CLINICAL STUDY PROTOCOL

PROTOCOL TITLE:	A Phase 2 Open-Label Study to Assess the Safety and Immunogenicity of an Alum-Adjuvanted Chikungunya Virus-Like Particle Vaccine (PXVX0317) in Prior Recipients of Other Alphavirus Vaccines Versus Alphavirus Naïve Controls
PROTOCOL NUMBER:	EBSI-CV-317-002
WRAIR IRB PROTOCOL NUMBER:	[redacted]
INVESTIGATIONAL PRODUCT:	PXVX0317 (CHIKV VLP Vaccine)
SPONSOR:	Emergent Travel Health Inc. [redacted]
FUNDING SOURCE:	U.S. Department of Defense and Emergent Travel Health Inc.
PROJECT OVERSIGHT AGENCY:	Walter Reed Army Institute of Research (WRAIR)
STUDY PHASE	2
SPONSOR MEDICAL MONITOR:	[redacted]
VERSION:	4.0
DATE:	25 JAN 2021

PROTOCOL SIGNATURE SHEET

This Clinical Study Protocol document and all information provided to you related to this protocol are the confidential and proprietary information of Emergent Travel Health Inc. ("Sponsor") and are subject to the terms of the Confidential Disclosure Agreement between you and Emergent Travel Health Inc.

By signing below, I hereby confirm the following:

I agree to abide by the terms of the Emergent Travel Health Inc. Confidential Disclosure Agreement.

I have read this protocol in its entirety and I agree to conduct the study according to this protocol. Any changes in procedure will only be made if necessary to protect the safety, rights, or welfare of subjects.

I agree to comply with the current International Conference on Harmonization Tripartite Guideline on Good Clinical Practice in addition to the appropriate FDA Code of Federal Regulations (CFRs), Department of Defense (DoD) and state and local regulations.

I agree to conduct the study in person or to supervise the study.

I agree to ensure that all who assist me in the conduct of the study have access to the study protocol, including any amendments thereto, and are also made aware of their responsibilities in meeting the foregoing obligations.

Principal Investigator
(Print Name)

Title

Signature **Date**

Site to send signed original to Emergent Travel Health Inc. and to keep a copy for files.

PROTOCOL SYNOPSIS

PROTOCOL TITLE	A phase 2 open-label study to assess the safety and immunogenicity of an alum-adjuvanted chikungunya virus-like particle vaccine (PXVX0317) in prior recipients of other alphavirus vaccines versus alphavirus naïve controls
SITES	Two US sites, Walter Reed Army Institute of Research (WRAIR) Clinical Trials Center (CTC) and US Army Medical Research Institute for Infectious Disease (USAMRIID) immunization clinic.
OBJECTIVES	It is currently unknown whether prior exposure to heterologous alphaviruses will enhance or interfere with immune responses to chikungunya virus (CHIKV) exposure or vaccination. The objective of this study is to evaluate the safety and immunogenicity of the chikungunya vaccine candidate PXVX0317 when administered to prior recipients of experimental alphavirus vaccines versus alphavirus naïve gender and age-matched controls. **Safety Objectives** • Evaluate the safety of PXVX0317 when administered to prior alphavirus vaccine recipients versus gender and age-matched controls. **Immunogenicity Objectives** Primary ▪ Evaluate the neutralizing antibody response to chikungunya virus induced by PXVX0317 when administered to prior alphavirus vaccine recipients versus gender- and age-matched controls. Secondary ▪ Evaluate the overall antibody responses to CHIKV and Venezuelan equine encephalitis virus (VEEV) induced by vaccination with PXVX0317 when administered to prior alphavirus vaccine recipients versus gender and age-matched controls. Exploratory ▪ Evaluate the cellular immune response to chikungunya virus induced by vaccination with PXVX0317 when administered

	to prior alphavirus vaccine recipients versus gender and age-matched controls. ▪ Evaluate the humoral immune responses to eastern equine encephalitis virus (EEEV) and western equine encephalitis virus (WEEV) induced by vaccination with PXVX0317 in selected subjects who previously received these vaccines versus alphavirus-naïve controls. ▪ Collect human immunoglobulin for use in a mouse challenge study. ▪ Evaluate the ability of CHIKV antibodies to PXVX0317 to neutralize diverse alphavirus strains.
PRIMARY IMMUNOGENICITY ENDPOINT	The anti-CHIKV seroconversion rate at Day 22 in prior alphavirus vaccine recipients versus alphavirus-naïve controls, where seroconversion is defined as a 4-fold rise over baseline in anti-CHIKV neutralizing antibodies as determined by a luciferase-based assay (NT_{80}).
SECONDARY AND SAFETY ENDPOINTS	**Secondary Immunogenicity Endpoints** • Anti-CHIKV neutralizing antibodies, determined by luciferase-based assay (NT_{80}), in prior alphavirus vaccine recipients versus alphavirus-naïve controls, assessed by - Geometric mean titer (GMT) and geometric mean ratio (GMR) on Days 1, 8, 22, 29, 57 and 182 - Seroconversion rates on Days 8, 29, 57 and 182 - The proportion of subjects with titers of at least 40, 160 or 640 on Days 1, 8, 22, 29, 57 and 182 • Anti-CHIKV total antibodies, determined by immunoassay, in prior alphavirus vaccine recipients versus alphavirus-naïve controls, assessed by - GMT and GMR on Days 1, 22 and 29 - Seroconversion rate on Days 22 and 29 where seroconversion is a 4-fold rise in titer over baseline - The proportion of subjects with titers of at least 40, 160 or 640 on Days 1, 22 and 29 • Anti-VEEV total and neutralizing antibody, as determined by immunoassay and Plaque-Reduction Neutralization Test ($PRNT_{80}$) respectively, in prior alphavirus vaccine recipients versus alphavirus-naïve controls, assessed by

	- GMT and GMR on Days 1, 22 and 29 - Seroconversion rate on Days 22 and 29 where seroconversion is a 4-fold rise in titer over baseline - The proportion of subjects with titers of at least 40, 160 or 640 at Days 1, 22 and 29 **Safety Endpoints** The safety and tolerability endpoints include local and systemic post-injection solicited events and other adverse events collected during the 7 days following the injection. Safety endpoints also include the occurrence of any unsolicited adverse events through Day 29, and serious adverse events and adverse events leading to withdrawal at any time during the study. All safety data will be tabulated according to pre-exposure status and at the time points of assessment.
Exploratory Endpoints	• Cellular immune responses to chikungunya antigens: PBMCs will be collected on Days 1, 8, 29, 57 and 182 in order to determine the nature and stability of the cellular immune response to PXVX0317. • Humoral immune responses at other time points, to other pathogens (including EEEV and WEEV) and using other thresholds than those specified above may be performed in selected subjects. • Neutralizing antibody titers to alphaviruses will be measured at three time points (Day 1 (pre-vaccination), Day 22, and Day 182).
INVESTIGATIONAL PRODUCT	PXVX0317, a purified CHIKV VLP vaccine, 40 mcg per 0.8 mL in Alhydrogel® adjuvant, will be administered intramuscularly (IM) on one occasion. The dose used in this study was selected based on the immunogenicity of this regimen in a previously conducted dose-finding Phase 2 trial, PXVX-CV-317-001.
CONTROL	All study participants will receive the same Investigational Product according to the same schedule. The alphavirus vaccine naïve subjects will serve as controls for determining the effect of pre-existing alphavirus immunity on vaccine safety and immunogenicity.
STUDY POPULATION	Up to 60 healthy adults (with replacements allowed for those who drop out prior to Day 29), including 30 prior recipients of

	investigational alphavirus vaccines, recruited from USAMRIID, and an equal number of gender and age-matched controls recruited from WRAIR.
INCLUSION CRITERIA	1. Age 18 to 65 years old (inclusive) 2. For women of childbearing potential, a negative pregnancy test at screening and on vaccination day, practicing highly effective contraception for at least 30 days prior to vaccination, and willing to use a highly effective method of contraception through study completion. 3. Able and willing to provide informed consent for study participation prior to screening procedures. 4. Free of obvious health problems as established by medical history and clinical examination at screening and enrollment. 5. Available to participate for the duration of the study (approximately 8 months). 6. For the cohort of prior alphavirus vaccine recipients, a documented history of prior alphavirus vaccination.
EXCLUSION CRITERIA	1. Acute disease or febrile illness at the time of screening or enrollment. 2. Clinically significant cardiac, respiratory, rheumatologic or other medical or psychiatric condition that, in the opinion of the Investigator, places the subject at increased risk or affects their ability to understand and comply with study procedures. 3. Abnormal screening lab test result that, in the opinion of the investigator, obscures interpretation of the safety data or suggests a clinically significant cardiac, respiratory, rheumatologic or other medical condition that places the subject at increased risk. 4. Pregnant, lactating or planning to become pregnant during the study period. 5. Laboratory evidence of infection with Hepatitis B, C or HIV. 6. History of naturally (non-laboratory) acquired chikungunya or other alphavirus infection or travel to a WHO-designated chikungunya-endemic region within 30 days prior to Day 1. 7. History of acute allergic reaction to any component of CHIKV-VLP vaccine or Alhydrogel®. 8. Current (30 days prior to Day 1) or anticipated use of systemic immunomodulatory or immunosuppressive medications.

	9. History of splenectomy, immunosuppressive condition, autoimmune disease, or immunodeficient condition. 10. Family history of congenital or hereditary immunodeficiency. 11. Suspected or known current alcohol or drug abuse that, in the opinion of the investigator, would interfere with the subject's ability to understand and comply with study procedures. 12. Current intravenous drug use. 13. Prior receipt of an investigational chikungunya vaccine. 14. Receipt or planned receipt of any licensed vaccine from 30 days prior to Day 1 through the Day 29 study visit. 15. Participation in another clinical trial during the study period in which an investigational product is administered. 16. For the cohort of alphavirus naïve vaccine recipients, history of prior alphavirus vaccination is exclusionary.
STUDY DESIGN	This is a Phase 2 parallel-group, open label study in healthy adults 18-65 years of age. A total of 60 subjects are planned to be enrolled with up to 30 at each of two sites. This study has a screening period of 60 days, a treatment and observation period from Day 1 to Day 29, and a follow-up period through Day 182. Subjects enrolled at USAMRIID will be matched by age (±3 years) and gender (both birth and self-identified) with alphavirus naïve subjects enrolled at WRAIR. This matching is done to ensure that demographic characteristics are comparable between the groups, not to pair data between two specific individuals. Details of visits, visit windows, and procedures are provided in the Schedule of Events (Appendix A). After signing the informed consent form, subjects will undergo screening procedures up to 60 days before the first injection. Subjects will be observed in clinic for 30 to 60 minutes after injection. Local and systemic solicited events occurring within 7 days after the injection will be recorded by the subject using a memory aid. Subjects will be specifically asked to record local injection site events (pain, redness, swelling) and systemic events (fever with oral temperature ≥100.4° F, chills, fatigue, malaise, headache, myalgia, arthralgia, and nausea). Any other adverse events and medications used through Day 29 will also be recorded. Blood will be collected at Day 1 (before the injection) and Days 8, 22, 29, 57 and 182.

	A final analysis of data collected throughout the study from all subjects will be performed after the last subject has completed the study and the immunogenicity and safety data have been locked.
STATISTICAL METHODS	The primary immunogenicity analysis will compare prior alphavirus vaccine recipients to alphavirus-naïve controls on the proportion in each group who seroconvert at Day 22 – three weeks after injection – where seroconversion is defined as a 4-fold or greater rise over baseline in anti-CHIKV neutralizing antibodies as assessed by a luciferase-based assay. The statistical significance of the comparison will be assessed using a Fisher's exact test. The percentage who seroconvert in each group will be presented along with its 95% CI calculated using the Wilson method. Similar methods will be used to compare groups on the percentage of subjects who achieve a titer of at least 40, 160, or 640 at Day 22 (primary time point) and on Days 8, 57 and 182 (secondary time points). GMT will be assessed using an analysis of covariance (ANCOVA) with logarithmically-transformed, anti-CHIKV neutralizing titers (log_{10}) as the dependent variable and pre-exposure group, age, gender as fixed effects and baseline titer as the covariate in the model. The least square means and 95% CIs estimated from the model will be back-transformed and reported as the group GMT values. The geometric mean ratio (GMR) of the two groups and its 95% CI will be estimated from a model contrast. Similar methods will be used to assess neutralizing and total antibody titers to other alphaviruses (VEEV, WEEV, EEEV) at other time points. Safety will be assessed primarily through the reporting of solicited and unsolicited AEs. Solicited AEs will be summarized for the 7-day period following the injection by the incidence of each type of AE, the first day of its onset, the number of days the AE was reported, and the maximum severity of the AE. Groups will be compared on the incidence of each type of solicited AE using pairwise Fisher's exact tests Unsolicited AEs will be summarized by System Organ Class and Preferred Term, by severity and by potential relationship to the vaccine. Additional displays will summarize any SAEs or AEs that lead to withdrawal or death.

	Displays of solicited and unsolicited AEs will present data separately for prior alphavirus vaccine recipients, alphavirus-naïve controls, and for all subjects combined. **Sample Size and Power Considerations** The sample size for this trial was based on the number of subjects in the Fort Detrick Special Immunization/Special Procedures Program who are available for screening at USAMRIID. Of the approximately 60 available subjects, up to 30 are expected to enroll in this trial along with an equal number of alphavirus naïve age- and gender-matched controls. Based on an interim analysis in Study PXVX0317-001, at least 95% of the alphavirus-naïve controls are expected to seroconvert in this trial. Assuming that the alphavirus-naïve group has a 95% seroconversion rate, a total sample size of 60 subjects has 80% power to detect a significant difference between groups if the seroconversion rate in the prior alphavirus vaccine recipients is 65% or lower. Or, in other words, power is 80% to detect a difference between groups if the seroconversion rate in the prior alphavirus recipients is 30 percentage points lower than in the alphavirus-naïve group. Previous studies have shown impaired immune responses to chikungunya vaccines in prior recipients of live attenuated and inactivated alphavirus vaccines. The decrease in seroconversion in two of these trials was 64% and 100% respectively (see section 1.3). Therefore, this study is adequately powered to detect immune interference on the order of one-half to one-third of what has previously been described. The power of other combinations of seroconversion rate and sample size can be seen in Table 3 in section 9.1. With respect to safety as a study endpoint, this study will have 80% power to detect a 30% difference between pre-exposure groups in the incidence of any single or aggregate solicited adverse event expected to occur in 5% of the subjects in one of the groups. With a total of 60 subjects receiving PXVX0317, there is a 95% chance that an uncommon AE – one expected to occur in 5% of vaccine recipients – will be observed at least once in the trial.

