrhythmias)
Hypotension (systolic) mm Hg	85 – 89	80 – 84	<80
Hypotension (diastolic) mm Hg	50 – 54	45 – 49	<45
Hypertension (systolic) mm Hg	141 – 150	151 – 160	>160
Hypertension (diastolic) mm Hg	91 – 95	96 – 100	>100

Pulse and blood pressure assessed on Screening will be considered as baseline.

Clinical Safety Laboratory Adverse Event Grading

Note: Ranges have been modified to reflect local laboratory normal ranges for the sites conducting this study

Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
WBC 10^3/UL (Decrease)	2.5 – 3.4	1.5 – 2.4	<1.5
WBC 10^3/UL (Increase)	10.9– 15.0	15.1 – 20.0	>20.0
HgB g/dL Female	10.5-11.4	8.5-10.4	<8.5
HgB g/dL Male	12.0-13.4	10.0-11.9	<10.0
HCT % decrease Female	31.5-34.2	25.5-31.4	<25.5
HCT % decrease Male	36.0-40.2	30-35.9	<30
HCT % increase	1.01-1.1 X ULN	1.11-1.2 X ULN	>1.2 X ULN
Platelets cell/10^3/UL (Decrease)	100-149	99-75	<75

Chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
ALT	1.25 – 3.0 x ULN	>3.0 – 5.0 x ULN	>5.0 x ULN
AST	1.25 – 3.0 x ULN	>3.0 – 5.0 x ULN	> 5.0 x ULN
Bilirubin– when ALT $\geq$3 x ULN	1.25 – 1.5 x ULN	>1.5 – 2.0 x ULN	>2.0 x ULN
Bilirubin	1.25 – 2.0 x ULN	>2.0 – 2.5 x ULN	>2.5 x ULN
BUN mg/dL	26-30	31-35	> 35
Creatinine mg/dL	1.1 – 1.7	1.8 – 2.0	>2.0

Urine Dipstick	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**

Protein	1+	2+	>2+
Glucose	1+	2+	>2+

9.2.4 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,
- Life-threatening adverse event*,
- Inpatient hospitalization or prolongation of existing hospitalization,
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- 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 patient or 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 vaccine and alternate etiology (if not related to study vaccine) by the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE form and CRF.
- Followed through resolution by the site principal investigator or sub-investigator.

- Reviewed and evaluated by an Independent Safety Monitor (ISM), the SMC (periodic review unless related), DMID, and the IRB.

9.2.5 Adverse Events of Special Interest

For this study Neurologic and Neuroinflammatory* Disorders after the first vaccination will be considered as Adverse Events of Special Interest (AESI). AESIs will be collected for this study through the end of study follow-up period and will be reported within 24 hours of site awareness. The study team will be notified by the data center when such an event is reported. Any AESI that meets a SAE criterion will be reported as SAE as well. All AESIs will be reviewed and evaluated by a study physician at the site, the DMID Medical Monitor, and the site ISM.

***Neurologic and Neuroinflammatory Disorders**: Acute Disseminated Encephalomyelitis, including site specific variants, Cranial Nerve Disorders (including paralyses/paresis), Guillain-Barré Syndrome (including Miller Fisher Syndrome and other variants), Immune-mediated Peripheral Neuropathies and Plexopathies, Optic Neuritis, Multiple Sclerosis, Narcolepsy, Transverse Myelitis, meningitis, or meningoencephalitis.

Subjects will be asked if they have any neurologic signs and symptoms at all follow-up visits after the first ZPIV vaccination.

9.2.6 Procedures to be Followed in the Event of Abnormal Clinical Findings

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

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

Any AE that meets a protocol-defined serious criterion 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 AdvantageEDC. 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 when they are provided to the DMID Pharmacovigilance Group. The ISM will provide a written assessment to DMID. The DMID Medical Monitor, DMID Medical Officer 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.

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

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 all participating 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 vaccine(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 AdvantageEDC on the Pregnancy Report form. No further study vaccinations will be administered to pregnant subjects, but with the subject’s permission all study mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome pending the subject’s permission.

9.4 Type and Duration of Follow-up of Adverse Events

Solicited injection site and systemic AEs (reactogenicity events) will be documented and reported from Day 1 through Day 8 after each study vaccination via memory aid, clinic visits on Day 2 and Day 8 after vaccination, and interview by telephone visit on Day 4 after each study vaccination.

Unsolicited AE will be documented from Day 1 through approximately 28 days after the last vaccination. Blood for safety laboratory tests will be collected on Day 8 and Day 15 after each study vaccination. All AEs will be assessed and followed through resolution or stabilization even if this extends beyond the study-reporting period.

