Appendix A. Study Protocol Approval Form

Study Protocol Approval Form

Protocol Title	A Phase 2 Parallel-Group, Randomized, Double-Blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], unadjuvanted or alum-adjuvanted)
Protocol Number	PXVX-CV-317-001
Protocol Version	4.0
Version Date	22 May 2020

This document represents the clinical and regulatory review and approval of the final protocol or protocol amendment referenced above.

Approved by:

CLINICAL STUDY PROTOCOL

PROTOCOL TITLE:	A Phase 2 Parallel-Group, Randomized, Double-Blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], unadjuvanted or alum-adjuvanted)
PROTOCOL NUMBER:	PXVX-CV-317-001
INVESTIGATIONAL PRODUCT:	PXVX0317 (CHIKV-VLP Vaccine)
SPONSOR:	[redacted]
SPONSOR MEDICAL MONITOR:	[redacted]
VERSION:	4.0
DATE:	**22May2020**

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) 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 Parallel-Group, Randomized, Double-Blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus-Virus Like Particle Vaccine [CHIKV-VLP], unadjuvanted or alum-adjuvanted)
SITES	Multi-center, up to 5 US sites
OBJECTIVES	The goal of this trial is to evaluate the safety, tolerability and immunogenicity of 8 formulation/schedule combinations of PXVX0317 to select one or more formulation(s) for further development. One formulation and dosing schedule will be further evaluated in an open-label Group 9. **Immunogenicity Objectives** Primary ▪ To assess the induction of anti-chikungunya virus (CHIKV) neutralizing antibody responses by different formulations and schedules, as measured at 28 days after the last injection (Day 57). Secondary ▪ To assess the kinetics of induction of anti-CHIKV neutralizing antibody responses by different formulations and schedules, as measured from 7 days after the first injection (Day 8) to 28 days after the last injection (Day 57). ▪ To assess the difference in persistence of neutralizing antibody responses induced by different formulations and schedules, as measured up to 731 days after the last injection (Day 760) relative to earlier time points. ▪ To assess the boosting of vaccine-induced neutralizing antibody responses by a booster dose of PXVX0317.

	Exploratory ▪ To obtain high-titer serum to support non-clinical studies (including passive transfer studies) by using plasmapheresis at Day 57 to collect plasma from subjects immunized with one dose and regimen from Group 9 subjects. ▪ To obtain high-titer serum to support non-clinical studies (including passive transfer studies) by using plasmapheresis at Day 22 to collect plasma from Group 10 subjects immunized with a 40/300 mcg single adjuvant dose. ▪ To further assess anti-CHIKV neutralizing antibody, including isotype(s) and epitope specificity, by using leukapheresis at Day 182 (Group 9 subjects) and from PBMC samples at Days 1, 8, 15 and 22 (Group 10 subjects). ▪ To characterize the anti-CHIKV cellular immune response by collecting PBMCs at Days 1, 29, 57, and 182 from Group 9 subjects and at Days 1, 8, 15 and 22 for Group 10 subjects. **Safety Objectives** To study the safety and tolerability of 8 different formulations and dosing schedule combinations of PXVX0317 in healthy adults. Safety will be assessed by measuring the incidence of local and systemic solicited adverse events, unsolicited adverse events, and serious adverse events.
INVESTIGATIONAL PRODUCT	PXVX0317, field-formulated from CHIKV-VLP with a Diluent and Alhydrogel® adjuvant as required
CONTROL	Diluent, used as a placebo control for Day 1 and/or Day 15 injections in certain treatment groups.
MODE OF ADMINISTRATION	Intramuscular (IM) injection
PRIMARY ENDPOINT	The geometric mean titer of anti-CHIKV neutralizing antibody determined by luciferase-based assay at Day 57.

STUDY POPULATION	At least 420 healthy US adults
INCLUSION CRITERIA	1. Male or female 2. Age 18 to 45 years old (inclusive) 3. Using an acceptable method of contraception (if female of childbearing potential). 4. Able and willing to provide informed consent for study participation.
EXCLUSION CRITERIA	• Current acute febrile illness. • Clinically significant cardiac, respiratory, or rheumatologic disease, in the opinion of the Investigator. • Pregnant or breast-feeding. • Laboratory evidence of infection with Hepatitis B/C or HIV. • History of chikungunya virus infection. • Travel to a WHO-designated chikungunya-endemic region within 30 days prior to Day 1. • History of allergic reaction to any component of CHIKV-VLP vaccine, Diluent, or Alhydrogel®. • Inability to discontinue systemic immunomodulatory or immunosuppressive medications 30 days prior to Day 1. • Received or plans to receive any licensed vaccine from 30 days prior to Day 1 through Day 57. • Received or plans to receive an investigational agent from 30 days prior to Day 1 through the duration of study participation. • Any other condition that, in the opinion of the Investigator, creates an unacceptable risk to the subject. • Any other condition that, in the opinion of the Investigator, may interfere with the conduct of the study or the validity of the data.