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LIST OF ABBREVIATIONS

Ab	Antibody
AE	Adverse Event
ANCOVA	Analysis of covariance
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
C	Capsule antigen
CBC	Complete blood count
CHIKV	Chikungunya virus
CHKVLP059	VRC-CHKVLP059-00-VP, the VLP vaccine produced by the VRC
CI	Confidence Interval
CRF	Case Report Form
CRP	C-reactive protein
CTC	Clinical Trial Center
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
E1, E2, E3	Envelope antigens on the chikungunya capsule
EC50	Half-maximal effective concentration
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EEEV	Eastern equine encephalitis virus
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immune absorbent spot
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
GRAS	Generally recognized as safe
HBV	Hepatitis B Virus
HBcAb	Antibody to Hepatits B Virus core antigen
HBsAb	Antibody to Hepatits B Virus surface antigen
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSPB	Human Subjects Protection Branch, WRAIR
IC50	Half maximal inhibitory concentration
ICF	Informed Consent Form

ICH	International Conference on Harmonization
IM	Intramuscular
IND	Investigational New Drug
IPD	Important Protocol Deviation
IRB	Institutional Review Board
IUD	Intrauterine device
Luc	Luciferase
MedDRA	Medical Dictionary of Regulatory Activities
MM	Medical Monitor
nAb	Neutralizing Antibody
NF	National Formulary and Drug Standards Laboratory
NIAID	National Institute of Allergy and Infectious Disease of the NIH
NT_{80}	Neutralization Titer showing 80% neutralization (of CHIKV in this study)
OHRO	USAMRIID Office of Human Research Oversight
Ph./Eur.	European Pharmacopoeia
$PRNT_{80}$	Plaque reduction titer showing 80% neutralization (of VEEV in this study)
SAE	Serious Adverse Event
SIP	USAMRIID Special Immunizations Program
SMP	Safety Management Plan
SOC	System Organ Class
SUSAR	Suspected unexpected serious adverse reaction
UPIRTSO	Unanticipated problem involving risk to self or others
US	United States
USAMRDC	US Army Medical Research and Development Command
USAMRIID	US Army Medical Research Institute for Infectious Diseases
USP	US Pharmacopoeia
VEEV	Venezuelan equine encephalitis virus
VLP	Virus-Like Particle
VRC	Vaccine Research Center of the NIAID
WEEV	Western equine encephalitis virus
WFI	Water for injection
WHO	World Health Organization
WHODRUG	World Health Organization Drug Dictionary
WRAIR	Walter Reed Army Institute of Research

DEFINITION OF TERMS

Protocol deviation	Any occurrence involving a procedure that did not follow the study protocol, applicable procedures, and/or regulatory requirements.
Important Protocol Deviation	The subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being
Adverse event	An adverse event (AE) is any untoward medical occurrence in a study participant, regardless of the suspected causal relationship with study vaccine.
Solicited adverse event	A solicited adverse event is a protocol-specified AE about which the Investigator or designee proactively asks the subjects during a protocol-specified time period. Solicited adverse events for this study will be collected for 7 days after injection and are local events of pain, redness, and swelling at the injection site and systemic events of fever with oral temperature ≥ 100.4 F, fatigue, chills, malaise, headache, myalgia, arthralgia, and nausea.
Unsolicited adverse event	An unsolicited adverse event is an AE that is spontaneously reported by the subject or discovered by the Investigator.
Suspected adverse reaction	Any AE for which there is a reasonable possibility that the drug caused the AE.
Unexpected adverse reaction	AE that is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

1 INTRODUCTION

1.1 Background

1.1.1 Chikungunya Virus and Chikungunya Fever

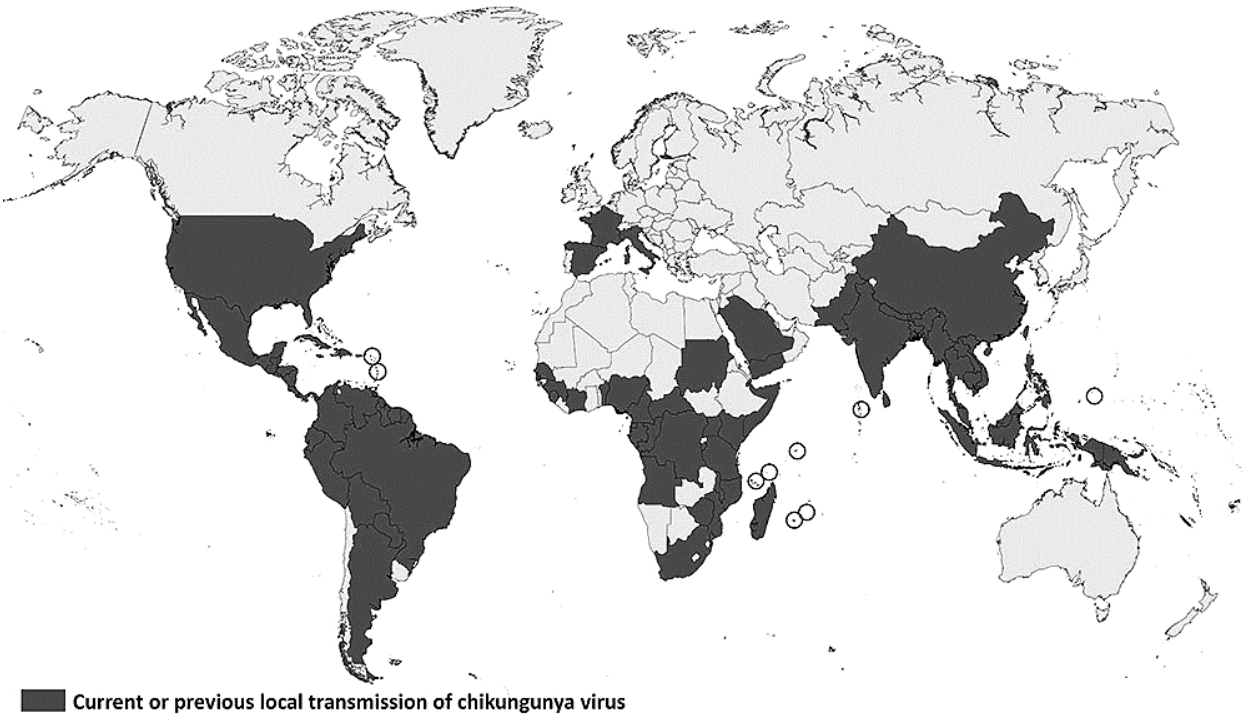
Chikungunya virus (CHIKV) is an arthropod-borne alphavirus of the family *Togaviridae*. The CHIKV virion contains a positive-sense single-strand RNA genome with a long open reading frame coding for Capsid © and Envelope (E1, E2, E3 and 6K) structural proteins, together with four non-structural proteins. Since the first case reports of CHIKV in a 1952-1953 outbreak in Tanzania (Ross 1956), this disease has been endemic in Africa and parts of Asia with transmission occurring through *Aedes aegypti* and more recently via *Aedes albopictus* (Powers 2007).

Beginning in 2014, CHIKV disease cases were reported among U.S. travelers returning from affected areas in the Americas and local transmission was identified in Florida, Puerto Rico, and the U.S. Virgin Islands. As of May 29, 2018, over 80 countries or territories have documented cases of CHIKV infection excluding those countries where only imported cases have been documented, as shown in Figure 1 (CDC 2018). Although mosquitoes are the primary mode of transmission of CHIKV, blood-borne transmission via needle stick is possible. Maternal-fetal transmission has been documented during pregnancy (Staples 2017).

Following an incubation period of 2 to 12 days, acute clinical manifestations include high fever, rash, gastrointestinal complications, headache, muscle pain, nausea, fatigue, myalgia, and joint pain (Borgherini 2007, Taubitz 2007, Pialoux 2007). The most classic symptom of chikungunya is a debilitating polyarthralgia that is present in greater than 90% of cases. This acute phase resolves within several weeks, but joint pain and arthritis may persist for months or years in over 30% of infected individuals (Schilte 2013).

There are currently no approved vaccines to prevent CHIKV infection or disease. However, protection against subsequent infection has been shown to correlate with the presence of anti-CHIKV antibodies that neutralize the virus *in vitro* (Yoon 2015). Such antibodies are readily induced by this and other vaccine candidates. But a major challenge stands in the way of licensing any chikungunya vaccine: the virtual impossibility of conducting a field efficacy trial, because outbreaks of chikungunya are so sporadic and short-lived. From the time the first case is detected until the epidemic begins to subside is often less than two months (Beesoon 2008).

Figure 1: CHIKV Global Burden as of May 29, 2018 (CDC 2018)

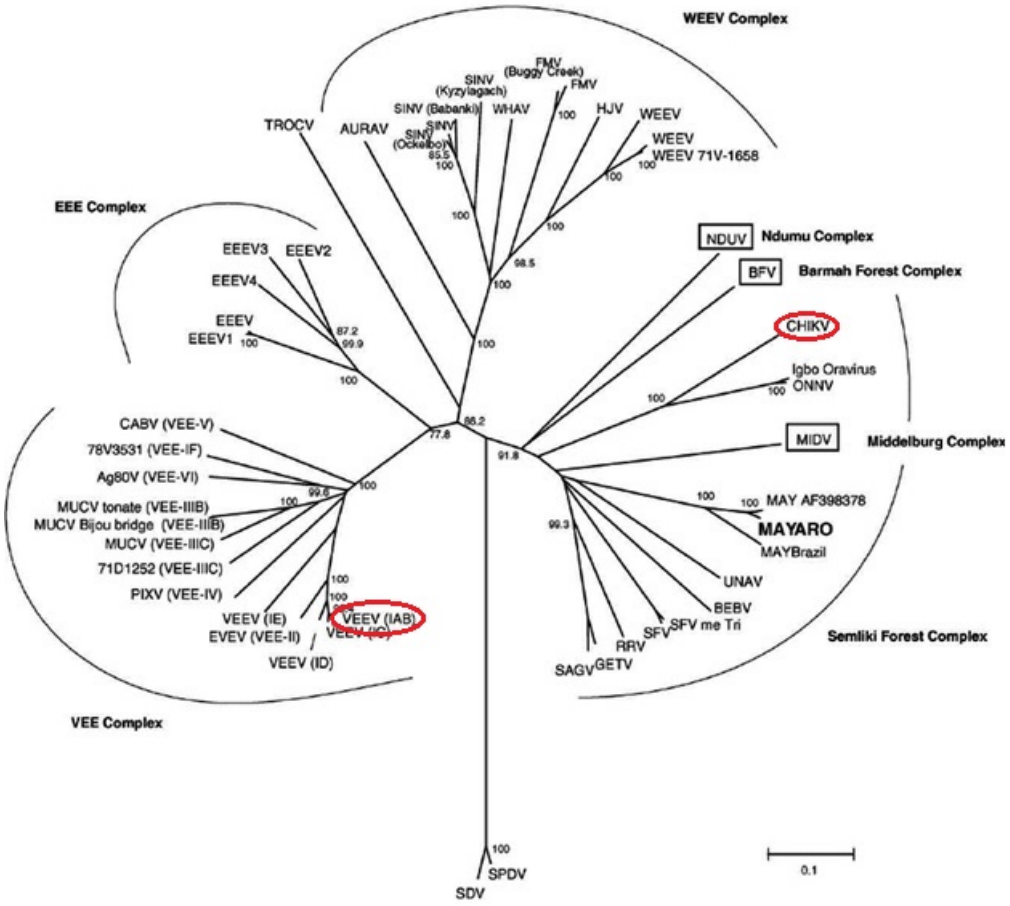


1.1.2 Venezuelan Equine Encephalitis Virus and Vaccines

Venezuelan equine encephalitis virus (VEEV) is an alphavirus related to CHIKV (Figure 2). Humans infected with VEEV develop a febrile illness, often with severe headache, averaging 1–5 days after exposure. Dissipation of febrile illness coincides with the production of neutralizing antibodies. The average case fatality rate of VEEV is <1%. Children have more severe disease, are more likely to develop encephalitis and can be left with severe neurological complications.

VEEV has been recognized as a potential biological weapon because of its high attack rate with a debilitating illness and its adaptability to aerosol dissemination. Accordingly, the US Army has invested much research in this pathogen and developed experimental vaccines to protect those who handle VEEV in the laboratory (Wolfe 2013).

Figure 2: Phylogenetic tree of alphaviruses showing the relative positions of CHIKV and the IAB strain of VEEV from which the TC83 vaccine was made.



Two vaccines for VEEV administered to laboratory workers at USAMRIID under IND are a live attenuated virus vaccine (TC83) and an inactivated virus vaccine (TC84). TC-83 was created by serially passaging the Trinidad Donkey VEEV strain in guinea pig heart cells and point mutations in E2 and the 5′ untranslated region are responsible for the attenuated phenotype of TC-83 (Taylor 2017). TC83 is the most widely used but has a significant (26%) non-response rate. Non-responders who require immunity are generally offered the inactivated TC84 vaccine.

Laboratory workers at USAMRIID may have also received investigational formalin-inactivated vaccines for Eastern Equine Encephalitis (EEEV) and/or Western Equine Encephalitis (WEEV) Viruses (Reisler 2012).

1.1.3 Alphavirus Immune Interference

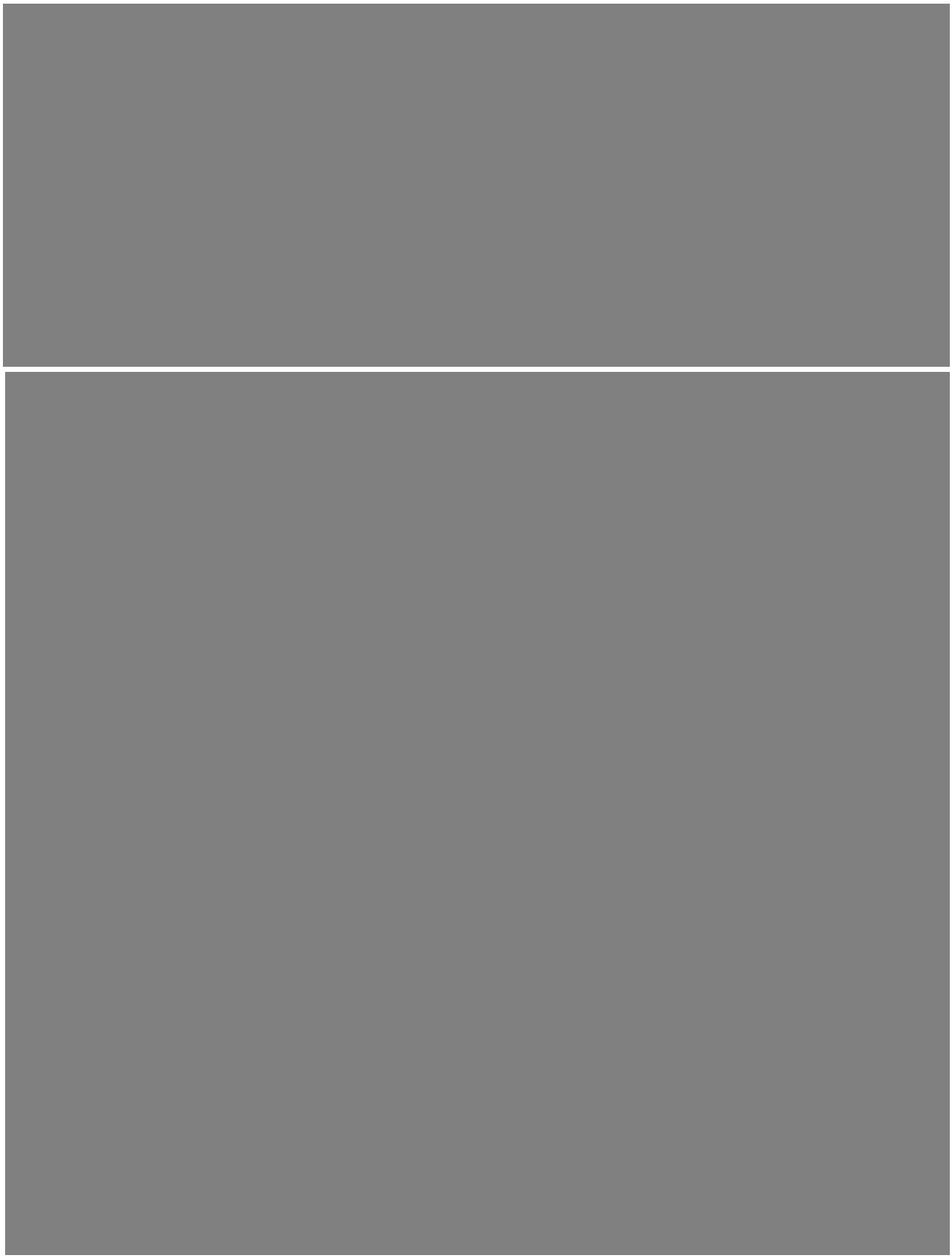
Over the many decades of experience with alphavirus vaccines at USAMRIID a number of studies have demonstrated immune responses to an alphavirus vaccine may be impaired among individuals who previously received a vaccine for a heterologous alphavirus. In one such study, recipients of the live attenuated chikungunya vaccine developed a GMT of neutralizing antibody of 300 (96.6% seroconversion) while those who received the TC83 vaccine a month prior developed only a GMT of 10 to CHIKV and only 36% seroconverted (McClain 1998). While the authors attributed this to impaired replication of the live attenuated CHIKV vaccine virus due to cross-reacting T cells or non-neutralizing antibodies, a similar phenomenon was also observed when a formalin inactivated CHIKV vaccine was given to prior recipients of VEEV, EEEV and WEEV vaccines (DeMeio 1979). This phenomenon has been dubbed "alphavirus immune interference". While antibody responses to chikungunya were impaired in these studies of prior recipients of TC83, there is no evidence that the non-responders were more susceptible to chikungunya. Horses also demonstrated impaired immune responses to the second of two sequential alphavirus vaccines (Van der Wagen 1975)(Calisher 1973). Challenge studies, though not consistently showing cross-protection, also do not show evidence that clinical outcomes are worse in prior recipients of heterologous alphavirus vaccines (Jochim 1974) or experimental infections (Byrne 1964).