AEs related to AFI/ARI will be collected and assessed from the time the ICF is signed through the end of the study, and followed until resolution or stabilization.

SAE, AESIs, and new-onset chronic medical conditions will be collected and assessed from the time of the first study vaccination through the end of the study, and followed until resolution or stabilization. Resolution of an AE and SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

AFI and ARI will be collected after signing ICF through the end of the study.

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

9.5 Halting Rules

9.5.1 Halting Rules for Decision to Enroll Non-sentinel Group

If any of the following conditions are met by either of the sentinel subjects in the 7 day follow up period after each vaccination (regardless of the relationship to the study vaccine), all new enrollments or 2nd vaccinations will be halted until the SMC reviews the safety data electronically (E-Review).

- If any sentinel subject experiences anaphylaxis, laryngospasm, bronchospasm, or generalized urticaria within 1 day after vaccination; *OR*
- If any sentinel subject experiences a Grade 3 AE lasting > 1 day not resulting from trauma or accident; *OR*
- If any two sentinel subjects experience a Grade 2* AE lasting > 1 day not resulting from trauma or accident; *OR*

  *The size of erythema, ecchymosis, or induration/swelling will not be used as a halting criterion and subjective systemic reaction must be corroborated by study personnel

- If any sentinel subject experiences a SAE or AESI other than the result from trauma or accident.

9.5.2 Halting Rules for Discontinuation from Second Vaccination

The following assessments should be performed prior to the administration of the second vaccination to determine if the subject remains eligible. A subject will not be given the boost vaccination if any of the following criteria are met:

1. Generalized urticaria, anaphylaxis, laryngospasm or bronchospasm within 3 days after administration of a study vaccine; *OR*
2. A serious adverse event/AESI that is considered to be related to any of the study vaccines; *OR*
3. A severe (grade 3) laboratory abnormality that is considered to be related to any of the study vaccines; *OR*
4. A severe (grade 3) unsolicited adverse event that is considered to be related to any of the study vaccines; *OR*
5. A severe (grade 3) solicited injection site reaction with a duration of 3 or more days; *(*The size of erythema, ecchymosis, or induration/swelling by itself will not be used as halting criteria*); OR*

6. A severe (grade 3) solicited systemic adverse event considered to be related to any of the study vaccines with a duration of 3 or more days; *OR*
7. Grade 2 or higher fever within 1 day after 1st study vaccination; *OR*
8. New or ongoing grade 2 or greater local, systemic, solicited or unsolicited adverse event, including safety lab abnormalities, at the time of 2nd dose of study vaccine administration (adverse events must decrease to grade 1 or less within the protocol-specified window for the 2nd study vaccination); *OR*
9. Subject becomes pregnant; *OR*
10. Subject develops a new illness or condition that meets the exclusion criteria; *OR*
11. Subject develops a new medical condition or medication change for which continued participation, in the opinion of the investigator, would pose a risk to the subject, would interfere with the subject's ability to complete the study, or would be likely to confound interpretation of the results; *OR*
12. Subject no longer willing and/or able to adhere to study restrictions outlined in the inclusion and exclusion criteria; *OR*
13. Subject has a fever (defined as an oral temperature ≥38.0°C) or a moderate or severe acute illness at the time of boost vaccination administration or in the 2 days prior to Day 29. The 2nd study vaccination can be deferred until the fever or illness resolves or only minor residual symptoms remain, that in the opinion of the investigator, will not interfere with the ability to assess safety parameters as required by the protocol, provided that the boost is given within the protocol-defined study window. Permission to give the 2nd study vaccination outside the protocol-defined study window must be obtained from DMID.

    Note: in case the boost vaccination is postponed, the timing of the safety/immunogenicity visits post-boost will be planned relative to actual vaccination day.

9.5.3 Study Halting Rules

If one or more of the halting rules, as outlined below, is met, further enrollment and vaccinations will be withheld until the SMC reviews the safety data electronically (E-Review).