	• Any other condition that, in the opinion of the Investigator, creates an unacceptable safety risk for apheresis (Group 9 and 10 subjects only). • Restricted venous access that would prevent the collection of PBMCs, plasma, and lymphocytes necessary for participation (Group 9 and 10 subjects only). • Weight < 110 pounds (Group 9 and 10 subjects only)
STUDY DESIGN	This is a Phase 2 parallel-group, randomized, double-blind study in healthy adults 18-45 years of age. At least 420 subjects are planned to be enrolled. This study has a screening period of 30 days, a treatment and observation period from Day 1 to Day 57, a follow-up period through Day 365 for all groups and an additional follow-up period through Day 760 for some groups. All subjects will be unblinded after their Day 365 visit. • Subjects in Groups 2, 3, 5, 6, and 7 will complete the study after the Day 365 visit. • Subjects in Groups 1 and 8 will continue to Day 760 without receiving a boost dose at Day 547. • Subjects in Group 4 will receive a boost dose of 40/300 mcg at Day 547 and will continue follow-up to Day 760. Treatment groups are shown in Table 4. Group 1 (the "Reference Group") receives a dose of 20 mcg CHIKV-VLP (unadjuvanted). Groups 2 through 8 receive CHIKV-VLP doses of 6, 10, 20, or 40 mcg in combination with Alhydrogel® 300 mcg. Group 9 receives CHIKV-VLP dose: 20/300 mcg and Group 10 receives CHIKV-VLP dose: 40/300 mcg. Groups 1 through 8 receive injections at Days 1, 15, and 29. Groups 1 through 4 receive PXVX0317 at Days 1 and 29 (placebo at Day 15); Groups 5 through 7 receive PXVX0317 at Days 15 and 29 (placebo at Day 1); and Group 8 receives PXVX0317 only at Day 29 (placebo at Days 1 and 15). Group 9 receives PXVX0317 at Days 1 and 29 and must participate in plasmapheresis collection at Day 57 (mandatory) and may participate in leukapheresis collection at Day 182 (optional). Group 10 receives PXVX0317 at Day 1 and must participate in plasmapheresis collection at Day 22 (mandatory).

	Details of visits, visit windows, and procedures are provided in the Schedule of Events for Groups 2, 3, 5, 6, and 7 (Appendix A1), for Groups 1, 4, and 8 (Appendix A2), for Group 9 (Appendix B1) and Group 10 (Appendix B2). After signing the informed consent form, subjects will undergo screening procedures up to 30 days before the first injection. Except for the open-label Groups 9 and 10, eligible subjects will be enrolled into a treatment group according to a randomization schedule in a 1:1:1:1:1:1:1:1 ratio. Group 9 will receive the same dosing schedule as subjects in Group 4, except no placebo at Day 15. Group 10 subjects will only receive a single dose on Day 1. Subjects will be observed in clinic for 30 minutes after each injection and vitals obtained at least 30 minutes but no more than 60 minutes after injection. Local and systemic solicited events occurring within 7 days after each 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 (oral temperature ≥100.4° F, chills, malaise, fatigue, headache, myalgia, joint pain, and nausea). Any other adverse events and medications used through Day 57 for Groups 1-9 and Day 29 for Group 10 will also be recorded. Blood will be collected at Day 1 (before the first injection) and Days 8, 15, 22, 29, 36, and 57 (Group 9 does not have a Day 15 or Day 22 visit and Group 10 does not have a Day 57 and 182 visit). An interim statistical analysis will be carried out on safety and immunogenicity data through the Day 57 visit. After the interim analysis, subjects will be enrolled in Group 9 (n =20). Subjects in Group 9 who discontinue study participation before Day 57 (or who do not undergo Day 57 plasmapheresis for other reasons) and subjects in Group 10 who discontinue study participation before Day 22 (or who do not undergo Day 22 plasmapheresis for other reasons) may be replaced. For Groups 1 through 8, follow-up visits will also be performed, and blood collected at Days 182 and 365. All subjects will be unblinded after their Day 365 visit; subjects in Groups 2, 3, 5, 6, and 7 will complete the study after the Day 365 visit. Subjects in Groups 1 and 8 will continue to Day 760 without receiving a boost dose at Day 547. Only subjects in Group 4 will receive a boost dose of 40/300 mcg at Day 547 and will continue follow-up to Day 760. Follow-up visits and blood collection for safety and immunogenicity will be

	performed at Days 575 (Group 4 only) and 760 for Groups 1, 4 and 8. 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.
SEROLOGY	Antibody (Ab) responses to CHIKV-VLP will be determined by a luciferase-based anti-CHIKV neutralization assay. Titers are expressed as the reciprocal of the serum dilution achieving 80% neutralization (NT80). Persistence of the induced response will be assessed by comparing anti-CHIKV neutralization responses at later time points with those obtained earlier. Ab responses will also be assessed by a commercial anti-CHIKV ELISA at Baseline and Day 57. Various PBMC compartment (e.g. memory B and helper T cells) responses will be assessed at specific time points during the study.
CRITERIA FOR EVALUATION (ENDPOINTS)	**Immunogenicity Endpoints** Anti-CHIKV neutralization response data for each treatment group will be evaluated and tabulated across the different time points. The primary analysis will be based on anti-CHIKV neutralization titers measured at 28 days after the last injection (Day 57). Analyses will then be conducted on immunogenicity data collected through Day 365 (Groups 2, 3, 5, 6, and 7) or Day 760 (Groups 1, 4, and 8). Data from Group 9 through Day 182, from Group 4 from the boost at Day 547 through Day 760 and Group 10 through Day 22 will be analyzed separately. The derived immunogenicity endpoints are listed briefly below. *Geometric Mean Titer (GMT) and Geometric Mean Ratio (GMR)* Anti-CHIKV neutralization titers will be logarithmically transformed (base10). The GMTs, GMRs and associated 95% confidence intervals for each treatment group will be computed by exponentiating the corresponding log-transformed (least squares) means and 95% confidence limits. The estimate of the random error used in calculating the confidence limits will be obtained from an ANOVA model with factors of treatment group and study site as