1.2 Name and Description of Investigational Vaccine

PXVX0317 is the Sponsor's research name for this Chikungunya Virus-Like Particle Vaccine (CHIKV-VLP vaccine). PXVX0317 is a field-formulated vaccine with two components: CHIKV-VLP and Alhydrogel® adjuvant. The final dose of the CHIK-VLP is 40mcg per vaccine dose. This dose was selected from preliminary data generated by the on-going PXVX-CV-317-001 Phase 2 study [unpublished data]. CHIKV-VLPs are comprised of three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997: Capsid (C, 35 kDa), Envelope 1 (E1, 55 kDa), and Envelope 2 (E2, 50 kDa) in sterile aqueous buffer. CHIKV-VLP is essentially identical to the VRC-CHKVLP059-00-VP vaccine used in the studies described below. Alhydrogel is a 2% (w/w) aqueous suspension of aluminum hydroxide.

PXVX0317 is described further in Section 3.

1.2.1 Summary of Findings from Nonclinical Studies



1.2.2 Summary of Findings from Clinical Studies

1.2.2.1 VRC 311

The safety and immunogenicity of CHKVLP059 were evaluated under BB-IND 14907 in VRC 311, a Phase 1 open-label, dose-escalation trial. (Chang 2014, www.clinicaltrials.gov: NCT01489358). Healthy adult participants 18 to 50 years old were assigned to sequential dose level groups to receive IM injections of 10 mcg, 20 mcg, or 40 mcg (without adjuvant) on weeks 0, 4, and 20, with follow-up for 44 weeks after enrollment. The primary endpoints were safety and tolerability of the vaccine. Secondary endpoints were CHIKV-specific immune responses assessed by neutralizing antibody assay and ELISA. Post-hoc analysis of neutralizing antibody (Nab) by luciferase-based assay was also performed by Emergent.

All injections were well tolerated, with no serious adverse events reported. The most common local reaction was mild injection site pain (36%) and the most common systemic reaction was mild malaise (24%). No moderate or severe reactogenicity was observed.

Neutralizing antibodies were detected in all dose groups after the second vaccination. The geometric mean titer (GMT) of the half maximum inhibitory concentration (IC50) was 2688 in the 10 mcg group, 1775 in the 20 mcg group, and 7246 in the 40 mcg group, and a significant boost occurred after the third vaccination in all dose groups (10 mcg group p=0·0197, 20 mcg group p<0·0001, and 40 mcg group p<0·0001). Four weeks after the third vaccination, the GMT of the IC50 was 8745 for the 10 mcg group, 4525 for the 20 mcg group, and 5390 for the 40 mcg group. These findings were confirmed by the Emergent luciferase-based assay to be used for the EBSI-CV-317-002 trial, demonstrating the suitability of the Emergent assay [Emergent, data on file].

1.2.2.2 VRC 704

The NIH's VRC 704 was a Phase 2 trial conducted at multiple CHIK-endemic sites in the Caribbean (Chen 2018). The study was a double-blind, placebo-controlled study with 200 subjects receiving 20 µg of CHIKV-VLP and 200 receiving placebo in a 2-dose series at Weeks 0 and 4. The study was initiated in 2016 and completed in 2018. Approximately 20% of subjects demonstrated detectable anti-CHIKV neutralizing antibodies at baseline. CHIKV-VLP appeared safe and well tolerated in subjects who were followed through Week 72, with no related SAEs or other safety concerns. CHIKV-VLP appeared highly immunogenic, with a GMT of 2004.5 and 99.5% of recipients having neutralizing antibodies at Week 8. A boosting effect of CHIKV-VLP was also observed in subjects with baseline anti-CHIKV neutralizing antibodies.

Specimens from VRC 704 were also analyzed at Emergent, using the CHIKV 181/25 luciferase assay. A sub-analysis was performed on subjects without pre-existing anti-CHIKV neutralizing activity. Using a more stringent 80% neutralization cutoff (NT_{80}), the GMT was 123 at Week 4 and 1701 at Week 8. Although antibody levels declined with time, as would be expected, the GMT

remained ≥100 for at least 18 months, indicating that long-term protection can potentially be achieved without the need for booster doses. These results demonstrated that CHIKV-VLP was safe and immunogenic in adults in CHIKV-endemic areas, including those with serologic evidence of previous CHIKV exposure. The most frequently reported local adverse event was pain/tenderness at the injection site reported as mild by 58 of 197 (29%) vaccine recipients who received at least one study injection and as moderate by 3/197 vaccine recipients (2.0%). The most frequently reported systemic adverse events were mild or moderate headache reported by 53 of 197 (27%) vaccine recipients, malaise (53/197, 25.6%), and myalgia (46/197, 23.4%). Placebo recipients reported these systemic reactogenicity symptoms at similar frequencies. One vaccine recipient (0.5%) experienced a headache graded as severe following the second vaccination. A total of 16 SAEs in 15 (3.8%) subjects were reported, all were assessed as unrelated to the study vaccine. All potentially related AEs resolved without clinical sequelae.

Taken together, the findings from VRC 311 and VRC 704 suggest that a single dose of 40 mcg CHKVLP059VP is well-tolerated and immunogenic in both CHIKV-exposed and CHIKV-naïve adults.

1.2.2.3 PXVX-CV-317-001

The Phase 2 PXVX-CV-317-001 trial is being conducted at multiple clinical trial sites in the US and compares multiple regimens of PXVX0317 in over 400 healthy adults. The dosages of CHIKV-VLP in that study range from 6 mcg to 40 mcg. These doses are below or approximately equivalent to those used in VRC 311 and VRC 704. Adverse reactions to PXVX0317 following any injection included: injection site pain (41.4% subjects), injection site redness (1.0%), injection site swelling (0.5%), malaise (14.2%), nausea (13.0%), headache (27.2%), joint pain (9.6%), fatigue (19.5%), chills (6.5%), fever (1.7%), and myalgia (22.7%). The majority of solicited AEs following any injection were mild or moderate; 6% of subjects reported at least one solicited AE that was graded as severe. No solicited events were potentially life threatening. Interim analyses of these data are the basis for the regimen proposed in this study: 40 mcg with alum adjuvant in a single dose. See Section 1.3.1.

1.3 Rationale for the Current Study and Military Relevance

This clinical trial is designed to develop our understanding of immunologic cross-reactivity between alphaviruses as it pertains to this chikungunya vaccine candidate. Specifically, this study will help us determine: (1) Does previous exposure to heterologous alphavirus antigens diminish or enhance the immune response to this chikungunya vaccine candidate? And: (2) Does this chikungunya vaccine candidate boost pre-existing or induce de novo immune responses to other alphaviruses?

If alphavirus immune interference is seen with this chikungunya vaccine, interpretation of post-vaccination titers in field studies will have to account for previous alphavirus exposure, and additional studies will be required to determine if this diminished response leads to decreased

efficacy in preventing chikungunya disease. The possibility of alphavirus immune interference is also of particular relevance to the US Department of Defense which has for years invested in the development of vaccines for VEEV, EEEV and WEEV because of their potential use as biological weapons (Wolfe 2013). If the entire force is to be vaccinated against these viruses, it will be important to know if and how concurrent or subsequent administration of a chikungunya vaccine will be impacted.

Regarding (2), the question of whether this vaccine candidate will boost or induce humoral and cellular immune responses to other alphaviruses is also relevant to the design of field studies. While conducting a field efficacy study of a vaccine against CHIKV appears epidemiologically impossible, it may be possible to design a study of that same candidate to show whether it impacts immunity to related alphaviruses that are considered potential pandemic threats (Hotez 2017) (Lwande 2015). Experts have recommended Iquitos, Peru, as a suitable location for a field study of a chikungunya vaccine based on recurring epidemics of dengue, which shares the same mosquito vector as CHIKV (Rezza 2019). In addition, transmission of VEEV and Mayaro viruses occurs in some districts of Iquitos, making this a potential site for testing the vaccine's impact on immunity to those viruses (Forshey 2010)(Morrison 2008).

The population available to participate in this study at Fort Detrick (USAMRIID) provides a unique opportunity to study both whether pre-existing immunity to alphaviruses impacts the anti-CHIKV immune response and whether the vaccine elicits cross-reactive immune responses to other alphaviruses. Researchers will have the opportunity to conduct in-depth immune analyses on individuals serially exposed to antigens from multiple alphaviruses. Serum will be collected for the determination of neutralizing, total, and cross-reacting antibodies. Resources are also available to collect peripheral blood mononuclear cells (PBMCs) for comparisons of cellular immune responses to this vaccine among those with and without prior alphavirus immunity.

In addition, this study allows us to closely study the safety of this vaccine in individuals with previous alphavirus immunity. This vaccine has already been used in several hundred individuals, including about 70 with previous chikungunya exposure, and no concerning safety signals have emerged. There is little reason to suspect that previous vaccination with a heterologous alphavirus will enhance the reactogenicity of this vaccine particularly among this group of participants, many of whom have already received multiple alphavirus vaccines without any unique safety signal emerging. Nevertheless, this study will provide additional safety data on this vaccine among those previously exposed to live attenuated VEEV to better assure the investigators and inform the consent of participants who will receive this vaccine in areas endemic for VEEV and other alphaviruses.

1.3.1 Rationale for Dosage and Route of Administration

The regimen of PXVX-0317 selected for this study is 40 mcg of VLP plus alum adjuvant administered in a single IM dose on Day 1. This regimen was selected by an interim analysis of the study PXVX-0317-001 referenced above. All regimens tested were given to groups of 50 to 53 healthy adults and all resulted in 100% seroconversion by Day 57. The group receiving 40 mcg plus alum showed the highest proportion developing and maintaining neutralization titers above 1:640 (44%) at Day 182 and was therefore selected for further development.

1.4 Benefits and Risks of Study Participation

1.4.1 Benefits of Study Participation

There is no expected benefit to subjects from this study. A potential benefit of participation in this clinical trial might be protection against chikungunya virus infection, however the clinical efficacy of this vaccine for that indication has not yet been proven. This study will accumulate additional safety data on this vaccine and uniquely provide a first glimpse at the safety and immunogenicity of this vaccine in the context of prior alphavirus exposure. This data will be important to the design of field studies. If, for example, the vaccine is less immunogenic in those with prior alphavirus immunity, field studies in areas where other alphaviruses are endemic should account for this in determining appropriate immunogenicity end-points. In addition, the in-depth immune analyses planned for the samples collected in this study may yield insights relevant to the VEEV vaccine development effort.

1.4.2 Risks of Study Participation

1.4.2.1 Risks with PXVX0317

The injection of PXVX0317, like other injections, can cause pain, redness, or swelling at the injection site. These types of reactions are generally mild. Less commonly, it can cause itching, bruising, or infection.

In an earlier study, the majority of side effects following any injection were mild (29.6%) or moderate (21%) and rarely were severe (7.2%). These types of reactions usually happen within a day after injection and typically last 1 to 3 days. The most recent data on the incidence of adverse events following PXVX0317 are provided in Section 1.2.2.3.

PXVX0317 does not contain any CHIKV virus and **cannot** cause CHIKV infection. There may be other side effects from PXVX0317 that are not common and that we do not yet know about. Participants will be advised to tell the study staff about any side effects they may think they are having.

For the study population who will continue to require investigational vaccines for potential occupational exposures, there is a hypothetical risk that PXVX0317 could influence the immune

responses to subsequent alphavirus vaccines. Whether this influence would be positive or negative is unknown. Considerable experience has accumulated at USAMRIID with repeated administration of multiple alphavirus vaccines in this population with no evidence yet of clinically significant adverse health effects (Pittman 2004).

1.4.2.2 Risk of Allergic Reaction

With any vaccine, there is a risk of severe allergic reaction. Although such reactions have not been reported with this vaccine, they have occurred with other vaccines containing some of the same ingredients. Symptoms of allergic reaction include:

- Rash
- Wheezing and difficulty breathing
- Difficulty swallowing
- Dizziness and fainting
- Swelling around the mouth, throat or eyes
- A fast pulse
- Sweating

Participants will be directed to inform study staff immediately if experiencing any of these symptoms.

1.4.2.3 Risks with Blood Draws

Blood drawing may cause pain, bruising, feeling faint, fainting, needle site infections, swelling, and rarely other infections. Bruising at the site of blood drawing can be prevented by applying pressure for several minutes. To reduce the risk of infection, we will wipe the area clean with alcohol and use sterile (germ-free) equipment.

1.4.2.4 Risks of Positive Screening Test for HIV, Hepatitis B and C

At the Screening visit, participants will be tested for hepatitis B and C and HIV. If blood tests show that a participant has HIV or hepatitis B or C, study staff are required by law to report this information to the Maryland Health Department. Participants will be informed of this. The test results reported to Maryland Health Department will contain the participant's name, contact information, including address and telephone numbers, and the type of testing that was done. If the participant is in the military, study staff are required to report the same kind of information to military preventive medicine service. As a result, this information may end up in the subject's military medical record and may be reported to their chain of command.

Receiving information that any health screening tests are abnormal or that tests for HIV and hepatitis B or C are positive may upset participants. The study doctors will discuss their health results face-to-face (and notify their primary doctor at their request). Counseling will be available to the study subject, if they wish. A positive HIV status could limit a participant's ability to obtain

future life and health insurance. In rare circumstances, a positive HIV test has led to discrimination in employment and housing, etc. Participants will be advised of this in the informed consent form.

1.4.2.5 Risks to Pregnancy

If potential participants are females who can become pregnant and want to take part in this study, they will be informed that PXVX0317 has not been thoroughly evaluated for potential risks to unborn or nursing children. Participants will be instructed not to get pregnant or breastfeed while taking part in this study. Female participants must have a negative pregnancy test at screening and again immediately before vaccination, however pregnancy tests are not 100% accurate. Participants will be advised that the only completely reliable methods of birth control are not having sex or surgical removal of the uterus. Other methods, such as the use of condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy.

If a participant becomes pregnant or feels she might be pregnant, she will be advised to contact her personal physician and the principal investigator of this study. If a participant becomes pregnant, the study team will ask permission to contact her regarding the outcome of her pregnancy.

1.4.2.6 Risks to Confidentiality

Efforts will be made to keep participants' personal health information confidential. In order to maintain confidentiality, paper study records will be stored in a secure location such as a locked office or locked cabinet. Electronic data will be password-protected. Electronic study records and samples taken will be coded with a number, not the participants' names. Records will only be shared with authorized personnel and only in connection with carrying out the obligations relating to the study.

Although efforts are made to protect the research study records, there is always a risk that someone could get access to the personal information in research records or other information researchers have stored about participants.

2 OBJECTIVES AND ENDPOINTS

2.1 Primary Objectives

The primary safety objective is to evaluate the safety of PXVX0317 when administered to prior alphavirus vaccine recipients versus gender and age-matched controls. Safety will be assessed by measuring the incidence of local and systemic solicited adverse events, unsolicited adverse events, and serious adverse events.