1. Death following vaccination and prior to the subject's last visit that was not the result of trauma or accident, regardless of the relationship to the study vaccine; OR

2. If any subject experiences anaphylaxis, laryngospasm, bronchospasm, or generalized urticaria within 1 day after vaccination; OR

3. If two subjects experience a Grade 3 AE in the same system organ class (SOC) including laboratory abnormalities within 30 days of vaccination that is related to vaccination and which does not resolve within 2 days; OR

4. If any subject experiences a SAE/AESI that is related to vaccination.

Note: If halting rule #3 has been met during the course of the study, subjects have been reviewed, and the study resumed, the following approach will be used after the first halt: If an additional grade 3 adverse event related to study product occurs, subsequent vaccinations will cease pending SMC review. The SMC will be provided the information electronically for a rapid review/assessment to be completed within 24 hours. The SMC will provide feedback regarding the relatedness to vaccine and recommendations regarding how to proceed (e.g, formal halt and/or ad hoc meeting of the SMC). If SMC review cannot be secured within 24 hours, the study will be halted until SMC review. If there is a grade 3 adverse event which is not related to vaccination but sufficient concern remains, then details will be provided to the SMC electronically. The SMC, when possible, will provide an opinion on the relatedness of the AE within 24 hours. In such a scenario the SMC will decide whether the study should be halted and/or an ad hoc SMC meeting scheduled.

9.6 Safety Oversight

9.6.1 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs and AESIs in real time and other AEs as needed and provide a written report to DMID. The ISM will have experience in infectious diseases or internal medicine, in close proximity to the participating VTEU site, and have the authority to readily access study subject records.

9.6.2 Research Monitor

An independent Research Monitor will be assigned to this study to monitor conduct of the study. The Research Monitor will work with the ISM to provide, evaluate, and report safety monitoring information to the IRB and, Sponsor, in a timely fashion.

The Research Monitor is a qualified physician not associated with this protocol and is responsible to oversee the safety of the research and report observations/findings to the site IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the site IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the site IRB can assess the monitor's report; and shall have the responsibility to promptly report their observations and findings to the site IRB and Sponsor.

9.6.3 Safety Monitoring Committee (SMC)

Safety oversight will be conducted by a SMC which is an independent group of experts that monitors subject safety and advises DMID. Members of the SMC will be separate and independent of study personnel participating in this study and should not have scientific, financial or other conflict of interest related to the study. The SMC will consist of at least 3 voting members with appropriate expertise to contribute to the interpretation of the safety data from this trial.

The SMC meetings for data review are as follows:

- Organizational meeting (prior to start of the study)
- Data Review Meetings (DRM) will be held during the study. The SMC will review and evaluate accumulated trial data for subject safety, trial conduct, and trial progress at the below timepoints. Unless a Halting rule has been met or there is a safety concern, screening, enrollment, and vaccination will continue while the data is being prepared and reviewed by the SMC for these DRMs.
- For Groups 1 and 2: 4 reviews of– all available safety data through Day 8 after 1st and 2nd vaccination.
- In addition, the SMC will convene to review each of the interim analyses as outlined in section 11.3.

- Annually for aggregate review of SAEs/AESIs.

- An ad hoc SMC meeting will be convened when a halting rule is met, or at the request of an investigator and/or DMID if there are safety concerns during the course of the study.

- Final Data Review Meeting: After clinical database lock to review the cumulative unblinded safety data for this trial. The data will be provided in a standard summary format.

The SMC will operate under the rules of a DMID-approved charter. Data reviews may include enrollment and demographic information, medical history, concomitant medications, physical assessments, dosing compliance, protocol adherence, clinical laboratory values, 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 will receive data in aggregate. The SMC may also be provided with observed rates of the AEs in an unblinded fashion, and may request 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 objective of the SMC is to make recommendations to the sponsors if the study should continue per protocol, be modified and then proceed, or be terminated. After each review/meeting the SMC will make recommendations as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

The DMID Medical Monitor is empowered to stop study enrollment and vaccine administration if adverse events that meet the halting criteria are reported or if any serious safety concerns arise. The DMID Medical Monitor and the ISM will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during the study.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject 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 standard operating procedures. 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, CRFs, source data (paper and electronic), audit trails, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the study 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:

The primary goal of this study is to assess and compare the safety and reactogenicity of a homologous prime boost regimen of ZPIV given at two different dose levels in a population living in a flavivirus/ZIKV endemic area. Secondary goals are to explore the humoral responses to ZIKV after each vaccination in subjects with and without pre-existing flavivirus/ZIKV immunity. Blood samples are also collected for measuring ZIKV-related cellular and humoral immunity and systems biology in future assays. Enhanced surveillance for febrile and rash illness after signing the ICF will be performed to obtain preliminary data on the incidence and clinical presentation of naturally occurring flavivirus infections after vaccination. This study, like other Phase I studies, is exploratory rather than confirmatory, and thus the objective is not to formally any test hypotheses, though comparisons between vaccine and placebo and between dose levels will be made. In addition, comparisons will be stratified by pre-vaccination flavivirus immune status of the volunteer and outcomes of flavivirus-naïve and flavivirus immune volunteers will be compared.