	fixed effects. Comparisons of treatment groups 2 through 8 to group 1 will be derived from contrasts based on this model. *Proportion (percentage) of subjects with anti-CHIKV titer exceeding defined cut-off values.* The proportion (percentage) of subjects with Ab responses exceeding stipulated cut-off values (for example, titers of 10 or 100), together with a two-sided 95% CI based on the Wilson method, will be tabulated for each treatment group at each time point. Fisher's exact tests will be performed to derive p-values for the pairwise treatment group comparisons. *Proportion (percentage) of subjects with at least a four-fold rise over baseline in anti-CHIKV titer.* The proportion (percentage) of subjects with post-vaccination titers at least four times higher than pre-vaccination, together with a two-sided 95% CI based on the Wilson method, will be tabulated for each treatment group at each time point. Fisher's exact tests will be performed to derive p-values for the pairwise treatment group comparisons. **Safety Endpoints** Subjects who receive a study injection will have data collected for safety analysis. The safety and tolerability of each treatment group will be compared in a descriptive fashion. The safety objectives will be assessed by analysis of all local and systemic post-injection solicited events and other adverse events collected during the 7 days following each injection. Safety analysis will also include the occurrence of any adverse events through Day 57 and for 28 days after boost, serious adverse events, and adverse events leading to withdrawal from study. In addition, any adverse events occurring within 30 minutes of the plasmapheresis procedure at Day 57 for Group 9 and Day 22 for Group 10 and leukapheresis procedure at Day 182 for Group 9 will be collected. All safety data will be tabulated according to treatment group and at different time points post injections. Data listings will include all subjects, including screen failures. The likely relatedness or lack of relatedness to vaccine as assessed by the principal Investigator will be provided.
DOSE SELECTION CRITERIA	The primary assessment will be based on anti-CHIKV neutralization titer measured at Day 57 after the first injection. GMTs for any two treatment groups will be considered different if the test is significant (two-sided 0.05 significance level), and if the percentage of subjects

	in the group that meet the specific response definition is no less than 75%. For formulations that appear indistinguishable, selection of a formulation will be based on a combined body of evidence obtained from the GMT values, percentage of responders, safety profiles and additional summary variables, if deemed necessary.
STATISTICAL METHODS	**Statistical Hypotheses** Pair-wise comparisons between relevant treatment groups will be based on the percentage of subjects with four-fold rise in anti-CHIKV titer and GMTs measured at Day 57. (28 days after the last injection). Specifically, we will be testing the null hypotheses: H_0: $P_i = P_j$ vs. H_1: $P_i \neq P_j$ where P_i and P_j denote the proportion of subjects with four-fold rise for any two of the treatment groups, and H_0: $\mu_i = \mu_j$ vs. H_1: $\mu_i \neq \mu_j$ where μ_i and μ_j denote the GMT for any two of the treatment groups. All tests will be carried out at a two-sided significance level of 0.05 and no adjustment for multiplicity will be applied, since the goal will be to rank different formulations and schedules rather than to establish inferential values. **Sample Size and Power Considerations** [redacted]

	[redacted]
INTERIM ANALYSIS	There will be a safety and immunogenicity interim analysis for the selection of a formulation and schedule based on the data collected for Groups 1 through 8 through Day 57. The results will be reported by treatment group preserving the double-blind status on the subject level.