The primary immunogenicity objective is to evaluate the neutralizing antibody response to chikungunya virus induced by PXVX0317 when administered to prior alphavirus vaccine recipients versus gender and age-matched alphavirus naïve controls.

2.2 Secondary Objectives

The secondary immunogenicity objective is to evaluate the overall antibody responses to CHIKV and VEEV induced by vaccination with PXVX0317 when administered to prior alphavirus vaccine recipients versus gender and age-matched controls.

2.3 Exploratory Objectives

The exploratory immunogenicity objectives are to evaluate the cellular immune response to CHIKV induced by vaccination with PXVX0317 when administered to prior alphavirus vaccine recipients versus gender and age-matched controls, and to evaluate the humoral immune responses to EEEV and WEEV viruses induced by vaccination with PXVX0317 in selected subjects who previously received these vaccines versus alphavirus-naïve controls. Additional exploratory objectives are to collect human immunoglobulin for use in a mouse challenge study, and to evaluate the ability of CHIKV antibodies to PXVX0317 to neutralize diverse alphavirus strains.

2.4 Endpoints

2.4.1 Safety Endpoints

The safety and tolerability endpoints include local and systemic post-injection solicited events and other adverse events collected during the 7 days following the injection. Safety endpoints also include the occurrence of any unsolicited adverse events through Day 29, and serious adverse events and adverse events leading to withdrawal at any time during the study. All safety data will be tabulated according to pre-exposure status and at the time points of assessment.

2.4.2 Primary Immunogenicity Endpoint

The anti-CHIKV seroconversion rate at Day 22 in prior alphavirus vaccine recipients versus alphavirus-naïve controls, where seroconversion is defined as a 4-fold rise over baseline in anti-CHIKV neutralizing antibodies as determined by a luciferase-based assay (NT_{80}). This time point was chosen based on data from an on-going phase 2 study of this vaccine candidate (EBSI-CV-317-001) in which the peak neutralizing antibody response for other single-dose regimens was observed to occur on study Day 22.

2.4.3 Secondary Immunogenicity Endpoints

- Anti-CHIKV neutralizing antibodies, determined by luciferase-based assay (NT_{80}), in prior alphavirus vaccine recipients versus alphavirus-naïve controls, assessed by
  - Geometric mean titer (GMT) and geometric mean ratio (GMR) on Days 1, 8, 22, 29, 57 and 182.
  - Seroconversion rates on Days 8, 29, 57 and 182.
  - The proportion of subjects with titers of at least 40, 160 or 640 on Days 1, 8, 22, 29, 57 and 182.
- Anti-CHIKV total antibodies, determined by immunoassay, in prior alphavirus vaccine recipients versus alphavirus-naïve controls, assessed by
  - GMT and GMR on Days 1, 22 and 29.
  - Seroconversion rate on Days 22 and 29, where seroconversion is a 4-fold rise in titer over baseline.
  - The proportion of subjects with titers of at least 40, 160 or 640 on Days 1, 22 and 29.
- Anti-VEEV total and neutralizing antibody, as determined by an immunoassay and a Plaque-Reduction Neutralization Test ($PRNT_{80}$) respectively, in prior alphavirus vaccine recipients versus alphavirus-naïve controls, assessed by
  - GMT and GMR on Days 1, 22 and 29.
  - Seroconversion rate on Days 22 and 29, where seroconversion is a 4-fold rise in titer over baseline.
  - The proportion of subjects with titers of at least 40, 160 or 640 on Days 1, 22 and 29.

2.4.4 Exploratory Immunogenicity Endpoints

- Cellular Immune Responses to Chikungunya Antigens: PBMCs will be collected on Days 1, 8, 29, 57 and 182 in order to determine the nature and stability of the cellular immune response to PXVX0317.
- Humoral immune responses at other time points, to other pathogens (including EEEV and WEEV) and using other thresholds than those specified above may be performed in selected subjects.

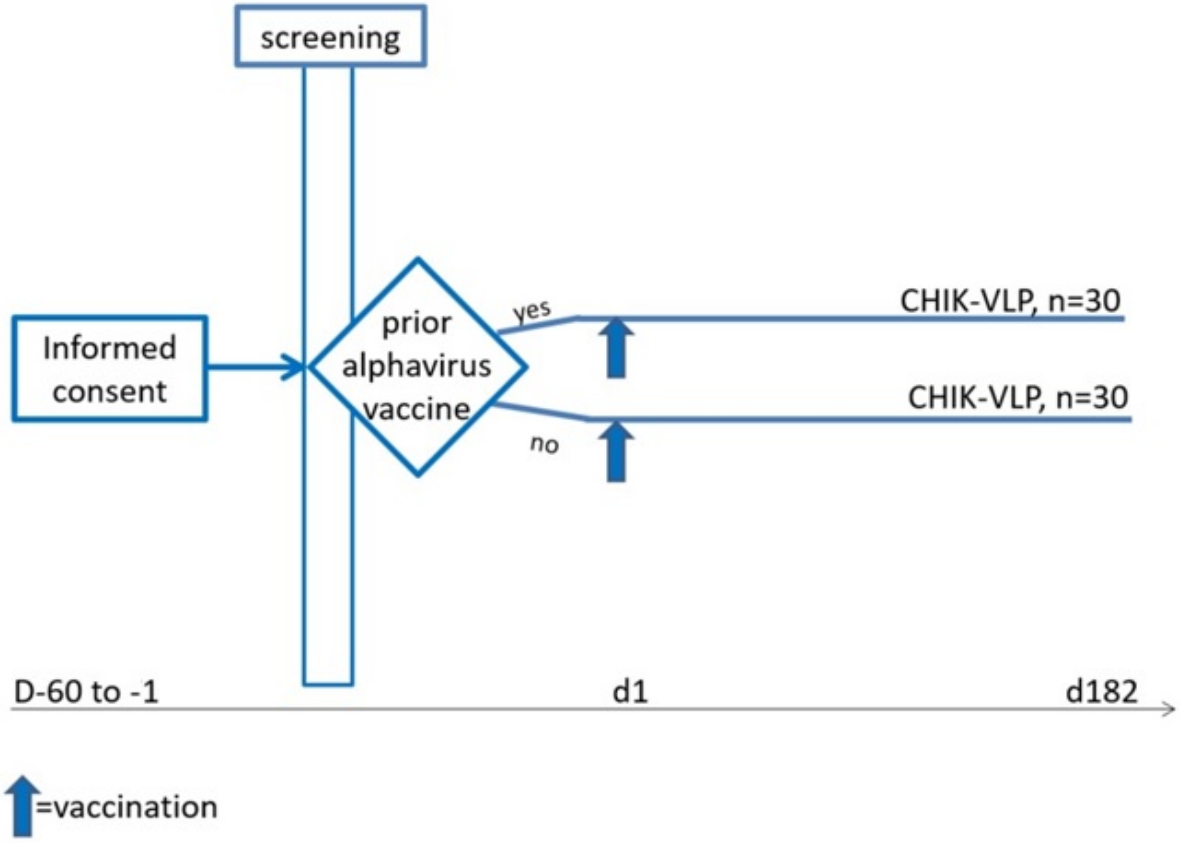
- Neutralizing antibody titers to alphaviruses will be measured at three time points (Day 1 (pre-vaccination), Day 22, and Day 182).

STUDY PLAN

2.5 Study Design

This is a Phase 2 parallel-group age- and gender-matched open label study in healthy adults 18-65 years of age (Figure 3).

Figure 3: EBSI-CV-317-002 Study Schema



2.6 Number of Study Participants

The study population will be composed of 60 healthy adults, 30 of which have previously received an investigational heterologous alphavirus vaccine and 30 age- and gender-matched controls who meet the eligibility criteria listed in Section 2.8. This number is based on the estimated number

of such individuals available for recruitment through the Fort Detrick Special Immunizations Program at USAMRIID. Replacements are allowed for subjects who drop out prior to Day 29. Refer to Section 4.18 for further details.

2.7 Estimated Study Duration

The study consists of a 60-day screening period, a treatment and observation period from Day 1 through Day 29, and a follow-up period through Day 182 (±14 days). Therefore, the maximum possible study duration for an individual subject is 256 days.

2.8 Study Population

2.8.1 Inclusion Criteria

Subjects must meet all of the following criteria in order to be enrolled:

1. Age 18 to 65 years old (inclusive)
2. For women of childbearing potential, a negative pregnancy test at screening and on vaccination day, practicing highly effective contraception for at least 30 days prior to vaccination, and willing to use a highly effective method of contraception through study completion.
3. Able and willing to provide informed consent for study participation prior to screening procedures.
4. Free of obvious health problems as established by medical history and clinical examination at screening and enrollment.
5. Available to participate for the duration of the study (approximately 8 months).
6. For the cohort of prior alphavirus vaccine recipients, a documented history of prior alphavirus vaccination.

2.8.2 Exclusion Criteria

Subjects who meet any of the following criteria cannot be enrolled:

1. Acute disease or febrile illness at the time of enrollment.
2. Clinically significant cardiac, respiratory, rheumatologic or other medical or psychiatric condition that, in the opinion of the Investigator, places the subject at increased risk or affects their ability to understand and comply with study procedures.

3. Abnormal screening lab test results that, in the opinion of the investigator, obscures interpretation of the safety data or suggests a clinically significant cardiac, respiratory, rheumatologic or other medical condition that places the subject at increased risk.
4. Pregnant, lactating or planning to become pregnant during the study period.
5. Laboratory evidence of infection with Hepatitis B, C or HIV.
6. History of naturally (non-laboratory) acquired chikungunya or other alphavirus infection or travel to a WHO-designated chikungunya-endemic region within 30 days prior to Day 1.
7. History of acute allergic reaction to any component of CHIKV-VLP vaccine, or Alhydrogel®.
8. Current (30 days prior to Day 1) or anticipated use of systemic immunomodulatory or immunosuppressive medications.
9. History of splenectomy, immunosuppressive condition, autoimmune disease, or immunodeficient condition.
10. Family history of congenital or hereditary immunodeficiency.
11. Suspected or known current alcohol abuse that, in the opinion of the investigator, would interfere with their ability to understand and comply with study procedures.
12. Current intravenous drug use.
13. Prior receipt of an investigational chikungunya vaccine.
14. Receipt or planned receipt of any licensed vaccine from 30 days prior to Day 1 through the Day 29 study visit.
15. Participation in another clinical trial during the study period in which an investigational product is administered.
16. For the alphavirus naïve group, history of prior alphavirus vaccination is exclusionary.

3 STUDY VACCINE

3.1 Investigational Vaccine (PXVX0317)

See also section 1.2.

3.1.1 CHIKV-VLP

CHIKV-VLP refers to the virus-like particle (VLP) component of PXVX-0317 produced by transient transfection of human embryonic kidney (HEK) 293 cells with a DNA expression plasmid encoding Capsid (C) and E3, E2, 6K, and E1 proteins. After expression of the plasmid-encoded proteins, VLPs self-assemble and are released into the cell culture medium as ~70 nm particles. The 6K and E3 proteins have not been specifically detected in the VLPs. No replication-capable viral genetic material is incorporated into the VLPs. VLPs are then concentrated from the cell supernatant and purified. After purification, the VLPs are diluted into a sucrose-containing aqueous buffer, sterile-filtered and filled into sterile single-dose glass vials to create the final CHIKV-VLP drug product. The production process does not use animal-derived raw materials or antibiotics. Process residuals (including host cell and recombinant DNA, host cell protein, and Benzonase®) were assessed to be at acceptable levels. All excipients are generally regarded as safe (GRAS).

The CHIKV-VLP drug product is a sterile aqueous buffered solution filled into 3 mL single-use glass vials with a 0.8 mL fill volume. The vial is sealed with a rubber stopper and an aluminum seal with a blue flip-off cap. The drug product is stored in a qualified, temperature-controlled freezer at ≤ –70°C. See the Pharmacy Manual for additional information on vaccine storage and preparation.

The composition of CHIKV-VLP drug product (Lot Number 1-FIN-2949) is shown in Table 2.

3.1.2 Alum Adjuvant

The adjuvant is a commercially available sterile, non-pyrogenic formulation of 2% w/w aluminum hydroxide gel (10.0 mg/mL aluminum), aqueous, branded as Alhydrogel® adjuvant 2%. It meets the requirements of the European Pharmacopoeia monograph for aluminium hydroxide and is hydrated for adsorption. The adjuvant is packaged in a 250 mL HDPE bottle with a rubber stopper and each bottle is for single-use only. The bottle should be shaken well before use.

The recommended storage condition for the adjuvant is room temperature not to exceed 30°C. Avoid freezing. See the Pharmacy Manual for additional information.

3.1.3 Study Vaccine Labelling

PXVX0317 will be shipped in individual vials labelled with the names of the Sponsor and product, the concentration, lot number, storage conditions and "Caution: New Drug – Limited by Federal Law to Investigational Use Only".

3.1.4 Study Vaccine Storage

PXVX0317 will be stored in monitored freezers at -70ºC or below until ready for use. Accountability will be maintained in accordance with Good Clinical Practices (GCP) to include dates on which vaccine was received, administered to subjects or returned to the sponsor. The study monitors will periodically check supplies held at the clinical sites to verify accountability. Templates for accountability records are included in the Pharmacy Manual.

The investigator will only approve administration of the Study Vaccine to subjects enrolled in this study according to the procedures described in this protocol. At the end of the study all unused Study Vaccine and containers will either be destroyed on site with appropriate documentation or returned to the Sponsor.

3.1.5 Study Vaccine Preparation

Alhydrogel® 0.03 mL is added to two separate Dose Vials and mixed by swirling. The Dose Vials are held at room temperature for 15 minutes. A total of 0.4 mL is withdrawn from the first Dose Vial into a syringe, which is then inserted into the second Dose Vial. A total of 0.8 mL is then withdrawn from the second Dose Vial.

For further information on Study Vaccine, including secondary packaging, receipt, accountability, and detailed preparation instructions, please refer to the Pharmacy Manual.

4 STUDY PROCEDURES

4.1 Recruitment and compensation for study participation

Informed consent will be obtained prior to any study procedures. Prior to agreeing to participate in the study, volunteers at either site will participate in an information session about the study. The investigator or designee will explain the study, outline participation requirements, review the consent form in detail with volunteers, and then answer any questions. Volunteers will then be afforded ample time to read the informed consent form and ask questions. If a volunteer decides to participate, he/she will sign and date the informed consent document.

The recruitment process will in general be similar for both military and civilian personnel. However, special effort will be made to ensure that military personnel are not unduly influenced or coerced into participating by individuals of superior rank or position. A signed copy of the informed consent will be provided to the volunteer before any study procedure is performed.

4.1.1 Recruitment at USAMRIID

Recruitment will be done via web-based advertising (USAMRIID E-news), recruitment flyers and email blasts. The target population for this study is not in the chain of command of the study team, and also very few (if any) Alpha-virus vaccinated personnel are active duty military. Thus, group briefings of military members (e.g. in formation), where influence of rank would possibly be of concern, are not planned.

4.1.2 Recruitment at WRAIR

Subjects will be recruited by the WRAIR CTC through the use of newspaper ads, flyers, and posters along with ads on Craigslist, Facebook, Instagram or other social media websites. Web-based advertising (www.clinicaltrials.army.mil), word of mouth, and the WRAIR CTC database will be used to contact clinical trial volunteers. Potential volunteers of any ethnic background, education, and income will be residents of the Washington, DC, metropolitan area. An institutional review board (IRB)-approved recruitment script will be read during all phone and face-to-face contacts with potential study subjects. All recruitment materials will be submitted to the WRAIR IRB for review and approval prior to use. Recruitment will be by non-coercive means.

4.1.3 Volunteer compensation for participation

Subjects will receive compensation for their participation in this study. Study volunteers will be provided a gift card for screening and debit card or direct deposit for enrolled visits from the WRAIR CTC or USAMRIID SIP study team as compensation. The schedule for compensation is detailed in the ICF.