11.2 Sample Size Considerations

The sample size for this study was selected to obtain preliminary estimates of vaccine safety and immunogenicity in a time sensitive manner. This study is not designed to test a specific null hypothesis. As this is a phase I study of ZPIV, it is primarily designed to collect initial information about safety and immune responses of two different doses of the vaccine. Due a natural disaster (hurricane Maria), 11 subjects had loss of serum samples at key timepoints and will not be included in any immunogenicity analyses, resulting in approximately 35 evaluable active subjects and 5 placebo subjects in each dose group for those analyses. These 11 subjects will be included in the safety analysis population resulting in approximately 45 active subjects in the 5.0 mcg dose group and 35 active subjects in the 2.5 mcg dose group, for a total of approximately 80 subjects receiving at least one study vaccination of ZPIV.

The sample size in each dose cohort is small, thus the precision of estimate for AEs is limited. Rare AEs associated with dose are not demonstrable in a clinical study of this size; however the probabilities of observing one or more AEs within a dose group at the minimum dose sample size of 35 and also among the approximately 80 subjects receiving ZPIV are presented in the table below. The minimum detectable event rates to achieve various levels of power are also displayed.

If there are AEs associated with a particular dose of ZPIV, this study will have at least 80% power to observe at least one such event in a dose cohort if the true rate is 4.5%. If there are AEs associated with ZPIV overall, independent of dose, this study will have approximately 80% power to observe at least one such event in all ZIKV vaccine subjects if the true rate is 2.0%.

Table 3a: Probability of observing at least one Adverse Event given various True Event Rates

Sample Size	"True" Unknown Event Rate	Probability of Observing an Event (%)	Sample Size	"True" Unknown Event Rate	Probability of Observing an Event (%)
35	0.1%	3.4	80	0.1%	7.7
	0.5%	16.1		0.5%	33.0
	1.0%	29.7		1.0%	55.2
	2.0%	50.7		2.0%	80.1
	3.0%	65.6		3.0%	91.3
	4.0%	76		4.0%	96.2
	5.0%	83.4		5.0%	98.3
	10.0%	97.5		10.0%	>99.9
	20.0%	>99.9		20.0%	>99.9

Table 3b: Minimum Detectable Event Rates given various levels of Power

Sample Size	Desired Power Level	Detectable Event Rate	Sample Size	Desired Power Level	Detectable Event Rate
35	0.80	4.5%	80	0.80	2.0%
	0.90	6.4%		0.90	2.8%
	0.95	8.2%		0.95	3.7%
	0.99	12.3%		0.99	5.6%

11.3 Planned Interim Analyses

11.3.1 Analysis Plan

This study has 3 planned interim analyses, all of which will be performed on monitored and locked datasets. The first two interim analyses will consider safety, reactogenicity, and immunogenicity data and will take place at the following time points:

- The first interim analysis will take place after the last subject in Group 1 has been followed through 28 days post-second ZPIV administration (Day 57) and the first interim database has been locked.

- The second interim analysis will take place after the last subject in Group 2 has been followed through 28 days post-second ZPIV administration (Day 57) and the second interim database has been locked.

In these first two interim analyses, safety and reactogenicity data will be summarized in aggregate by enrollment group, while humoral immunogenicity data will be presented by study treatment and pre-vaccination flavivirus immune status. Note that safety/reactogenicity data and immunogenicity data may be presented separately, as results become available.

- The third interim analysis will describe the incidence and severity of AFI/ARI after all subjects have completed one-year follow-up after the 2nd vaccination. In this interim analysis, data will be summarized in aggregate by enrollment group and pre-vaccination flavivirus immune status.

No individual data will be released in any interim analyses. However, these analyses will be made available to the study team and may impact the course of this study, be published, or be used in the planning of future studies.

The interim safety analyses may include tables summarizing demographics, study status, and protocol adherence information. Tables and figures will present unsolicited adverse events by MedDRA System Organ Class (SOC) and Preferred Team (PT), severity, and relationship to study product. Solicited adverse events will be summarized by symptom, severity, and study day. Laboratory results will be summarized by parameter, severity, and study day. Listings of early terminations, protocol deviations, and clinical data may be included.

The interim immunogenicity analyses **may** include summaries of geometric mean titers (GMTs) and their associated 95% confidence intervals (CIs) by visit and treatment group as well as peak GMT by treatment group and by pre-vaccination flavivirus immune status. The geometric mean fold rise (GMFR) and its associated 95% CI as well as the proportion of subjects that seroconvert to ZIKV will be summarized by visit and treatment group and pre-vaccination

flavivirus immune status. Titer results will also be displayed in reverse cumulative distribution (RCD) curves by visit and treatment group.