TABLE OF CONTENTS

Page

PROTOCOL SIGNATURE SHEET **2**

PROTOCOL SYNOPSIS **3**

TABLE OF CONTENTS **12**

1 INTRODUCTION **18**

1.1 Background 18

1.2 Name and Description of Investigational Vaccine 19

1.2.1 Summary of Findings from Nonclinical Studies 19

1.2.2 Summary of Findings from Clinical Studies 22

1.2.2.1 VRC 311 22

1.2.2.2 VRC 704 22

1.3 Rationale for the Current Study 23

1.3.1 Rationale for Dosage and Route of Administration 24

2 OBJECTIVES AND ENDPOINTS **25**

2.1 Primary Objectives 25

2.2 Secondary Objectives 25

2.3 Exploratory Objectives 25

3 STUDY PLAN **27**

3.1 Study Design 27

3.2 Estimated Study Duration 28

3.3 Study Population 28

3.3.1 Inclusion Criteria 28

3.3.2 Exclusion Criteria 28

4 STUDY VACCINE **30**

4.1 Investigational Vaccine (PXVX0317) 30

4.1.1 CHIKV-VLP 30

4.1.2 Diluent 31

4.1.3 Alhydrogel Adjuvant 31

4.1.4 Other Supplies 32

4.1.5 Formulations and Doses of PXVX0317 32

4.1.6 Study Vaccine Preparation 32

4.2 Control Vaccine (Diluent) 33
5 STUDY PROCEDURES 34
5.1 Informed Consent 34
5.2 Screening 34
5.3 Medical History 34
5.4 Physical Examination 34
5.5 Vital Signs 35
5.6 Laboratory Tests 35
5.7 Pregnancy Testing and Contraception 36
5.8 Randomization 36
5.9 Study Vaccine Administration 37
5.10 Acute Observation 38
5.10.1 Observation Following Apheresis 38
5.11 Solicited Adverse Events 38
5.12 Unsolicited Adverse Events 39
5.13 Prior and Concomitant Medications 39
5.14 Prohibited Medications 39
5.15 Emergency Unblinding 39
5.16 Protocol Deviations 40
5.17 Study Completion: For Individual Subjects 40
5.18 Early Discontinuation 40
5.19 Subject Replacement 41
5.20 Study Completion: Overall 41
6 STUDY PROCEDURES BY VISIT 42
6.1 Scheduled Study Visits 42
6.1.1 Screening (-30 days to Day 1) – All groups 42
6.1.2 Day 1 – All groups 42
6.1.3 Day 8 (+3 days) – All groups 43
6.1.4 Day 15 (± 1 day) – Groups 1 through 8 only 43
6.1.5 Day 15 (± 2 days) – Group 10 only 44
6.1.6 Day 22 (+3 days) – Groups 1 through 8 only 44
6.1.7 Day 22 (+ 5 day) – Group 10 only 44
6.1.8 Day 29 (± 1 day) – Groups 1-9 44

6.1.9 Day 29 (-1/+5 days) – Group 10 only (phone call follow-up) 45
6.1.10 Day 36 (+3 days) – Groups 1-9 45
6.1.11 Day 57 (± 3 days for Groups 1-8) and (-14/+3 days for Group 9) ... 45
6.1.12 Day 182 (± 7 days) – Groups 1-9 46
6.1.13 Day 365 (± 7 days) – Groups 1 through 8 only 46
6.1.14 Day 547, Boost Visit (± 7 days) – Group 4 only 46
6.1.15 Day 547, without boost (± 7 days) – Groups 1 and 8 only 47
6.1.16 Day 554, phone visit (+ 3 days) – Group 4 only 47
6.1.17 Day 575 (± 3 days) – Groups 4 only 47
6.1.18 Day 760 (-14/+90 days) – Groups 1, 4 and 8 only 47
6.2 Early Discontinuation Visit 47
6.3 Unscheduled Visits 48
6.4 Summary of Apheresis Collection Procedures 48
6.4.1 Plasmapheresis Procedure 48
6.4.2 Leukapheresis Procedure 49
7 SAFETY 50
7.1 Definitions 50
7.1.1 Adverse Event 50
7.1.2 Solicited Adverse Event 50
7.1.3 Unsolicited Adverse Event 51
7.1.4 Serious Adverse Event 51
7.2 Severity Grading 51
7.3 Causality Assessment 52
7.4 Follow-up of Adverse Events 52
7.5 Reporting of Adverse Events 53
7.5.1 Reporting Periods 53
7.5.2 Documentation 56
7.6 SAE Reporting 56
7.7 Other Events Requiring Immediate Reporting 57
7.7.1 Pregnancy 57
7.7.2 Dosing Errors 57
7.7.3 Early Discontinuation for Safety Reasons 57
7.7.4 Emergency Unblinding for Safety Reasons 57

7.7.5 Safety Events Related to Apheresis 58
7.8 Medical Monitor 58
7.9 Study Stopping Rules 58
8 DATA HANDLING 59
8.1 Source Documentation 59
8.2 Case Report Forms 59
8.3 Retention of Study Documentation 59
8.4 Data Monitoring 59
8.5 Laboratory Data 59
8.6 Audit Compliance 60
9 STATISTICAL ANALYSIS 61
9.1 Sample Size Calculation 61
9.2 Treatment Period 61
9.3 Treatment Groups 61
9.4 Populations for Analysis 62
9.5 Safety Analysis 63
9.5.1 Solicited AEs 63
9.5.2 Unsolicited AEs 64
9.5.3 Analysis of Other Safety Data 65
9.6 Immunogenicity Analysis 65
9.7 Interim Analyses 67
10 ADDITIONAL INFORMATION 68
10.1 Ethical Conduct of the Study 68
10.1.1 Future Use of Stored Specimens and Data 68
10.2 IRB Oversight 68
10.3 Informed Consent 68
10.4 COVID-19 related considerations 69
10.5 Subject Confidentiality 69
10.6 Compensation for Injury 69
10.7 Clinicaltrials.gov 69
10.8 Public Disclosure and Publication Policy 69
10.9 Amendments 69
APPENDIX A1: SCHEDULE OF EVENTS (GROUPS 2, 3, 5, 6, AND 7) 71

APPENDIX A2: SCHEDULE OF EVENTS (GROUPS 1, 4, AND 8) 72
APPENDIX B1: SCHEDULE OF EVENTS (GROUP 9 ONLY) 74
APPENDIX B2: SCHEDULE OF EVENTS (GROUP 10 ONLY) 75
APPENDIX C: TOXICITY GRADING SCALE..... 76
REFERENCES..... 78

LIST OF ABBREVIATIONS

Ab	Antibody
AE	Adverse Event
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
CHIKV	Chikungunya Virus
CI	Confidence Interval
CRF	Case Report Form
DMP	Data Management Plan
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IM	Intramuscular
IND	Investigational New Drug
IPD	Important Protocol Deviation
IRB	Institutional Review Board
MedDRA	Medical Dictionary of Regulatory Activities
MM	Medical Monitor
nAb	Neutralizing Antibody
NT	Neutralization Titer
PBMC	Peripheral Blood Mononuclear Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMP	Safety Management Plan
VLP	Virus-Like Particle
US	United States
WHO	World Health Organization

1 INTRODUCTION

1.1 Background

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 (C) 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).