4.2 Informed Consent

The Investigator must obtain informed consent from study participants prior to starting any study-related activities. All prospective subjects must sign and date an Institutional Review Board (IRB)-approved informed consent form (ICF) following receipt of the study informational briefing and an opportunity for question and answer from study investigators and staff. In order to participate in the study, volunteers must also sign a separate consent form for HIV testing. For further details on informed consent, refer to Section10.4.

4.3 Screening

Screening procedures are listed in the Schedule of Events in Appendix A.

Each subject who signs an ICF will receive a sequential three-digit identification number unique to the site (e.g., 001, 002…). When screening information is entered into the EDC a subject will be assigned a subject identification number with the following format: CV317002-two-digit site number-three digit identification number, e.g., CV317002-01-001. Subject numbers will also be sequential since screen failed subjects will be entered into the EDC system.

Each site will maintain a screening enrollment log to record the enrollment or the reason(s) for screen failure for all subjects who receive a subject identification number. Reason for screen failure will also be captured in the EDC disposition eCRF.

Re-screening:

Subjects who meet exclusion criterion 1 (acute febrile illness) at the time of their scheduled enrollment may be re-screened after resolution of their acute illness. Subjects who meet exclusion criteria 6, 8, 14 or 15 (prohibited travel, medications, vaccines or participation in another clinical trial) may be re-screened after the appropriate duration has passed. There may be situations in which an eligible subject is not able to be vaccinated within 60 days of their screening period. Their participation will require undergoing all screening procedures again, including re-consenting and use of the same subject ID number.

4.4 Medical History

Medical history information will be collected from subjects at the Screening Visit and confirmed at the Day 1 (Baseline) Visit and will include (but not be limited to) demographic information, current and past medical conditions, and prior and concomitant medications taken within 30 days of Day 1. For subjects at USAMRIID, records of all vaccines received through the SIP will also be included.

4.5 Physical Examination

A physical examination will be performed on subjects during the Screening Visit.

The examination should include:

- Height
- Weight
- General physical exam

A directed physical exam may be performed on subjects at additional time points if indicated by AE reporting.

4.6 Vital Signs

Vital signs collected from subjects will include blood pressure, heart rate, and temperature. The first set of screening vitals are to be collected and transcribed into the screening eCRF for inclusion of the subject into the study. Vital signs will be checked up to the Day 29 visit and at the investigator's discretion thereafter.

4.7 Laboratory Tests

The schedule of sample collection is shown in Appendix A. Phlebotomy will observe the American Red Cross limit of no more than 450 mL in any 8-week period. Further details regarding specimen collection, processing and shipping will be provided in the Laboratory Manual.

4.7.1 Safety labs

At screening, blood will be collected for serum testing for HBV, HCV and HIV and seropositive subjects excluded. Overall health will be assessed with a complete blood count, creatinine, and liver associated enzymes (AST/ALT) at screening and Days 1 and 8.

4.7.2 Assays of humoral immunity

Neutralizing antibody to CHIKV will be assessed by a luciferase-based anti-CHIKV neutralization assay (NT_{80}) at Day 1 (pre-vaccination) and on Days 8, 22, 29, 57 and 182. Total antibody to chikungunya will also be assessed by immunoassay on Days 1 (pre-vaccination), 22 and 29. Neutralizing and total antibody to VEEV will be determined by $PRNT_{80}$ and an immunoassay, respectively, on Days 1, 22 and 29. Additional sera will be collected for exploratory analyses including assays of humoral immunity to EEEV and WEEV in selected subjects. Collected samples will also be used to evaluate neutralizing antibody titers to alphaviruses at Day 1 (pre-vaccination), Day 22, and Day 182.

4.7.3 Assays of cellular immunity

PBMCs for assays of cell mediated-immunity will be collected on Days 1, 8, 29, 57 and 182. Cellular immune response characterization will focus on the magnitude and quality of anti-CHIKV and anti-VEEV responses. Peptides have been designed to cover the structural polyprotein (15mers, 10 overlap) of both pathogens. ELISPOT (Mabtech) analysis of samples will be

conducted with large peptide pools in order to identify pools eliciting IFNγ responses. Once immunogenic large pools have been identified, a more refined re-stimulation with overlapping peptide pools will be conducted to identify specific epitopes eliciting either response. Identification of those peptides will inform on immunogenic regions of the viral proteins, which may assist in determining whether there are conserved epitopes in the alphaviruses that elicit a potentially cross-reactive immune response.

Concurrent with ELISPOT studies, supernatant will be collected after re-stimulation to assess a broader cytokine and chemokine panel in response to the peptide pools. The panel will include IL4, IL15, and IL12, which are suggestive of Th2 and Th1 profile immune responses. A MagPix multiplex cytokine and chemokine array will be used for this analysis. These data will provide a preliminary readout of virus-specific immunity and may help identify peptide pools with T helper epitopes missed by the IFNγ readout.

4.7.4 Samples collected for future research

Coded sera, plasma, and PBMCs collected but not used for the above assays will be retained for a minimum of five years. These will be stored at WRAIR, USAMRIID, and Emergent Travel Health Inc. under appropriate storage conditions. Samples will be labeled with, at a minimum, a subject ID, time point, and protocol information but will not be labeled with any personal identifying information. Samples may be shared during the course of the study for protocol-defined analyses or storage. Research not defined in the protocol or transfer of samples after study completion or to other institutions, will only occur with the approval of the storing institution's appropriate oversight authority.

4.8 Pregnancy Testing and Contraception

Female subjects of childbearing potential will undergo a urine pregnancy test at screening and prior to the injection, and again at Day 29 after the injection. The Investigator must report any pregnancies as described in Section 6.7.1. Female subjects of childbearing potential must also use a highly effective method of contraception (failure rate <1% per year) throughout the study period. Highly effective forms of contraception include combined estrogen and progestogen containing or progestogen-only hormonal contraception associated with inhibition of ovulation, IUD, intrauterine hormone-releasing system, bilateral tubal occlusion, abstinence, or vasectomized partner. The Investigator must confirm that contraception methods were initiated at least 30 days prior to Day 1 (e.g. hormonal contraception) to be considered fully effective.

4.9 Study Vaccine Administration

On Day 1 before study vaccine administration, the interim medical history will be reviewed and updated in the subject file, a targeted clinical exam will be performed, blood samples will be collected, and females of childbearing potential will have a urine pregnancy test. Immediately prior to study vaccine administration, staff will verify that the subject is still eligible. Once these

procedures are performed and if the subject is still eligible study vaccine will be administered. Study vaccine is 0.8 mL in volume and administered by intramuscular injection as described in the Pharmacy Manual, using universal precautions and sterile technique. The injection will be administered into the deltoid muscle by a staff member delegated by the Investigator. Which deltoid to use will be at the discretion of the administering staff member with consideration for subject preference and features that may obscure interpretation of any local AEs such as tattoos, scars or other lesions.

4.10 Acute Observation

The subject will be monitored by study staff for signs of an acute adverse reaction for 30 minutes after the injection and vital signs will be repeated between 30 and 60 minutes after injection.

4.11 Solicited Adverse Events

Solicited AEs will be collected for 7 days after the injection. AEs that correspond to solicited AE terms but occur outside of (or continue past) the solicited AE collection period are also collected through the unsolicited AE reporting period. Solicited adverse events for this study are local events of pain, redness, and swelling at the injection site and systemic events of fever with oral temperature ≥ 100.4 F, chills, malaise, fatigue, headache, myalgia, arthralgia, and nausea.

Subjects will be trained to complete a memory aid to observe, measure, and record these solicited AEs. To measure oral temperature, a digital thermometer will be provided to the subject to measure their temperature each day and record them in their memory aid. To record injection site local reactions, a ruler will be provided to the subject to measure the diameter of redness or swelling at the largest point of the reaction each day and record them in their memory aid.

Study staff will review the signs and symptoms recorded on the memory aid and the action taken for the event at subsequent visits. The memory aid will be collected and maintained in the subject file but the final determination of each adverse event name, onset and resolution dates, severity and causality will be made by the Investigator. Severity will be graded according to the Toxicity Grading Scale (Appendix B). The results of the Investigator's assessment will be recorded as a separate source document from the memory aid and will be entered on the corresponding (solicited) adverse event eCRF.

4.12 Unsolicited Adverse Events

Unsolicited adverse events (any AEs not listed on the memory aid) will be collected for this study and details on definition, evaluation, reporting periods and documentation are outlined in Section 6.

4.13 Prior and Concomitant Medications

At the screening visit, the details of prior and concomitant medications (through 30 days prior to Day 1) usage will be collected. For subjects at USAMRIID, this will include all vaccines received

through the SIP. The details of all concomitant medications taken from Day 1 through Day 29 will be captured. Concomitant medications associated with a SAE will be collected through the end of the study.

4.14 Prohibited Medications

Subjects must not have received or be planning to receive:

- Systemic immunosuppressant or immunomodulatory medications (e.g. chemotherapeutics, oral corticosteroids) from 30 days prior to Day 1 through Day 29.
- Licensed vaccines from 30 days prior to Day 1 through Day 29.
- Investigational agents from 30 days prior to Day 1 through the duration of study participation.

The history of all prohibited medications at any time during study participation (regardless of association with an AE) will be collected.

4.15 Protocol Deviations

A protocol deviation (PD) is defined as any occurrence involving a procedure that did not follow the study protocol, applicable procedures, and/or regulatory requirements. The Investigator is responsible for conducting the study in accordance with the protocol. Any deviation from the protocol must be documented in the study file. In addition, deviations must be reported to the IRB as applicable. Subject-specific deviations must be recorded in the subject's source documents and in the subject's protocol deviation eCRF. The Sponsor will review all protocol deviations on an ongoing basis and will be responsible for categorizing protocol deviations as Important Protocol Deviations (IPDs) - the subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being. PDs will be promptly reported to the Sponsor in accordance with the Clinical Monitoring Plan.

All staff involved in the conduct of a clinical trial will be aware of the specific protocol requirements for completing study visits and notify the PI in the event of any breach of protocol according to the following procedures.

- Notify the PI as soon as possible if they are not present when the deviation is discovered.
- Document in the volunteer's study chart all protocol deviations directly involving or affecting the volunteer, and document a thorough explanation of the circumstances leading up to the deviation.
- The requirement and timeline for reporting protocol deviations to the HSPB/WRAIR IRB is determined by the categorization of the deviation: important protocol deviation or

protocol deviation.

- Knowledge of any instances of serious or continuing non-compliance with the regulations or requirements will be reported immediately to the WRAIR IRB.

- Important protocol deviations are departures from protocol that have a potential to affect the rights and the research participant, to increase the risk to the participant, to change the willingness of the volunteer to continue participation or to compromise the integrity of the study data in such a way that the study objectives may not be achieved. IPDs that occur in greater than minimal risk protocol will be reported to the WRAIR IRB through HSPB by the PI or designate, within 48 hours of becoming aware of the event and recorded in the deviation study log, and a written report submitted within 10 working days of becoming aware of the deviation. All reports will be submitted with a cover memo naming the protocol, description of the event, summary of any harm to study participant(s) and steps to prevent further deviations. A summary of IPDs occurring within the reporting period should also be included in the continuing review reports.

- Protocol deviations are routine departures that typically involve a volunteer's failure to comply with the protocol. Example: missing scheduled visits. Protocol deviations that occur in greater than minimal risk protocols will be reported to the WRAIR IRB through HSPB in the Continuing Review Report(s).

4.16 Study Completion: For Individual Subjects

An individual subject is considered to have completed study participation after completion of the Day 182 visit and any required safety follow-up.

4.17 Early Discontinuation

An individual subject is considered to undergo Early Discontinuation if they stop study participation before the Day 182 visit.

An enrolled subject may voluntarily withdraw consent for further participation at any time before study completion. The Investigator will request (but cannot require) such subjects to provide the reason(s) for withdrawal of consent and to undergo an Early Discontinuation visit. Samples and data already collected will be analyzed as part of the ITT population.

In addition, the Investigator, at his or her discretion, may withdraw a subject from further vaccination or from any further participation in the study.

4.17.1 Criteria for withdrawal from further study participation

The following are criteria for withdrawing subjects from further study participation.

- Loss to follow-up – Loss to follow-up requires failure to show up for a visit, and unable to be reached to re-schedule. Documentation will be made of at least 3 unsuccessful attempts to contact subjects.
- Noncompliance with the protocol
- Other reason(s) which, in the opinion of the Investigator, indicate(s) that further participation in the study is not in the best interest of the subject or study staff.

4.18 Subject Replacement

Subjects who undergo Early Discontinuation at either site before Day 29 may be replaced at the Sponsor's discretion. Should a subject who previously received an alphavirus vaccine withdraw, a replacement for this cohort will be enrolled. If an age and gender-matched alphavirus-naïve subject has already been enrolled for the withdrawn subject, they will not be compelled to leave the study. Should an alphavirus naïve subject who has been age and gender-matched to a prior alphavirus vaccine recipient withdraw, they will be replaced by a new alphavirus naïve subject that is age and gender-matched to the same prior alphavirus vaccine recipient.

4.19 Study Completion: Overall

The study is planned to be completed after all subjects have completed the Day 182 visit (or Early Discontinuation, as appropriate), all necessary safety follow-up has been completed, and all data has been monitored and queries are resolved. The Sponsor reserves the right to terminate the study prior to the planned study completion.

5 STUDY EVENTS BY VISIT

The overall summary of evaluations by visit is given in the Schedule of Assessments in Appendix A. All visits are relative to the first day of vaccine administration, Day 1. Acceptable time windows for the visit schedule are indicated. Note that evaluation for SAEs will occur throughout the study even if not specifically stated.

5.1 Scheduled Study Visits

5.1.1 Screening (Day -60 to Day -1)

The following will take place during the visit, which will occur within 60 days prior to Day 1:

- Informed consent
- Demographics collection
- Review of eligibility criteria
- Medical history to include prior and concomitant medications and drug/vaccine allergies
- Vital signs
- Physical exam
- Blood Collection for:
    - HBV, HCV, HIV testing
    - CBC
    - Creatinine, AST and ALT
- Urine pregnancy test (females of childbearing potential)

5.1.2 Day 1

The following will take place during the visit and ***prior*** to study vaccine administration:

- Vital signs
- Interim medical history (to include concomitant medications)
- Clinical check
- Blood Collection (pre-dose) for:
    - CBC
    - Creatinine, AST, ALT;
    - Sera for $PRNT_{80}$ (VEEV), NT_{80} (CHIKV); total antibody to VEEV and CHIKV;
    - Sera for future use;
    - PBMCs.

- Urine pregnancy test (females of childbearing potential)
- Confirmation Inclusion/Exclusion criteria are met
- Study Vaccine Administration

The following will take place during the visit and ***after*** study vaccine administration:

- Observation for at least 30 minutes
- Vital signs after 30 minutes and no later than 60 minutes
- AE evaluation (solicited and unsolicited)
- Clinical check
- Memory aid, ruler and thermometer distribution and training

5.1.3 Day 8 (+3 days)

- Vital signs
- Collection of the memory aid
- AE evaluation (solicited and unsolicited)
- Clinical check
- Blood collection for:
    - CBC
    - Creatinine, AST/ALT;
    - Sera for antibody assays/future use;
    - PBMCs.

5.1.4 Day 22 (±3 days)

- Vital signs
- AE (unsolicited) evaluation
- Clinical check
- Blood collection for:
    - Sera for antibody assays/future use.

5.1.5 Day 29 (±3 days)

- Vital signs
- AE (unsolicited) evaluation
- Clinical check
- Blood collection for:

- Sera for antibody assays/future use;
- PBMCs.

- Urine pregnancy test (females of childbearing potential)

5.1.6 Day 57 (± 7 days)

- Vital signs may be collected at the discretion of the investigator
- Clinical check
- Blood collection for:
  - Sera for antibody assays/future use;
  - PBMCs.