11.4 Final Analysis Plan

The primary Clinical Study Report (CSR) will summarize all safety and protocol-specified humoral immunogenicity results, including all primary and secondary endpoint data, collected through Day 750. Given the urgency to obtain data in a timely manner, additional humoral and cellular immunogenicity assessments will be summarized in one or more addenda to the main CSR. Prior to the interim database lock, a formal Statistical Analysis Plan that specifies all planned analyses will be developed. In the following an abbreviated version of the planned primary and secondary analysis is provided.

11.4.1 Analysis Populations

The Safety Analysis population includes all subjects who received at least one study vaccination.

The immunogenicity population includes all subjects who received at least one study vaccination and contributed both pre- and at least one post-study vaccination samples for immunogenicity testing for which valid results were reported. Subjects with a loss of samples at key timepoints due to the natural disaster (hurricane Maria) will be excluded from the immunogenicity population.

The Per Protocol (PP) population includes all subjects in the immunogenicity population 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:
  - Second vaccination not received,
  - Second vaccination received out of window,
  - Receipt of non-study licensed live vaccine within 30 days prior to or after each study vaccination,
  - Receipt of non-study licensed inactivated vaccine within 14 days prior to or after each study vaccination,
  - Receipt of immunosuppressive therapy (e.g., corticosteroids) within 30 days prior to or after each study vaccination.
- Data from any visit that occurs substantially out of window.

In the case of mis-randomization, subjects in all analysis populations will be analyzed according to the study vaccine actually received.

Subjects will additionally be classified by baseline flavivirus immune status, defined as follows:

- ZIKV and DENV naïve (no detectable antibodies to ZIKV or DENV pre-vaccination)
- ZIKV seropositive (detectable antibodies to ZIKV pre-vaccination).
- DENV seropositive (detectable antibodies to DENV pre-vaccination)
- ZIKV and DENV seropositive (detectable antibodies to ZIKV and DENV pre-vaccination)

11.4.2 Safety Analysis

The number, percentage (observed rate), and exact two-sided 95% CI for subjects reporting each solicited injection site reaction and solicited systemic reaction within 7 days following vaccination will be summarized by treatment group for each vaccination as well as overall (following any vaccination). In addition, maximum severity and duration of local and systemic reactions will be determined for each subject and summarized by group following each vaccination (as well as overall) and the resulting number and percentage of subjects will be summarized by severity grade (none, mild, moderate, severe). The number and percentage of subjects overall and in each group with laboratory AEs or abnormal laboratory values from the time of first vaccination through 28 days after the last vaccination will be listed by study visit and by toxicity grade.

For formal group comparisons of local reactogenicity, subjects will be assigned to “none” and “mild, moderate or severe (Grade 1, 2 or 3)” severity groupings based on their maximum severity of any solicited injection site reaction following 7 days after any vaccination. Local “mild, moderate, or severe” rates for subjects receiving moderate dose ZPIV and subjects receiving high dose ZPIV will be compared using a Fisher’s Exact Test. In addition, rates for any severe (Grade 3) local, systemic or laboratory toxicity reaction following any vaccination will be compared between the groups using a Fisher’s Exact Test.

The occurrence of SAEs and AESIs (see Section 9) will be presented by relationship to study vaccination, number and percentage of subject as well as by MedDRA® same System Organ Class (SOC) and Preferred Term (PT) for each study group as well as combined across study groups. Related SAE rates will be compared between the groups using a Fisher’s Exact Test.

The proportion of subjects reporting at least one related unsolicited AE until the end of the study will be summarized by MedDRA SOC and PT. The number, percentage (observed rate) and exact two-sided 95% CI for subjects reporting a related unsolicited AE overall, by same SOC, and by PT will be summarized by group for each vaccination.

The number and percentage (observed rate) of subjects that withdrew or discontinued their vaccination series due to any reason will be tabulated. Withdrawal/discontinuation rates between dose groups will be compared using a Fisher’s Exact Test.

Please see the separate Statistical Analysis Plan for additional information.

11.4.3 Immunogenicity Analysis

The percentage of subjects overall, by baseline flavivirus immune status, and by treatment group that seroconvert to ZIKV (as measured by ELISA and neutralization assay) will be described using exact 95% confidence intervals (CI). In addition, the geometric mean fold rise (GMFR) compared with baseline (Visit 00) will be summarized by baseline flavivirus immune status, treatment group and visit.

Subject-specific peak and per-visit results will be described. In addition, peak and per-visit GMTs and 95% CIs for each treatment group and baseline flavivirus immune status will be provided.