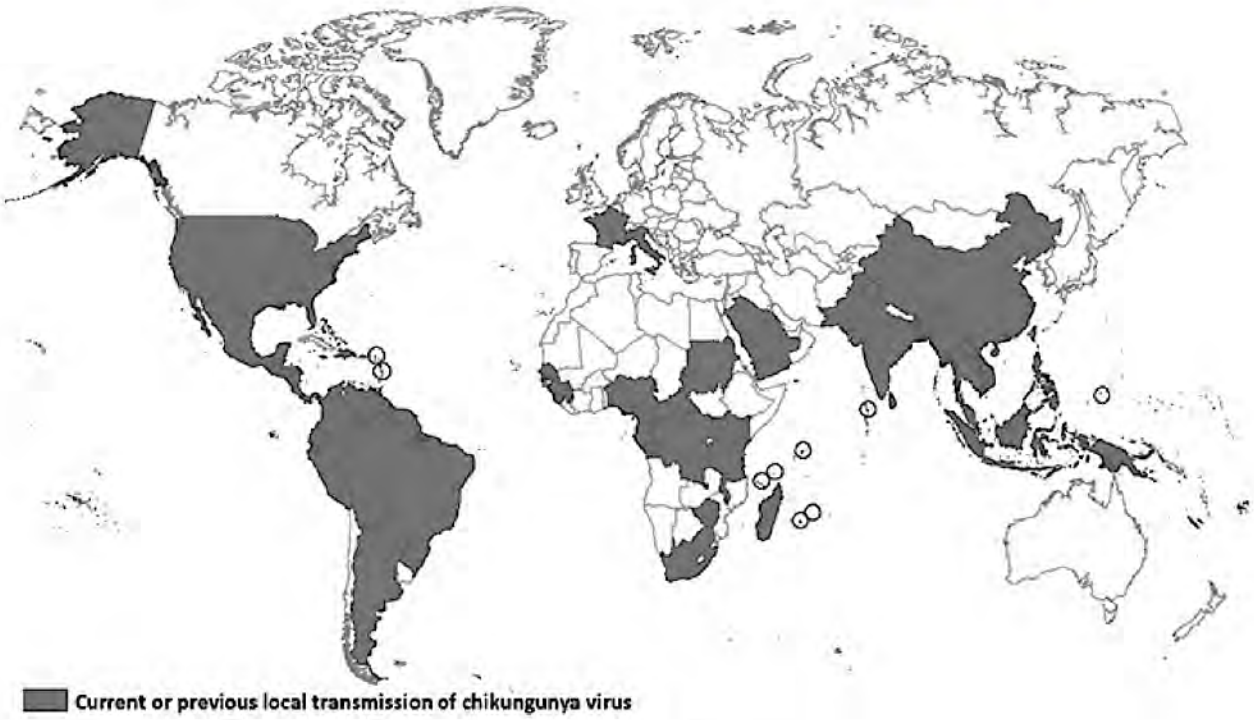
Beginning in 2014, CHIKV disease cases were reported among U.S. travelers returning from affected areas in the Americas and local transmission was identified in Florida, Puerto Rico, and the U.S. Virgin Islands. As of April 22, 2016, 103 countries or territories have documented cases of CHIKV infection excluding those countries where only imported cases have been documented (CDC 2017). 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 up to 10% of infected individuals.

Diagnosis of CHIKV infection can be based on either serology or presence of virus. During the acute phase of disease, there are generally high levels of viremia and reverse transcriptase-polymerase chain reaction (RT-PCR) or culture can frequently be used to detect infection. Viral RNA can be detected by RT-PCR. Immunoglobulin M (IgM) antibodies develop within 1 week after infection and persist for several months. Immunoglobulin G (IgG) antibodies typically develop after the onset, persisting for weeks to months and perhaps longer. Protection against subsequent infection has been shown to correlate with the presence of anti-CHIKV antibodies that neutralize the virus *in vitro*. (Yoon 2015)

There are currently no approved vaccines to prevent CHIKV infection or disease.

Figure 1: CHIKV Global Burden as of April 22, 2016 (CDC 2017)



1.2 Name and Description of Investigational Vaccine

PXVX0317 is the Sponsor's research name for Chikungunya Virus (E1, E2 and capsid Proteins, HEK 293 cells) Virus-Like Particle Vaccine (CHIKV-VLP vaccine). PXVX0317 is a field-formulated vaccine with three components: CHIKV-VLP, Diluent as required for each dose level, and Alhydrogel® for the adjuvanted formulations. CHIKV-VLP is comprised of three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997: Capsid/core (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 used in the studies described below. Diluent is identical to the buffer of the CHIKV-VLP. Alhydrogel is a 2% (w/w) aqueous suspension of aluminum hydroxide.

PXVX0317 is described further in Section 4 .

1.2.1 Summary of Findings from Nonclinical Studies

VRC-CHKVLP059-00-VP (CHKVLP059)

Administration of CHKVLP059 protected monkeys from CHIKV challenge (VRC "Study No. 2"). Infusion of serum from CHKVLP059-immunized monkeys also protected immune-deficient mice from CHIKV challenge (VRC "Study No. 3"). These studies provided nonclinical evidence of the immunogenicity and protective efficacy of CHKVLP059 and suggested that anti-CHIKV neutralizing antibodies are an important component of protection.