5.1.7 Day 113 (± 10 days)

- Vital signs may be collected at the discretion of the investigator
- Clinical check

5.1.8 Day 182 (±14 days) End-of-Study Visit

- Vital signs may be collected at the discretion of the investigator
- Clinical check
- Blood collection for:
  - Sera for antibody assays/future use;
  - PBMCs.

5.2 Early Discontinuation Visit

All subjects who discontinue study participation before the Day 182 visit will be requested to undergo an Early Discontinuation visit.

- AE evaluation if indicated (solicited if within 7 days of vaccination; unsolicited if within 28 days of vaccination);
- Collection and review of the Memory aid (if within 7 days of vaccination);
- Vital signs may be collected at the discretion of the investigator
- Clinical check;
- Blood Collection for:
  - CBC (if discontinuation occurs within 7 days of vaccination)

- creatinine, AST, ALT (if discontinuation occurs within 7 days of vaccination)
- Sera for antibody assays/future use.

- Urine pregnancy test for females of child-bearing potential (if discontinuation occurs before Day 29).

5.3 Unscheduled Visits

Any study procedure, excluding study vaccination, may be conducted at an unscheduled visit as needed and recorded on the unscheduled visit eCRF. Examples include repeat specimen collection and additional safety follow-up for an adverse event.

6 SAFETY

6.1 Definitions

6.1.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence in a study participant, regardless of the suspected causal relationship with study vaccine.

The definition of an AE includes:

- A new-onset symptom or disease
- An exacerbation of a pre-existing symptom or disease
- A new-onset laboratory abnormality considered by the Investigator to be clinically significant
- A new-onset symptom or disease that occurs as a result of a protocol-specified procedure

The definition of an AE does **not** include:

- A pre-existing symptom or disease that does not worsen during the study (even if first disclosed by the subject after the start of the study)
- A medical or surgical intervention such as surgery, endoscopy, tooth extraction, or transfusion (although the condition leading to the procedure or a complication from the procedure may be an AE)
- An uncomplicated pregnancy
- A dosing error without any resulting signs or symptoms
- Any other situation where an untoward medical occurrence has not occurred (e.g. hospitalization for cosmetic or other elective medical or surgical procedure)

The Investigator will attempt to establish a diagnosis based on signs, symptoms, and other clinical information. Whenever possible, the Investigator will report an AE as a diagnosis rather than one or more signs or symptoms. If a clinically significant laboratory abnormality meets the definition of an AE, a diagnosis or clinical signs and symptoms rather than the abnormal laboratory finding should be reported if possible. If no diagnosis is known and clinical signs and symptoms are not present, but the laboratory abnormality is clinically significant by itself, then it should be reported as the AE.

6.1.2 Solicited Adverse Event

A solicited adverse event (solicited AE) is a protocol-specified AE about which the Investigator or designee proactively asks the subjects during a protocol-specified time period. Solicited adverse events for this study are local events of pain, redness, and swelling at the injection site and systemic events of fever with oral temperature ≥ 100.4 F, fatigue, chills, malaise, headache, myalgia, arthralgia, and nausea.

6.1.3 Unsolicited Adverse Event

An unsolicited adverse event (unsolicited AE) is an AE that is spontaneously reported by the subject or discovered by the Investigator.

6.1.4 Serious Adverse Event

An SAE is an AE (either solicited or unsolicited) which meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires hospitalization or prolongs an existing hospitalization
- Results in a persistent clinically significant disability or incapacity
- Is a congenital anomaly or birth defect
- Requires medical or surgical intervention to prevent one of the above outcomes
- Important medical event

The Investigator will evaluate all AEs for seriousness using the above criteria.

"Life-threatening" means that, in the opinion of the Investigator, the subject was at immediate risk of death from the event as it occurred. It does not mean that the event might have caused death had it occurred in a more severe form.

Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an SAE.

Important medical events may be considered serious at the discretion of the Investigator.

These seriousness criteria also apply to the Study Stopping Rules in Section 6.9.

6.2 Severity Grading

The Investigator will grade all adverse events for severity. Adverse events listed in the Toxicity Grading Scale in Appendix B will be graded according to the criteria in the table. Adverse events not listed in the Toxicity Grading Scale will be graded as follows:

- Mild (Grade 1) – No interference with activity
- Moderate (Grade 2) – Some interference with activity
- Severe (Grade 3) – Significant; prevents daily activity
- Potentially Life-Threatening (Grade 4) – as determined at ER visit or hospitalization

6.3 Causality Assessment

The Investigator will assess all AEs, including solicited AEs, for causality (relationship to study vaccine), assigning one of these three categories: Not Related, Possibly Related, and Probably Related.

An AE will be considered "Not Related" to study vaccine if **any** of the following conditions are met:

- An unreasonable temporal relationship between administration of the study vaccine and the onset of the AE (e.g., the event occurred either before, or too long after administration of the study vaccine for it to be considered related);
- A causal relationship between the study vaccine and the AE is biologically implausible (e.g. injury as a passenger in an automobile accident);
- A clear alternative causality for the AE is present (e.g. typical adverse reaction to a concomitant medication).

An AE will be considered "Possibly related" if there is a reasonable possibility that the AE may have been caused by the study vaccine.

An AE will be considered "Probably related" if there is evidence that the AE was caused by the study vaccine.

6.4 Follow-up of Adverse Events

The Investigator must follow all AEs until resolution, until the condition stabilizes or is no longer clinically significant, or until the subject is lost to follow-up.

The Investigator is responsible for ensuring the conduct of any supplemental investigations considered necessary to evaluate the AE. These may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

Subjects will be instructed to contact the Investigator (or designee) immediately in the event of a non-fatal SAE. All subjects experiencing an SAE will be evaluated by the Investigator or designee as soon as is feasible following the report of the SAE by the subject. In the event of a fatal SAE, the Investigator must provide the Sponsor with any available post-mortem findings, including histopathology.

Additionally, the Sponsor may request that the Investigator perform or arrange for the conduct of supplemental investigations for one or more AEs.

6.5 Reporting of Adverse Events

6.5.1 Reporting Periods

The reporting period for solicited AEs begins immediately after the injection and continues for 7 days. The reporting period for unsolicited AEs begins immediately after study vaccine administration on Day 1 and continues for 28 days afterwards. AEs that correspond to solicited AE terms but occur outside of (or continue past) the solicited AE collection period are also collected through the unsolicited AE reporting period. The reporting period for SAEs begins at the time of informed consent and continues for the duration of study participation.

6.5.2 Documentation

The Investigator or designee will document all AEs in the subject's source documents and enter all AEs in the adverse event eCRF within 3 calendar days of awareness.

All AEs should include:

- Event term,
- Start and stop date,
- Severity,
- Serious and if so, by which criteria,
- Relationship to study vaccine,
- If medical care required for the AE (medically attended).

6.6 SAE Reporting

Serious Adverse Events will be reported in a prospective manner during the period starting from the day of the enrollment of each subject until completion of the last study visit. The Investigator or designee must report all SAEs to the Medical Monitor (MM) and DoD Research Monitor (DoDRM) within 24 hours of their awareness of the event, using the SAE Form. The Investigator or designee must also enter SAEs in the adverse event eCRF. Related SAEs should be promptly (within 48 hours) reported by the Investigator to the WRAIR IRB as a UPIRTSO (related SAEs are unexpected and are presumed to place subjects at risk until proven otherwise) and by the Sponsor to the FDA as a SUSAR. Immediate and prompt notifications should be in the form of email, phone, or fax.

The SAE Form should be completed as thoroughly as possible and signed by the Investigator or designee before reporting to the MM. The SAE Form must include an assessment of causality and should include a preliminary diagnosis if possible. All SAEs assessed as not related must include an alternate causality.

In order to avoid delays in initial reporting, additional information regarding the SAE may be provided as a follow-up report. The Investigator may also modify the diagnosis, seriousness, and/or causality assessment based on this information.

The investigator should not wait to receive additional information to fully document the event before notifying the HSPB/WRAIR IRB of a related SAE. In addition to fax, the complete report can be delivered to the address of the WRAIR HSPB at usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil. This initial notification should give sufficient information to permit identification of:

- The reporter;
- The subject identification number, age, gender;
- Date of vaccination dose, number (first or second) and group (previously vaccinated versus alphavirus naive);
- Diagnosis (MedDRA terminology);
- Description of adverse event (date of onset, signs/symptoms and severity);
- Assessment of relatedness to study vaccination;
- Action taken;
- Outcome of the event;
- Concomitant medications (dose, route, duration).

As new information is obtained, updated reports should be submitted, and if available, should include copies of relevant hospital case records, autopsy reports, and other documents where applicable.

The MM will make the determination whether an SAE is a "suspected unexpected serious adverse reaction" (SUSAR) as defined in 21 CFR 312.32.

- *Suspected adverse reaction* means any AE for which there is a reasonable possibility that the drug caused the AE.
- *Unexpected adverse reaction* means an AE that is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

The Sponsor will report adverse events to FDA in accordance with 21 CFR 312.32. Specifically, the Sponsor will report fatal or life-threatening SUSARs no later than 7 calendar days after initial receipt of the information and will report non-life-threatening SUSARs no later than 15 calendar days after determining that the information qualifies for expedited reporting.

The MM will notify the Investigator of SUSARs and any other events that meet criteria for expedited reporting to regulatory authorities. The Investigator will notify the applicable IRB of these events and adhere to any other applicable local reporting requirements.

For further information regarding SAE reporting, please refer to the EBSI-CV-317-002 Safety Management Plan (SMP).

6.7 Other Events Requiring Immediate Reporting

6.7.1 Pregnancy

The Investigator or designee must report all pregnancies to the MM or designee within 24 hours of their awareness of the pregnancy, using the Pregnancy Report Form. All pregnancies will be followed to outcome. Additional information regarding the pregnancy may be provided as a follow-up report.

An uncomplicated pregnancy is not considered an AE. Complications of pregnancy may qualify as AEs or SAEs and would therefore be documented and reported as specified above.

The following should always be considered as an SAE:

- Spontaneous pregnancy loss, including:
  - spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation)
  - ectopic and molar pregnancy
  - stillbirth (intrauterine death of fetus after 22 weeks of gestation).
- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect (as per CDC MACDP guidelines) identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the fetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the investigational vaccine(s)/product(s) will be reported to the sponsor. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

The reports for pregnancies will be summarized in the continuing review reports that are submitted to the WRAIR IRB as well.

6.7.2 Dosing Errors

The Investigator or designee must report any error in the dosing of study vaccine to the MM or designee within 24 hours of their awareness of the error. Additional information regarding the dosing error may be provided as a follow-up report. A dosing error without signs or symptoms is not considered an AE but may be determined to be an important protocol deviation (IPD).

6.7.3 Early Discontinuation for Safety Reasons

The Investigator or designee must report any early discontinuation from study participation or further vaccination for safety reasons (as determined by the Principal Investigator) to the MM or designee within 24 hours of discontinuation. Additional information regarding ongoing adverse events may be provided as a follow-up report.

6.8 Study Stopping Rules

Enrollment and dosing will be stopped for any SAE assessed by the Investigator as possibly or probably related to study vaccine.

Enrollment may be resumed following review of available safety data by the Sponsor.

For further information regarding stopping rule reporting procedures, please refer to the Safety Management Plan (SMP).

7 STUDY MONITORING PROCEDURES

The Sponsor has ethical, legal, and scientific obligations to conduct this study in accordance with established research principles and ICH GCP guidelines. As such, in order to fulfill these obligations, study monitors (CRAs), a DoD Research Monitor, and a Medical Monitor, will be employed for the duration of the study.

7.1 Study Monitors

Clinical Research Associates (CRAs) will monitor study progress by scheduling and performing on-site study visits throughout the study. A CRA will conduct site initiation visits, several interim monitoring visits and a site close out visit. They will communicate with the sites via phone, email and formal visit confirmation and follow-up letters. The CRA will be responsible for 100% source document verification of study data, to include reviewing all UPIRTSOs associated with the protocol. The CRA may escalate any critical subject safety or GCP finding to the Investigator, Sponsor and Medical Monitor and direct the site to contact the IRB and the DoD Research Monitor immediately if required. Regular inspection of the eCRFs will be conducted by the CRA in order to assess subject enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the eCRFs, verification of eCRF data against original source documents, and occurrence of AEs. A full description of the responsibilities of the CRA, which will include reviewing the Investigational Site File on a routine basis, will be documented in the Monitoring Plan. The CRA will also verify appropriate accountability and storage of the IVP and ensure site pharmacy staff understand and conduct Study Vaccine preparation procedures according to the protocol.

7.2 DoD Research Monitor

The DoD Research Monitor is a physician independent of the Sponsor who is responsible for serving as advocate for the medical safety of volunteers in accordance with Department of Defense Instruction (DoDI) 3216.02. As such, he/she may:

- Perform oversight functions and report their observations to the IRB or other designated officials on recruitment/enrollment procedures, the consent process, other study interventions and interactions, data matching, data collection, and analysis.
- Review ‘unanticipated problem involving risk to subjects or others’ (UPIRTSO) reports.

The DoD Research Monitor is authorized to:

- Interview and examine subjects and their clinical data.
- Remove individual subjects from the study.
- Stop the research protocol in progress.

- Promptly report their observations and findings to the IRB or other designated official as required.
- Take any other steps necessary to protect the safety and well-being of human subjects until the IRB can assess their report.

The DoD Research Monitor must review all unanticipated problems involving risks to subjects or others (UPIRTSOs), serious adverse event (SAE) reports, and all subject deaths. The Research Monitor will provide an unbiased written report of these events promptly (within 48 hours) to the WRAIR IRB by phone [redacted], or by email ([redacted]), or by facsimile [redacted]. The DoD Research Monitor or their approved alternate will then submit written reports within 10 working days to the WRAIR IRB at the following address: [redacted] All DoD Research Monitor reports for unrelated SAEs and deaths should be kept with the corresponding SAE reports at the study site.

The DoD Research Monitor or their approved alternate at a minimum must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comments on the relationship to participation in the study. They must also indicate whether he/she concurs with the details of the report provided by the principal investigator.

The DoD Research Monitor or their approved alternate should review all initial, follow up, and final reports for SAEs, unanticipated problems involving risks to subjects or others, and all subject deaths in a timely manner, and provide their own independent report to the study team.

7.3 Medical Monitor

The Medical Monitor (MM) will provide safety oversight for the study. The MM will review de-identified (coded) adverse event reports and assess causality of SAEs on behalf of the Sponsor. In addition, the MM will communicate safety signals detected in other studies of this vaccine that bear on the conduct of this study or the safety and consent of its participants.

Name: [redacted]

8 DATA HANDLING

8.1 Source Documentation

The Investigator must maintain source documentation of all study conduct data and observations relevant to the study. This source documentation includes, but is not limited to, ICFs, original medical records, progress notes from the Investigator and study staff, laboratory reports, memory aids for solicited adverse events, and documentation of study vaccine accountability.

The paper source documents will be stored in locked cabinets in the respective clinical sites when not actively in use. Only the study coordinators, clinical trial manager, and clinic senior enlisted officer will have access to the keys to the storage containers for paper documents at the clinical sites CTC. Both clinical sites are located on military installations with restricted access.

8.2 Case Report Forms

This study will employ eCRFs provided by the Sponsor. Certain clinical information requested in this protocol will be recorded on these eCRFs. The Investigator is responsible for the adequacy and accuracy of all data entered on the eCRFs. The Investigator is also responsible for signing all eCRFs, after which they will be locked by the Sponsor to prevent further data entry or modification. When the Final Clinical Study Report is completed, the data manager/designee will initiate the process for archiving the study data.

For further information on eCRFs, please refer to the CRF Completion Guidelines. Details on data handling will be described in the Sponsor's Data Management Plan (DMP).