11.4.4 Surveillance Analysis

We will describe the number and proportion of subjects who develop an AFI or an ARI who have ZIKV or DENV RNA or other evidence of acute ZIKV or DENV infection detected within 14 days of symptom onset. We will analyze these endpoints for all subjects overall, and by treatment group and pre-vaccination flavivirus immune status. We will also describe the severity of these laboratory confirmed ZIKV and DENV infections overall, by treatment group, and pre-vaccination immune status. Severity endpoints for ZiKV infection severity will be defined using duration of symptoms, grade of symptoms, use of concomitant medications, and need for hospitalization or other medical care. Severity endpoints for DENV infections will be based on these items as outlined in the 1997 and 2009 World Health Organization (WHO) guidelines. Please see the separate Statistical Analysis Plan for additional information.

11.4.5 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

Each participating 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. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, monitoring, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data.

Source data are all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records 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, photography, 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 clinical trial. Data collection forms for use as source documents will be derived from the CRFs and be provided by the SDCC.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating VTEU site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all trial-related sites, source data/ case report forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and 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(s) 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 Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

A Central Institutional Review Board/Independent Ethics Committee (IRB/IEC) will approve all study-related materials prior to their use. The IRB of the VTEU site with oversight of the subcontract site (Saint Louis University) will also approve all materials and have oversight of the study.

Prior to enrollment of subjects into this trial, the approved protocol and ICFs will be reviewed and approved by the appropriate IRB (Central IRB for subcontract site, site IRB for VTEU site) listed on its FWA.

The responsible official for each 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 Federal Wide Assurance numbers will be provided to DMID.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the site principal investigator for submission to the respective IRBs.

14.3 Informed Consent Process

14.3.1 Informed Consent

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 21 CFR Part 50 and 45 CFR 46 and the local IRB.

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, including the nature and risks of this trial, alternate therapies, any known AEs, the investigational status of the components, 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 trial, after having the nature and risks 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.

An ICF describing in detail the study interventions/products, study procedures, risks and possible benefits are given to subjects. The ICF will be translated into Spanish. 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 the research study 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/interventions being done specifically for this trial, including administering study vaccine. .

DMID will provide the site principal investigator, in writing, any new information that significantly impacts the subjects' risk of receiving the investigational vaccine. 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.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the subjects consent; however, before any study procedures are performed to determine protocol eligibility an ICF must be signed. Subjects will be given a copy of all ICFs that they sign.

By signing the ICF, subjects agree to complete all evaluations 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.

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

This trial will be inclusive of all subjects who are 21 to 49 years of age who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, gender, gender orientation, or ethnic

background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations of subjects.

14.5 Subject Confidentiality

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

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 participating VTEU and subcontract sites as part of this trial (other than a subject's medical records) will be kept confidential by the site principal investigator and other study personnel to the extent permitted by law. This information and data will not be used by the site principal investigator 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 investigator 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. The study monitor, Department of Defense, 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 investigator. This includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this trial. The participating VTEU subcontract site will permit access to such records.

14.6 Study Discontinuation

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

14.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for taking part in this trial.

Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the central IRB's policies and procedures, and subject to IRB approval.

If it is determined by the participating VTEU subcontract site and the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating VTEU subcontract site, such as giving emergency medications to stop immediate allergic reactions to the study vaccine. No financial compensation will be provided to the subject by the participating VTEU subcontract site for any injury suffered due to participation in this trial.

14.8 Future Use of Stored Specimens

Subjects must give concurrent consent for collection and storage of samples for use in Zika/flavivirus-related future research studies examining humoral, cellular, and other immunological assessments including systems biology to be enrolled and receive first vaccination. These studies are in addition to the protocol-specified humoral immunity studies. Samples collected for the future assessment of immunity to ZIKV/flaviviruses and systems biology will not be destroyed until all assessments are completed. Any residual/remnant samples remaining after all ZIKV/flavivirus-related immunogenicity assessments are completed may be used in additional future research studies of immune responses to infectious microbes. Samples will be stored at a central clinical storage facility indefinitely for future use and may be shared with investigators at the participating VTEU sites and with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality. Subjects may withdraw their consent for future use of stored samples at any time after receipt of 1st study vaccination (section 5.3.4), however all protocol-specified humoral immunity studies to flaviviruses will be completed. Withdrawal of consent for future use of samples must be in writing.

HLA Typing may be performed on future use PBMCs collected prior to vaccination on Day 1, to understand immune response to ZPIV. This testing will be conducted only on blood samples of subjects who have consented for genetic testing.

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

There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will NOT be kept in their health records.