In addition, the safety of the proposed doses and regimens in this study is supported by the well-known safety record of aluminum salt-based adjuvants in humans and a repeat-dose rabbit toxicology study performed in compliance with Good Laboratory Practices (GLP). Rabbits received four 1 mL intramuscular (IM) injections of a nominal dose of 40 mcg of CHKVLP059 or Diluent (placebo) on Days 1, 22, 43, and 64. Injections were very well tolerated locally, with no systemic toxicity. Transient elevations of fibrinogen and C-reactive protein were observed in the animals which received CHKVLP059, consistent with an acute phase inflammatory response. There were no treatment-related effects on in-life parameters, including body temperature and joint range of motion. All animals survived to necropsy, which revealed mixed cell infiltrates at injection sites and no other treatment-related macroscopic or microscopic findings.

PXVX0317 Mouse Immunological Study PTR-TRD-CHIK-004

This study compared the immunogenicity of CHIKV VLP material made by Emergent Travel Health with material made by the VRC and also explored the benefit of adjuvant. Treatment groups are shown in Table 1. C57Bl/6 x Balb/c F1 hybrid mice were immunized at Days 0 and 35 with PXVX0317 (Groups 1 to 6) or CHKVLP059 (Groups 11 to 16). VLP doses were 0.2, 2, or 20 mcg of VLP with or without 250 mcg of alum (Alhydrogel). All of the above doses were prepared immediately before dosing. Additionally, doses of 2 and 20 mcg of PXVX0317 with 250 mcg of alum were prepared 4 hours (Groups 7 and 8) or 24 hours (Groups 9 and 10) before dosing. Control groups received CHIKV DNA plasmid (Group 17), alum (Group 18), or no treatment (Group 19). There were 6 animals per group. All doses were administered in 0.1 mL total volume by the intramuscular (IM) route. Serum was taken at Days 14, 28, and 56. Day 14 sera were pooled by group and Day 28 and 56 sera were analyzed individually.

Anti-CHIKV-VLP antibodies were measured by ELISA of pooled sera at Day 14 (Table 1). ELISA antibody titers were calculated as the 50% endpoint response of the dilution curve (EC50). Antibody responses to CHIKV-VLP vaccine were evident at Day 14. Responses were similar between PXVX0317 and CHKVLP059, increased with increasing VLP dose, and increased at all doses with the addition of alum. Responses to the PXVX0317 doses prepared 4 or 24 hours prior to immunization were also similar to those to the same doses immediately before dosing. Negligible EC50 values were observed in all control groups.

CHIKV-specific neutralizing antibodies were measured by CHIKV-Luc neutralization assay at Days 14, 28, and 56. Neutralization titers were calculated as the maximum serum dilution that neutralizes 80% of luciferase activity following infection of Vero cells by a recombinant CHIKV-Luc virus (NT80). Geometric mean titers (GMTs) were calculated by group. Neutralization was detected in all groups treated with VLP at Day 14. As with ELISA, the neutralizing antibody response was similar between PXVX0317 and CHKVLP059, increased with increasing VLP dose, and increased at all doses with alum. In addition, a boosting of the response was observed after the second dose, with all groups exhibiting higher NT80 GMTs at

Day 56 than Day 28. Responses to all PXVX0317 2 mcg doses with alum were similar. The PXVX0317 20 mcg dose prepared 24 hours before dosing elicited lower GMTs at Days 28 and 56 than the doses prepared immediately or 4 hours before dosing.

These data supported the comparable and dose-dependent immunogenicity of the PXVX0317 and CHKVLP059 VLPs after both primary and booster immunization, with or without alum. The addition of alum elicited a marked and potentially dose-sparing increase in antibody titers. Also, there appeared to be minimal reduction of immunogenicity up to at least 4 hours prior to immunization. Taken together, these findings support the regimens planned in CV-317-001.

Table 1: PTR-TRD-CHIK-004 Treatment Groups and Results

Group	Treatment	Dose mcg	Alum mcg	Day 14 (pooled)		Day 28 (individual)	Day 56 (individual)
				ELISA EC50	CHIKV-Luc NT80	CHIKV-Luc NT80 GMT (Range)	CHIKV-Luc NT80 GMT (Range)
1	PXVX0317	0.2	-	209	80	98 (85-210)	1070 (261-2346)
2	PXVX0317	2	-	1417	385	551 (348-1031)	7313 (3824-14134)
3	PXVX0317	20	-	3899	1754	655 (445-1090)	17416 (7295-39518)
4	PXVX0317	0.2	250	1669	1168	1190 (486-6000)	5555 (3348-15033)
5	PXVX0317	2	250	4145	2975	1428 (855-2567)	11840 (6502-28819)
6	PXVX0317	20	250	10423	7075	2897 (2243-4404)	14995 (6705-31434)
7	PXVX0317	2	250	3815	662	1203 (309-2609)	13197 (5326-44969)
8	PXVX0317	20	250	11636	2587	4191 (2175-8065)	31384 (24270-76294)
9	PXVX0317	2	250	2904	1728	1856 (967-3203)	11317 (5587-24270)
10	PXVX0317	20	250	9874	3302	1642 (1385-2391)	6524 (4284-10163)
11	CHKVLP059	0.2	-	156	85	119 (90-222)	1615 (527-5224)
12	CHKVLP059	2	-	495	650	363 (113-1155)	4819 (3199-8750)
13	CHKVLP059	20	-	4396	1503	909 (285-1503)	11619 (5408-19999)
14	CHKVLP059	0.2	250	1079	1175	972 (336-13116)	5655 (2307-14134)
15	CHKVLP059	2	250	5064	1706	1300 (794-2832)	12345 (6989-28710)
16	CHKVLP059	20	250	13170	3172	3236 (1556-7796)	10212 (4284-18343)
17	CHIKV DNA	50	-	<100	31	20 (<20-57)	38 (<20-193)
18	Alum only	-	250	109	<10	<20	<20
19	Naïve	-	-	<100	<10	<20 (<20-21)	<20

Source: PTR-TRD-CHIK-004. - no treatment or not applicable
Groups 7 and 8: prepared 4 hours before dosing; Groups 9 and 10: prepared 24 hours before dosing.
EC50 = 50% endpoint response; NT80 = maximum dilution achieving 80% neutralization

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 (unadjuvanted) 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 Travel Health.