8.3 Database

Source data will be entered and stored in a web-enabled Medrio EDC system. The Medrio EDC System is an integrated Software as a Service (SaaS) platform with a fully hosted Electronic Data Capture (EDC) system. All data entered will be hosted in Medrio's hosting provider data center. This study was built in Version #R40 and the system version will undergo upgrades over the course of the study.

Medrio is an approved Emergent Travel Health vendor under [redacted] Contract Service Providers Qualification Procedure. The system is compliant with 21 CFR Part 11.

8.3.1 Database Quality Control

The study database will be tested by the database developer, data managers and other users prior to approval for production use. All changes made to the database after the database is in production

are documented with a change order request submitted to the Database Developer. All changes will be communicated through the data management team. The data manager will act as the official requester for any database changes. All changes are tested and documented by the requester (data manager/designee) prior to release into production.

Documentation of the database structure is produced, in the form of data definition report, after the database is tested and approved by the database developer and DM/Designee. This document provides the definition of the database structure and SAS or variable names for programming.

8.3.2 Data Entry and QC

The Medrio EDC system will be accessed on government-issued computers at the clinical trial sites with compliant security systems and institutional firewall as specified by their installation's Information Management Directorate (IMD). These CAC-enabled government computers have the most up to date McAfee antivirus software automatically uploaded and updated on an ongoing basis. All devices meet DoD 8570 and AR 25-2 requirements to maintain data.

Data Management will perform and document three types of Quality Control checks to ensure Study Database Accuracy:

- Early database QC
- Ongoing database QC
- Query QC

All issues found during the quality accuracy assessment will be resolved for the database quality to be accepted. Prior to initiating the study database quality evaluation, the data manager will assure that:

1) all data are in the study database
2) all known data discrepancies have been resolved
3) all required medical coding has been completed, if applicable
4) all SAEs have been reconciled with the safety repository, and any discrepancies resolved, if applicable

8.3.3 Database Access

Access to the database is granted to personnel based on specific security roles associated with particular privileges related to the role they have in the study. Access levels and their associated privileges are defined in the finalized Data Management Plan, and user access will be administered via the Emergent Data Management System Administrator.

- Data Management privileges include access to view data, generate and manage queries, lock and unlock eCRF pages, generate listings and create reports (including ad-hoc reporting).
- Clinical Monitor privileges include access to generate and manage queries, generate listings, run reports and to mark pages as source document verified. Access to add/change data is limited to study site personnel.
- Study Coordinator privileges are limited to data entry, report generation and query resolution.
- Principal Investigator privileges are limited to applying a signature to the data as well as read-only access to all of the eCRFs.

8.3.4 Database Security

Emergent Data Management system administrator controls user access per user roles and privileges. Only qualified study personnel will be granted database access. Password is required for user access. Password must have minimum of ten characters, must contain at least three of the following four items:

- contains one or more uppercase letters
- contains one or more lowercase letters
- contains one or more numbers
- contains one or more special characters

Passwords expire after 90 days. To reset the password, the user will need to provide the old password and correct answers to security questions in the user profile.

8.3.5 Database Locking

The database is locked when all expected data are entered, monitored, cleaned (known discrepancies resolved) and signed by the PI. See Section 8.3.2 and 8.5.

8.3.6 Database Back-up

Disaster mitigation measures are as follows:

- All data is encrypted and transferred nightly to Medrio's hosting provider servers
- All backups are stored on secure network based file storage on machines separate from the server being backed up
- All electronically stored Corporate Data is backed up nightly

- Full backups are taken every night and stored in a secure facility separate from the production environment
- Incremental backups occur every 15 minutes to a secure facility that is separate from the production environment.

If a device containing study data is lost or stolen, the loss will be reported through the respective installation's chain of command within 24 hours of the event's discovery, if occurring on a weekday, or within 24 hours of the next business day. The loss will also be reported to the study sponsor within the same 24 hour interval.

8.4 Retention of Study Documentation

The Investigator will maintain all study documentation, including copies of ICFs, eCRFs, and documentation of study vaccine accountability for either 2 years following FDA or other regulatory approval of PXVX0317, or 2 years after clinical development of PXVX0317 is discontinued, unless a longer period is required by applicable law or regulation. The Investigator will destroy study documentation only upon instruction by the Sponsor and must notify the Sponsor upon completion of such destruction.

All paper source document data including the entire regulatory study file will be stored and disposed of in accordance with WRAIR CTC SOP [redacted] *Establishment and Maintenance of a Regulatory Document File*.

Electronic data will be stored by the sponsor and disposed of in accordance with EBSI SOPs.

8.5 Data Monitoring

The Sponsor or designee will monitor completed eCRFs against source documentation at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The Investigator must make source documentation accessible to the Sponsor or designee as needed to verify the information in the eCRFs. The Investigator agrees to cooperate with the Sponsor or designee to ensure that any problems detected in the course of data monitoring are resolved.

An audit trail will capture a history of all data entry transactions and query responses, notes, author(s), and date/time stamps based on the server time. Any changes including reason for change will be electronically captured for each question in the eCRFs. System validation, recovery, security, and compliance were documented.

The DM/Designee will periodically run reports to facilitate data cleaning and to provide progress reports at project meetings as needed.

The DM/Designee will work with the SAS programmer(s) and the statisticians to provide data listings and/or snapshots for SAE reconciliation, laboratory data reconciliation, Safety reporting, FDA IND Annual Report and other data reviews and/or reports if applicable as needed.

8.6 Laboratory Data

This study will employ electronic transfers of laboratory data generated from clinical specimens collected by the Investigator. The Investigator is responsible for the adequacy and accuracy of data associated with collection of these specimens. The Sponsor is responsible for the adequacy and accuracy of the data generated by external laboratories.

8.7 Audit Compliance

The Investigator must permit the Sponsor and/or designee, regulatory agencies, the WRAIR IRB, and the USAMRIID Office of Human Research Oversight direct access to facilities and study documentation for the purpose of auditing study conduct. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are needed for the evaluation of the study.

9 STATISTICAL ANALYSIS

9.1 Sample Size Calculation

The sample size here was chosen based on the number of subjects in the Fort Detrick Special Immunization/Special Procedures Program (USAMRIID) who are available for screening. Of the approximately 60 available, 30 are expected to enroll in this trial along with an equal number of alphavirus naïve age- and gender-matched subjects. Based on an interim analysis of this vaccine lot and regimen in Study PXVX0317-001, at least 95% of the alphavirus-naïve controls are expected to seroconvert in this trial. Assuming that the alphavirus-naïve group has a 95% seroconversion rate, a total sample size of 60 subjects affords 80% power to detect a significant difference between groups if the seroconversion rate in the prior alphavirus vaccine recipients is 65% or less. Or, in other words, power is 80% to detect a difference between groups if the seroconversion rate in the prior alphavirus recipients is 30 percentage points lower than in the alphavirus-naïve group. Compared to the previously cited studies in which decrements in the proportion of seroconversion of 64% (McClain 1998) and 100% (Harrison 1971) (DeMeio 1979) were seen, this study is adequately powered to detect immune interference on the order of one-half to one-third of what has previously been described.

In the event that we have overestimated the willingness of this pool of eligible subjects to participate, the ability to detect differences in the proportion of seroconversion of the same order as was previously shown by McClain (36% versus 95% seroconversion), 10 subjects per group would afford 71% power to detect a significant difference. The power of other combinations of seroconversion rate and sample size can be seen in Table 3. For example, 10 subjects per group afford 81% power to detect a difference between groups if the seroconversion rate is a low as 30% in the prior alphavirus vaccine recipients, while 20 subjects per group provide 89% power to detect a difference if the seroconversion rate is 50% or lower among prior alphavirus recipients. This is certainly less than ideal in terms of designing a field study in an area endemic for other alphaviruses. The decision to conduct such a study will be dependent on many factors and the residual possibility that a lesser degree of immune interference may have gone undetected will have to be considered. If, one the other hand, immune interference on the order of that reported by DeMeio is seen in which a neutralizing antibody response is entirely abrogated, data from this study would be sufficiently compelling to avoid recruiting for field studies in areas co-endemic for other alphaviruses.

Table 3: Power of Detecting a Significant Difference between Prior Alphavirus Vaccine Recipients and Alphavirus-naïve Vaccine Recipients with a 95% Seroconversion Rate

Number of Subjects per Arm	Seroconversion Rate among Prior Alphavirus Vaccine Recipients				
	30%	40%	50%	60%	70%
10	81%	63%	43%	24%	9%
20	> 99%	97%	89%	72%	45%
30	> 99%	> 99%	98%	89%	63%

Power of each combination estimated from a Fisher's exact test comparing the seroconversion rate observed among prior alphavirus vaccine recipients to the rate observed among alphavirus-naïve vaccine recipients assuming the true rate in the latter group is 95%.

With respect to safety as a study endpoint, this study will have 80% power to detect a 30% difference between pre-exposure groups in the incidence of any single or aggregate solicited adverse event expected to occur in 5% of the subjects in one of the groups. With a total of 60 subjects receiving PXVX0317, there is a 95% chance that an uncommon AE – one expected to occur in 5% of vaccinees – will be observed at least once in the trial.

9.2 Treatment Period

The treatment and observation period begins at the time of vaccine administration and extends through the Day 29 visit. The follow-up period spans the time following the Day 29 visit through the end-of-study visit on Day 182 (±14).

9.3 Treatment Groups

Subjects will all receive the same vaccine regimen in an open label fashion. There will be no randomization and no blinding as the primary endpoint (immune response at Day 22) is not subject to subjective interpretation. The participants themselves already know whether or not they have previously received another alphavirus vaccine as such vaccines have only been available through IND and their administration required informed consent. The safety profile of this vaccine is well-established for a phase 2 candidate and is not expected to differ between the two groups enough to justify the inclusion of a placebo group. Laboratory workers at USAMRIID have been exposed to multiple alphavirus and other vaccines in the past without evidence of any clinically significant adverse health effects (Pittman 2004).

Subject matching will be done by age (±3 years) and gender, both of which have been shown to play roles in vaccine immunogenicity in general (Klein 2016) and alphavirus immune interference in particular (Reisler 2012), (Pittman 2009).

9.4 Populations for Analysis

Study Population: All screened subjects who provide informed consent and provide demographic and other baseline screening measurements, and are assigned a study subject ID.

Exposed Population: All subjects in the Study Population who receive study vaccination.

Safety Population: All subjects in the Exposed Population who provide safety assessment data. This generally includes anyone who was not lost to follow-up at Day 1 as they will be at risk for reporting an SAE.

Immunogenicity Evaluable Population (IEP): The IEP includes all subjects in the Exposed Population who:

- Have no important protocol deviation or other reason to be excluded as defined prior to database lock.
- Have not received a prohibited medication.
- Provide evaluable serum sample results for baseline, the relevant post-vaccination time points, and within the required time frames:
    - Baseline: Day 1 or within 60 days before study vaccine administration
    - Day 22: Day 19 through Day 25, inclusive

Analysis of demographic and baseline characteristics: The demographic and baseline characteristics will be summarized according to prior alphavirus vaccine exposure group and overall.

Age, height, weight, and body mass index at enrollment will be summarized by reporting the mean, standard deviation, median, minimum and maximum, and calculated by prior alphavirus vaccine exposure group and overall.

The frequencies and percentages of subjects by sex, race, and ethnicity will be presented by vaccine exposure group and overall. Demographic data will be tabulated for the Study, Immunogenicity Evaluable, and Safety populations.

9.5 Safety Analysis

Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations, and the timing of the vaccination, will be summarized by pre-exposure group for the Study Population.

All safety analyses will be based on the Safety Population.

9.5.1 Solicited AEs

With the exception of redness and swelling, all solicited AEs will be summarized according to severity grading scales defined in Section 6.2 from "mild" to "potentially life-threatening."

Solicited AEs will be recorded daily until 7 days post-injection using a structured memory aid. The analyses of solicited AEs (any event, after any injection, and after each injection) will be performed by maximum severity and by treatment group. In addition, solicited AEs ongoing after 7 days post-injection will also be recorded as unsolicited AEs.

Summary tables tallying the incidence, timing, duration and severity of any local or systemic solicited AE will be presented. The pre-exposure groups will be compared on the incidence of each type of solicited AE using pairwise Fisher's exact tests. The percentage of subjects experiencing each solicited AE will also be presented by maximum severity.

The severity of redness and swelling recorded as diameters (mm) will be summarized according to categories based on the largest diameter linear measurement when the local reaction is present:

- Grade 0/absent = 0 to 24 mm.
- Grade 1/mild >24 to ≤ 50 mm.
- Grade 2/moderate >50 to ≤100 mm
- Grade 3/severe >100 mm or <100 mm with blister formation or skin breakdown

Events reported as not present (0 mm is entered) will be reported as Grade 0.

The following classifications are used in the summaries:

Grade 0 (0-24 mm), Any (>24-50 mm, >50-100 mm, >100 mm)

The following summaries of solicited events will be performed:

1. Solicited events by day post-injection, for each event and for any event.
2. Time of first onset of solicited adverse events, after injection, for each event and any event.
3. Solicited adverse events by maximum event severity, after injection, for each event and for any event.
4. Duration of solicited adverse events (defined as the number of total days during the post-vaccination collection period with the event), after injection, for each event and any event.
5. Solicited adverse events, occurrence of at least one event by category (local, systemic), after injection.

For each of the time points or time intervals presented in the summaries, only subjects with at least one observation (i.e., any non-missing values but excluding "Not done/unknown") for the solicited adverse events will be summarized.

9.5.2 Unsolicited AEs

All the unsolicited AEs occurring during the study, will be recorded, regardless of their assessment of relatedness by the Investigator.

The original verbatim terms used by Investigators to identify AEs in the eCRFs will be mapped to preferred terms using the MedDRA dictionary. The unsolicited AEs will then be grouped by MedDRA preferred terms into frequency tables according to system organ class (SOC). All reported AEs, as well as AEs judged by the Investigator as at least possibly related to study vaccine, will be summarized by pre-exposure group, according to SOC and preferred term within SOC. When an unsolicited AE occurs more than once for a subject, the maximum severity and strongest relationship to the pre-exposure group will be counted.

Only treatment-emergent AEs will be summarized, i.e., excluding those after a subject has given informed consent, but before vaccination. The selection of unsolicited AEs and their assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

Unsolicited AEs will be summarized by alphabetic SOC and preferred term as follows:

- Any unsolicited AE
- Possibly or probably related unsolicited AEs
- SAEs
- Possibly or probably related SAE
- Unsolicited AE leading to withdrawal
- Any AE leading to death

Listings of all AEs will be provided by subject.

Combined Solicited and Unsolicited Adverse Events

Solicited AEs continuing beyond 7 days after injection will be coded by MedDRA and combined with the unsolicited AEs. A summary of subjects with all combined solicited and unsolicited AEs, by SOC and preferred term, will be provided as well.

9.5.3 Analysis of Other Safety Data

The frequencies and percentages of concomitant medications will be tabulated overall and by treatment group. Medications will be coded using the WHODRUG dictionary.

9.6 Immunogenicity Analysis

All tests will be carried out at a 2-sided significance level of 0.05 and no adjustment for multiplicity will be applied.

Seroconversion Analysis:

The primary immunogenicity analysis will compare prior alphavirus vaccine recipients to alphavirus-naïve controls on the proportion in each group who seroconvert by anti-CHIKV neutralizing antibody at Day 22. Seroconversion is defined as a 4-fold or greater rise over baseline in anti-CHIKV neutralizing antibodies as assessed by a luciferase-based assay.

Specifically, we will test the hypotheses:

$H_0: P_1 = P_2$ vs. $H_1: P_1 \neq P_2$

where P_1 and P_2 denote the proportion of subjects who seroconvert in the prior alphavirus vaccine recipients and the alphavirus-naïve controls, respectively.