14.9 Disclosure of Study Related Information

All subjects can choose to receive written disclosure of the treatment assignment and dose of study vaccine received after the final database lock.

15 DATA HANDLING AND RECORD KEEPING

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

Case report forms will be created by the SDCC to record and maintain data for each subject enrolled in this study.

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

15.1 Data Management Responsibilities

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 case report form and provided by the SDCC to record and maintain data for each subject enrolled in the study. 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 CRF should be consistent with all source documents or the discrepancies should be explained.

The sponsor will provide guidance to investigators on making corrections to the data collection forms and CRFs.

15.2 Data Capture Methods

Clinical (including, but not limited to, AE and SAEs), reactogenicity, concomitant medications, medical history, screening and safety lab values, and physical assessments), 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 primarily be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Clinical (including, but not limited to, AE and SAEs, reactogenicity, concomitant medications, medical history, safety lab values, outcome measures, and physician assessments), viremia by qRT-PCR, and immunogenicity data will be collected.

15.4 Timing/Reports

A final report will be prepared following the availability of all the clinical, safety, reactogenicity, and immunogenicity data. Interim statistical reports may be generated as deemed necessary and appropriate by DMID. 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 and subcontract site with a summary of results by treatment group and/or subject treatment assignments. In this regard, the VTEU site and subcontract site requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines. See Section 14.9 for further details.

15.5 Study Records Retention

Study records and reports, including, but not limited to, CRFs, source documents, ICFs (except for future use ICFs), laboratory test results, and medication inventory records, shall be retained for 2 years following the date 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 such indication, until 2 years after the investigation is discontinued and FDA is notified. The site must contact DMID for authorization prior to the destruction of any study records. ICFs for future use will be maintained as long as the samples exist.

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 the supervisory VTEU site and to DMID, via the SDCC's AdvantageEDC^{SM}.

All protocol deviations, as defined above, must be addressed in study subject case report 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 central IRB/IEC 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 Office of Extramural Research (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

17 LITERATURE REFERENCES:

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SUPPLEMENTS/APPENDICES

APPENDIX A

SCHEDULE OF PROCEDURES AND EVALUATIONS

Study Visit	V00	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14	V15	V16	V17	Fever /Rash	Early Term	Unsch Visit
Overall Study Day	Screen D-28 to--1	D1 Vaccination	D2	D4 +1 Phone call	D8 ±1	D15 ±1	D29 ±3	D30	D32 +1 Phone call	D36 ±1	D43 ±2	D57 ±3	D107 ± 14 Phone call	D157±14 Phone call	D209 ±14	D268 ±14 Phone Call	D328 ±14 Phone call	D388 ±14			
Study Day after Second Vaccination							D1 Vaccination 2	D2	D4+1 Phone call	D8 ±1	D15 ±2	D29 ±3	D79±14 Phone call	D129±14 Phone call	D181 ±14	D240±14 Phone call	D300 ±14 Phone call	D360 ±14			
Informed Consent[1]	X																				
Review Eligibility Criteria[2]	X	X					X														
Medical History[3]	X	X[2]	X	X	X	X	X[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (T,P,& BP)[5]	X	X	X		X	X	X	X		X	X	X							X	X[15]	X[15]
Height & Weight	X																				
Abbreviated Physical Exam[6]	X																				
Serology (HIV-1/2, HBV, HCV)	X																				
Serology (ZIKV, DENV)	X																		X[19]		
Randomization/Enrollment		X																			
Evaluate Pre-administration reactogenicity		X					X														
Study Product Administration and observation for 30 minutes		X					X														

Study Visit	V00	V01	V02	V03	V04	V05	V06	V07	V08	V09	V10	V11	V12	V13	V14	V15	V16	V17	Fever /Rash	Early Term	Unsch Visit
Overall Study Day	Screen D-28 to--1	D1 Vaccination	D2	D4 +1 Phone call	D8 ±1	D15 ±1	D29 ±3	D30	D32 ±1 Phone call	D36 ±1	D43 ±2	D57 ±3	D107± 14 Phone call	D157±14 Phone call	D209±7	D268 ±14 Phone Call	D328 ±14 Phone call	D388 ±14			
Study Day after Second Vaccination							D1 Vaccination	D2	D4+1 Phone call	D8 ±1	D15 ±2	D29 ±3	D79 ±14 Phone call	D129±14 Phone call	D181 ±7	D240±14 Phone call	D300 ±14 Phone call	D360 ±14			
Give Memory Aid[9]		X					X														
Evaluate vaccine site[11]		X	X		X	X	X	X		X	X	X								X[13]	X[13]
Targeted Physical Exam[6]		X	X		X	X	X	X		X	X	X			X			X	X	X	X
Serum or Urine Pregnancy Test[7]	X	X					X					X								X[13]	X[13]
Safety Lab Evaluations[8]	X				X	X				X	X									X[14]	X[14]
Urine for protein and glucose	X				X	X				X	X									X[14]	X[14]
Concomitant Medications	X	X	X	X	X	X	X[2]	X	X	X	X	X							X	X	X
Review Memory Aid			X	X	X			X	X	X										X[12]	X[12]
AE Collection		X	X	X	X	X	X	X	X	X	X	X							X	X[15]	X[15]
AFI/ARI/ SAE/AESI/new medical conditions[22, 20]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum for Humoral Immunity		X[16]				X	X[16]				X	X			X			X		X[17]	X[17]
CPT tubes for future immunity/systems biology[21]		X[16,18,]	X[18]		X[18]	X[18]	X[16,18]	X[18]		X[18]	X[18]	X			X			X		X[17]	X[17]
Paxgene tubes for future systems biology		X[16]	X		X	X	X[16]	X		X	X									X[17]	X[17]