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 $p=0{\cdot}0197$, 20 mcg group $p<0{\cdot}0001$, and 40 mcg group $p<0{\cdot}0001$). 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 Travel Health luciferase-based assay to be used for the PXVX-CV-317-001 trial, demonstrating the suitability of the Emergent Travel Health assay [Emergent Travel Health, data on file].

1.2.2.2 VRC 704

VRC 704 is a multi-center, randomized, placebo-controlled, double-blind Phase 2 study to evaluate the safety and immunogenicity of a regimen of CHKVLP059 20 mcg administered by IM injection at week 0 and week 4 in 400 healthy adults in CHIKV-endemic areas. Vaccinations are complete. The study was completed on 06 March 2018 and results have been posted on clinicaltrials.gov.

Overall all injections were well tolerated. The most frequently reported local reactogenicity symptom was pain/tenderness at the injection site reported as mild by 77 of 395 (19.5%) subjects who received at least one study injection and as moderate by 3/395 subjects (0.8%). The most frequently reported systemic reactogenicity symptoms were mild or moderate headache reported by 112 of 395 (28.4%) subjects, malaise (101/395, 25.6%), and myalgia (82/395, 20.8%). No related SAEs have occurred and no clinically significant patterns of laboratory abnormalities have been identified.

Preliminary blinded analysis using a luciferase-based assay has demonstrated that approximately 30 percent of subjects had pre-vaccination neutralizing antibodies consistent with prior CHIKV infection. In addition, approximately 50 percent of subjects with no pre-vaccination antibodies demonstrated a robust induction of neutralizing antibodies 4 weeks after the second dose of (blinded) study vaccine, consistent with the 1:1 randomization ratio.

Taken together, the findings from VRC 704 suggest that a regimen of 2 doses of 20 mcg CHKVLP059VP administered 4 weeks apart is well-tolerated and immunogenic in both CHIKV-exposed and CHIKV-naïve adults.

1.3 Rationale for the Current Study

The current study builds on the findings of VRC 311 and VRC 704, specifically to compare the immunogenicity of regimens of various doses of CHIKV-VLP with Alhydrogel to the unadjuvanted regimen used in VRC 704. It is possible that doses of 20 mcg or lower with Alhydrogel, given in a 4-week, 2-week, or single-dose regimen, may achieve faster, higher, and/or more durable immune responses than those achieved in VRC 311 and VRC 704. Specifically, for durability, the immune response in the 40/300 mcg group (the final selected dose for the PXVX0317 development program) will be assessed through 104 weeks (2 years) of follow-up, compared with a maximum of 78 weeks (18 months) in VRC704.

In addition, this study will evaluate the safety and immunogenicity of booster doses of PXVX0317 administered at 78 weeks. The existing data from VRC 311 includes only booster doses administered at 20 weeks.

This study will also substantially expand the combined safety database of CHIKV-VLP (PXVX0317 and CHKVLP059VP) recipients, with all subjects planned to receive at least one dose of PXVX0317.

An open label group given a CHIKV-VLP dose of 20/300 mcg (Group 9, n=20) will be implemented to allow evaluation of cellular immune responses and to provide anti-CHIKV antibodies for use in studies in animals. Peripheral blood mononuclear cells (PBMCs) will be collected at specific time points listed in Section 2.3 of the protocol. Plasmapheresis will be used to collect plasma for antibody extraction to support non-clinical animal passive transfer studies.

An open label group given a CHIKV-VLP dose of 40/300 mcg (Group 10, n=10) will be implemented to allow evaluation of cellular immune responses and to provide anti-CHIKV antibodies for use in studies in animals. Peripheral blood mononuclear cells (PBMCs) will be collected at specific time points listed in Section 2.3 of the protocol. Plasmapheresis will be used to collect plasma for antibody extraction to support non-clinical animal passive transfer studies.

1.3.1 Rationale for Dosage and Route of Administration

The dosages of CHIKV-VLP in this study range from 6 mcg to 40 mcg. These doses are below or approximately equivalent to those used in VRC 311 and VRC 704. Based on the findings from those studies and the addition of Alhydrogel, these doses are expected to be safe and immunogenic.

The Alhydrogel dose of 300 mcg is within the range of doses of alum adjuvants used in many licensed vaccines, including Engerix-B®. This dose also creates a concentration ratio to CHIKV-VLP that achieves high (~90%) levels of adsorption, thought to enhance both immunogenicity and short-term stability.

The route of administration (intramuscular – IM) is consistent with that in VRC 311 and VRC 704.

Group 9 will receive a dose of 20/300 mcg on Days 1 and 29. This dose was selected following the interim data analysis regarding all doses and their impact on subject seroconversion rates (at Days 29 and 57), GMT levels, and safety-related events.