The significance of the comparison of the pre-exposure groups will be assessed using a Fisher's exact test. The percentage who seroconvert in each group will be presented along with its 95% CI calculated using the Wilson method. A sensitivity analysis of the comparison will be performed using a logistic regression model that incorporates age, gender, and baseline titer as potential predictive factors. Fisher's test comparing seroconversion rates at other time points may also be performed. Similar analyses of the proportion who seroconvert will be conducted on Days 8, 29, 57 and 182 for anti-CHIKV neutralizing antibody and on Days 22 and 29 for total anti-CHIKV, total anti-VEEV and neutralizing anti-VEEV antibodies (secondary endpoints).

Threshold analyses:

The percentage of subjects achieving specified thresholds (e.g., ≥ 40, 160, and 640 anti-CHIKV antibody titers), and associated Wilson 95% CIs, will also be calculated for Days 1, 8, 22, 29, 57 and 182 for neutralizing antibody and at Days 1, 22 and 29 for total anti-CHIKV, total anti-VEEV and neutralizing anti-VEEV antibodies (secondary end-points). The groups will be compared by pairwise Fisher's exact tests on the percentage achieving each threshold.

GMT analyses:

For the secondary comparisons based on GMT, the hypotheses tested will be

$H_0: \mu_1 = \mu_2$ vs. $H_1: \mu_1 \neq \mu_2$

where μ_1 and μ_2 denote the GMT for the prior alphavirus vaccine recipients and the alphavirus-naïve controls, respectively.

The GMTs on Days 22 and 29 will be compared for differences between the pre-exposure groups. The GMTs and Geometric Mean Ratios (GMRs) will be analyzed via linear model. The primary model is an analysis of covariance (ANCOVA), with logarithmically-transformed titers ($\log_{10}$) as

the dependent variable and pre-exposure group, age, gender, as fixed effects and baseline log-transformed titer as the covariate in the model. The least square means estimated from the ANCOVA and their 95% CIs will be back-transformed and reported as the group GMT values. The GMR comparing the pre-exposure groups will be calculated by back-transforming the difference estimated from a linear contrast in the model. The 95% CI for the GMR will be calculated analogously.

The GMTs and GMRs based on antibody titers measured at all other protocol-specified time points will be analyzed as described above for Day 22. The difference in persistence of the antibody response induced after first vaccination at each time point will be visually assessed.

Summaries of both seroconversion and GMT will be presented by age and gender.

Exploratory endpoints (both categorical and continuous) for the heterologous alphavirus analyses will be analyzed descriptively.

10 ADDITIONAL INFORMATION

10.1 Ethical Conduct of the Study

The study will be performed in accordance with the protocol and consistent with ICH Good Clinical Practice (GCP) Guidelines and applicable DoD and local regulatory requirements and laws.

10.2 IRB Oversight

The study (protocol, informed consent form, recruiting materials, and any documents seen by the subject) will be reviewed and approved by the WRAIR IRB. Subjects will not be recruited, consented, screened, or enrolled until the IRB has approved the required documentation. In addition, the IRB will review amendments to the protocol before their implementation.

The Investigator will retain all correspondence with the IRB in the trial master file (TMF) and forward copies of all IRB approvals to the Sponsor.

10.3 Informed Consent

The Sponsor or designee will provide a master informed consent form (ICF) template to each site for development of a site-specific ICF.

All site-specific ICFs must be approved by the Sponsor or designee and the IRB and must be in compliance with ICH GCP, DoD and local regulatory and legal requirements. The Sponsor or designee will advise the Site of required changes to the master ICF template during the course of the study.

The Investigator will ensure that each potential study participant is fully informed about the nature and objectives of the study and possible risks associated with participation. Before informed consent is obtained, the Investigator, or a qualified person designated by the Investigator, will provide the potential study participant with ample time and opportunity to inquire about the details of the trial, and will answer all relevant questions to the potential study participant's satisfaction. The potential study participant will then decide whether or not to participate in the trial. The Investigator, or a qualified person designated by the Investigator, will obtain written informed consent from each study participant before any study-specific activity is performed.

The Investigator will retain the original and any amended signed and dated Informed Consent Form(s) at the study site and provide a copy to each study participant.

All informed consent forms also address the subject of the Genetic Information Nondiscrimination Act of 2008 (Pub. L. 110-233), also known as GINA. Participants will be advised that GINA is a federal law that prohibits discrimination in health insurance coverage and employment based on genetic information. However, GINA does not apply to employers with fewer than 15 employees.

GINA's protections in employment do not extend to the US military. Nor does it apply to health insurance through the TRICARE military health system, the Indian Health Service, the Veterans Health Administration, or the Federal Employees Health Benefits Program. Lastly, the law does not cover long term care insurance, life insurance or disability insurance.

The language in the study ICFs state as follows: "Though not currently planned, some future research studies may include genetic testing of research samples. This means that some of your DNA may be sequenced in order to, for example, learn how our DNA influences how we respond to vaccines. Using new technology, information about your DNA structure (genetic information) gained from your samples could be used to indicate your risk for developing certain diseases. This genetic information is unique to you and may indicate changes in your future health status or life expectancy, or that of your children and other relatives. There is growing concern that such information could be used by employers or insurance companies to discriminate against you. However, if we do this sort of testing, we will not share the results with you or knowingly make your samples available to your future employers or insurance companies."

10.4 Subject Confidentiality

The Investigator will ensure that each subject's anonymity is maintained. On eCRFs and other documents submitted to the Sponsor and/or its designee, subjects must be identified by subject number only. For documents that are not for submission to the Sponsor and/or its designee (e.g., signed ICFs), the Investigator must maintain these documents securely and in compliance with all federal laws and regulations, and ICH GCP Guidelines.

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

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

10.5 Compensation for Injury

All nonexempt research involving human subjects shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6). Namely, the Informed Consent document will not "include any exculpatory language through which the subject or the legally authorized representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution, or its agents from liability for negligence."

If a subject is injured because of participation in this research, whether or not they are a DoD healthcare beneficiary, the subject is entitled to medical care for that injury at a military hospital or clinic; medical care charges for care at a military hospital or clinic will be waived. It cannot be determined in advance which military hospital or clinic will provide care. If the subject obtains care for research-related injuries outside of a military hospital or clinic, the subject or the subject's insurance will be responsible for medical expenses. Transportation to and from DoD hospitals or clinics will not be provided, except in emergencies or situations where a non-DoD healthcare beneficiary requires a military escort for access to said hospitals or clinics. No reimbursement is available if the subject incurs medical expenses to treat research-related injuries. No compensation is available for research-related injuries. The subject is not waiving any legal rights. The subject should contact the PI if the subject believes he or she has sustained a research-related injury. The subject should contact the PI for any questions.

10.6 Clinicaltrials.gov

For purposes of reporting to clinicaltrials.gov, the Sponsor is the responsible party and will provide information regarding this study in accordance with applicable regulations.

10.7 Public Disclosure and Publication Policy

All publication rights are delineated in the Clinical Study Agreement.

10.8 Amendments

The protocol may be amended only by the Sponsor (Emergent Travel Health Inc.). All amendments and modifications will be reviewed and approved by the principal investigator, the sponsor's representative. Amendments and modifications will then be submitted through the WRAIR HSPB for review by the WRAIR IRB and, if necessary, by the WRAIR Scientific Review Committee (SRC). WRAIR IRB approval of protocol amendments is required prior to implementation. A copy of all approval memos and approved protocol documents, including those associated with future protocol amendments, will also be submitted to USAMRIID for review and filing by the local Office of Human Research Oversight (OHRO) as well as review by and filing in the SIP Clinic on site regulatory files.

The informed consent must be revised to ensure alignment with any amendment as appropriate and must also be reviewed and approved by WRAIR IRB. If a significant change is made to the

protocol and informed consent, any subject already enrolled in the study will be informed about the revision and asked to sign the revised informed consent. A copy of the revised, signed, and dated informed consent will be given to the subject. All original versions of the informed consent will be retained in the protocol regulatory file and a copy will be retained in the clinic medical record.

APPENDIX A: SCHEDULE OF EVENTS

Study Event[1]	**Screen**	**Day 1**	**Day 8**	**Day 22**	**Day 29**	**Day 57**	**Day 113**	**Day 182**	**Early DC**
Compliance Ranges	-60 to -1	NA	+3 days	±3 days	±3 days	±7 days	±10 days	±14 days	
Informed Consent	X								
Vital signs	X	XX[8]	X	X	X	X[9]	X[9]	X[9]	X[9]
Review Inclusion/Exclusion Criteria	X	X							
Screening Medical History to include prior/concomitant medications	X								
Physical Examination	X								
Interim medical history, solicited[2] and unsolicited[3] AEs, and concomitant medications		X	X	X	X				X
Memory aid distribution		X							
Memory aid collection and review			X						If < D8
Clinical check[4]		XX[8]	X	X	X	X	X	X	X
CBC[5]	X	X	X						If < D8
Creatinine, AST, ALT[5]	X	X	X						If < D8
Anti-HIV-1/2, Anti-HCV, HBsAg, HBcAb, HBsAb	X								
Urine pregnancy test[6]	X	X			X				If < D29
Sera for antibody assays/future use[7]		X	X	X	X	X		X	X
Collection of blood for Peripheral Blood Mononuclear Cells (PBMCs)		X	X		X	X		X	
Minimum volume of blood required	24.5 mL	84.5 mL	84.5 mL	17 mL	57 mL	77 mL	0 mL	77 mL	0 to 20 mL
Cumulative volume of blood required (approximate mL)	30	120	210	230	290	370	370	450	n/a
Vaccination		X							

[1] SAE's will be collected throughout the study
[2] Solicited AEs collected through Day 8 only.
[3] Unsolicited AEs collected through Day 29 only.
[4] Clinical checks: targeted physical exams based on AEs/SAEs identified
[5] CBC, Creatinine, AST, ALT will be collected but the results will not be included in the database.
[6] Urine pregnancy tests only for women of child-bearing potential.
[7] Antibody assays vary with time point but include NT_{80} to CHIKV, $PRNT_{80}$ to VEEV, total antibodies to CHIKV and VEEV, and exploratory assays of humoral immunity.
[8] On vaccination day, vital signs and clinical checks will occur twice: before and 30 to 60 minutes after vaccination.
[9] Vital signs may be collected at the discretion of the investigator past Day 29 but will not be included in the database.

APPENDIX B: TOXICITY GRADING SCALE

EVENT	MILD (Grade 1)	MODERATE (Grade 2)	SEVERE (Grade 3)	POTENTIALLY LIFE THREATENING (Grade 4)
Fever	> 100.4 – 101.1°F (≥ 38.0 – 38.4°C)	≥ 101.2 – 102°F (≥ 38.5 – < 39°C)	≥ 102.1°F – 104°F (≥ 39°C – 40°C)	> 104°F (> 40°C)
Headache	No interference with activity	Some interference with activity, may require repeated use of non-narcotic pain reliever for more than 24 hours	Significant, prevents daily activity, any use of narcotic pain reliever	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Nausea	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization for hypotensive shock
Vomiting	1–2 episodes/24 hours	> 2 episodes/24 hours	Requires IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms / 24 hours	4 – 5 stools or 400-800 gms/24 hours	6 or more watery stools or > 800 gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Injection site pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Use of any narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Injection site erythema/redness	25-50 mm	>50 mm – 100 mm	> 100 mm	Necrosis or exfoliative dermatitis
Injection site induration/swelling	25-50 mm and does not interfere with activity	>50 mm – 100 mm or interferes with activity	> 100 mm or prevents daily activity	Necrosis
Hgb (decrease from baseline value in gm/dL)	FEMALE: Any decrease – 1.5 MALE: Any decrease – 1.5	FEMALE: >1.5 – 2.0 MALE: >1.5– 2.0	FEMALE: >2.0 – 5.0 MALE: >2.0 – 5.0	FEMALE: > 5.0 MALE: > 5.0
WBC increased	10,800 – 15,000 cells/mm^3	> 15,000 – 20,000 cells/mm^3	> 20,000 – 25,000 cells/mm^3	> 25,000 cells/mm^3
WBC decreased	2500 – 3500 cells/mm^3	1500 – < 2500 cells/mm^3	1000 – < 1500 cells/mm^3	< 1000 cells/mm^3
Lymphocytes decreased	750 – 1000 cells/mm^3	500 – < 750 cells/mm^3	250 – < 500 cells/mm^3	< 250 cells/mm^3

(cont'd)

APPENDIX B: TOXICITY GRADING SCALE (cont'd)

EVENT	MILD (Grade 1)	MODERATE (Grade 2)	SEVERE (Grade 3)	POTENTIALLY LIFE THREATENING (Grade 4)
Neutrophils decreased	1500 – 2000 cells/mm^3	1000 – < 1500 cells/mm^3	500 – < 1000 cells/mm^3	< 500 cells/mm^3
Platelets	125,000 – 140,000 cells/mm^3	100,000 – < 125,000 cells/mm^3	25,000 – < 100,000 cells/mm^3	< 25,000 cells/mm^3
Liver function tests (AST, ALT)	1.1 – 2.5 × ULN	> 2.5 – 5.0 × ULN	> 5 – 10 × ULN	> 10 × ULN
Alkaline phosphatase	1.1 – 2.0 × ULN	> 2 – 3.0 × ULN	> 3 – 10.0 × ULN	> 10 × ULN
Albumin	2.8 – 3.1 g/dL	2.5 – < 2.8 g/dL	< 2.5 g/dL	—
Bilirubin – with increased LFTs	1.1 – 1.25 × ULN	> 1.25 – 1.5 × ULN	> 1.5 – 1.75 × ULN	> 1.75 × ULN
Bilirubin – with normal LFTs	1.1 – 1.5 × ULN	>1.5 – 2.0 × ULN	> 2.0 – 3.0 × ULN	> 3.0 × ULN
Sodium –Hyponatremia	132 – 134 mEq/L	130 – <132 mEq/L	125 – < 130 mEq/L	< 125 mEq/L
Sodium – Hypernatremia	144 – 145 mEq/L	> 145 – 147 mEq/L	> 147 – 150 mEq/L	> 150 mEq/L
Potassium – Hyperkalemia	5.1 – 5.2 mEq/L	> 5.2 – 5.4 mEq/L	> 5.4 – 5.6 mEq/L	> 5.6 mEq/L
Potassium – Hypokalemia	3.5 – 3.6 mEq/L	3.3 – < 3.5 mEq/L	3.1 – < 3.3 mEq/L	< 3.1 mEq/L
Blood Urea Nitrogen (BUN)	23 – 26 mg/dL	> 26 – 31 mg/dL	> 31 mg/dL	Requires dialysis
Serum creatinine	1.5 – 1.7 mg/dL	> 1.7 – 2.0 mg/dL	> 2.0 – 2.5 mg/dL	> 2.5 mg/dL or requires dialysis
Urine protein	Trace	1+	2+	Hospitalization or dialysis
Urine glucose	Trace	1+	2+	Hosp. for hyperglycemia
Urine blood (RBC/hpf)[b]	1–10 (clean catch, not menstruating)	11–50 (clean catch, not menstruating)	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion
	1–10 (clean catch, F)	11–50 (clean catch, F)	> 50 and/or gross blood	
	1–10 (M)	11–50 (M)		

LLN = Lower limit of normal. ULN = Upper limit of normal. [b]A positive test for blood will not be considered Clinically Significant in a female subject who is menstruating, and microscopic analysis will not be performed unless clinically indicated. If the subject is not menstruating a repeat Urine Analysis will be performed.

When developing this Toxicity Grading Scale, Emergent referred to the recommendations in the FDA's Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventive Vaccine Clinical Trials (2007) and adjusted some parameters to close gaps between the toxicity grades.

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12 ATTACHMENTS

12.1 Study Personnel Roles and Responsibilities

12.2 Recruitment Materials and Scripts

12.3 Consent to Participate in Research for WRAIR CTC

12.4 Consent to Participate in Research for USAMRIID Division of Medicine

12.5 Consent for HIV Antibody Blood Test

12.6 Information Sheet Regarding Compensation of Federal Personnel

12.7 Active Duty Military Personnel Statement of Supervisor's Approval