Study Visit	V18	V19	V20	V21	V22	V23															
Overall Study Day	D448±14 Phone Call	D 508±14 Phone Call	D569 ±14	D629±14 Phone call	D689±14 Phone Call	D750±14															
Study Day after Second Vaccination	D420±14 Phone Call	D 480±14 Phone Call	D541 ±14	D601 ±14 Phone Call	D661 ±14 Phone Call	D722±14															
AFI/ARI /SAE/AESI/new medical conditions[22, 20]	X	X	X	X	X	X															
Medical History[3]	X	X	X	X	X	X															
Serum for Humoral Immunity			X			X															
CPT tubes for future immunity/systems biology			X			X															
Targeted Physical exam[6]			X			X															

Definitions: AE = adverse event; BP = blood pressure; DENV = dengue virus; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV 1/2 = human immunodeficiency virus types 1 and 2; HR = heart rate; T = body temperature; SAE = serious adverse event; AESI = Adverse event of Special Interest; ZIKV = Zika virus.

[1] Must describe study, administer informed consent, and have informed consent form (ICF) signed prior to initiation of any study-related procedures.

[2] Subjects must meet eligibility criteria; these criteria should be reviewed prior to administration of prime and boost dose of study vaccine or placebo.

[3] Obtain complete medical history from subject at first screening visit and update it at Day 1 prior to vaccination; interim medical history obtained at follow-up visits.

[5] Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Repeat of vital signs allowed once if found to be abnormal on Day 1 and Day 29.

[6] Abbreviated physical examination is a complete exam with no genitourinary and rectal exam performed. Targeted physical exams are done at scheduled clinic visits if indicated based on review of medical history.

[7] Females of childbearing potential will have serum pregnancy test done at screening and urine pregnancy test done in the 24 hours prior to each study vaccination; Results must be negative to enroll subject and negative prior to each study vaccination. Urine pregnancy test may be done if subject withdrawals from study early or has an unscheduled visit.

[8] Screening laboratory tests, including WBC, hemoglobin, hematocrit, platelet count, ALT, AST, bilirubin, BUN, and creatinine, may be repeated once on a second screening visit If test is out of acceptable range, provided there is an alternative explanation for the out of range value; All screening tests need to be done within 28 days of Day 1, otherwise all tests, except for serology for HIV 1/2, Hepatitis C virus, or Hepatitis B surface antigen, will need to be repeated within the screening period. Safety laboratories include WBC, hemoglobin, hematocrit, platelet count, ALT, AST, bilirubin, BUN, and creatinine.

[9] Distribute memory aid to subjects and give them study materials.

[11] Assess vaccination site.

[12] If visit occurs ≤8 days after 1st of 2nd vaccination. Includes local and systemic reactogenicity assessment

[13] If prior to ≤28 days after the last study vaccination.

[14] If visit occurs ≤15 days after 1st or 2nd vaccination.

[15] If visit occurs on or prior to Day 57.

[16] Obtained prior to vaccination.

[17] If visit occurs within window of a study Day when these labs would be collected.

[18] Part of the plasma from the CPT tubes will be saved for systems biology.

[19] Baseline serology will include testing for DENV and ZIKV. AFI/ARI assessment will include testing for DENV, ZIKV, and CHIKV.

[20] If the subject reports history of AFI or ARI within the past 14 days at any time after signing the ICF, conduct an AFI/ARI assessment and sample collection as per Section 8.7,

[21] PBMCs for HLA typing only on Day 1.

[22]. Assess for SAEs and AESIs from time of first ZPIV administration until end of study.