Group 10 will receive a single dose of 40/300 mcg on Day 1. This dose was selected for future development following the Day 182 interim data analysis regarding all doses and their impact on subject seroconversion rates, GMT levels, and safety-related events.

The boost dose for Group 4 will be 40/300 mcg (based on the final selected dose for the PXVX0317 development program).

2 OBJECTIVES AND ENDPOINTS

2.1 Primary Objectives

The primary safety objective is to evaluate the safety and tolerability of 8 formulation/schedule combinations of PXVX0317 in healthy adults and to select one or more formulation(s) for further vaccine development. 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 assess the induction of anti-CHIKV neutralizing antibody responses by different formulations and schedules, as measured at 28 days after the last injection (Day 57).

2.2 Secondary Objectives

The secondary immunogenicity objectives include the following:

- To assess the kinetics of induction of anti-CHIKV neutralizing antibody responses by different formulations and schedules, as measured from 7 days after the first injection (Day 8) to 28 days after the last injection (Day 57).
- To assess the difference in persistence of neutralizing antibody responses induced by different formulations and schedules, as measured up to 731 days after the last injection (Day 760) relative to earlier time points.
- To assess the boosting of vaccine-induced neutralizing antibody responses by an additional dose of PXVX0317.

2.3 Exploratory Objectives

The exploratory immunogenicity objectives include the following:

- To obtain high-titer serum to support non-clinical studies (including passive transfer studies) by using plasmapheresis at Day 57 to collect plasma from subjects immunized with one dose and regimen from Group 9 subjects.
- To obtain high-titer serum to support non-clinical studies (including passive transfer studies) by using plasmapheresis at Day 22 to collect plasma from Group 10 subjects immunized with 40/300 mcg single adjuvant dose.
- To further assess anti-CHIKV neutralizing antibody, including isotype(s) and epitope specificity, by using leukapheresis at Day 182 (Group 9 subjects) and from PBMC samples at Days 1, 8, 15 and 22 (Group 10 subjects).

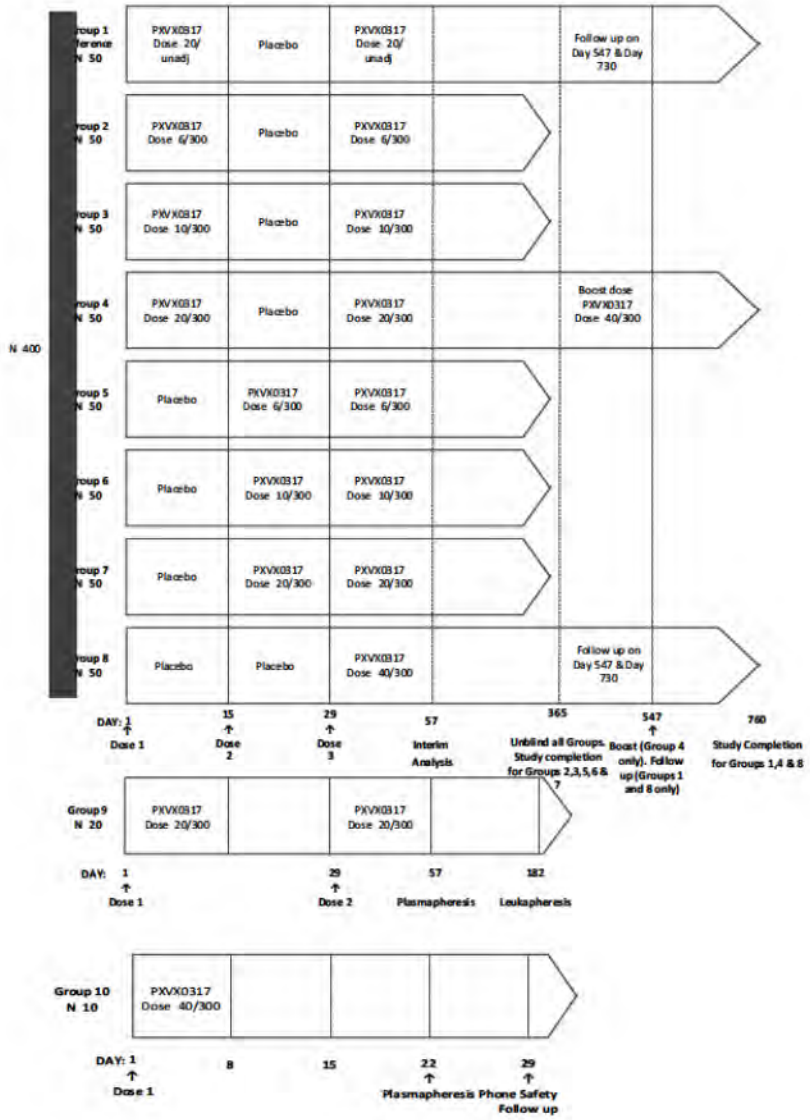
- To characterize the anti-CHIKV cellular immune response by collecting PBMCs at Days 1, 29, 57, and 182 from Group 9 subjects and at Days 1, 8, 15, and 22 for Group 10 subjects.

3 STUDY PLAN

3.1 Study Design

This is a Phase 2 parallel-group, randomized, double-blind study in healthy adults 18-45 years of age for Groups 1 through 8, and two additional open label groups (Group 9 and 10) (Figure 2).

Figure 2: PXVX-CV-317-001 Study Schema



Dose shown as mcg CHIKV-VLP/unadjuvanted or mcg CHIKV-VLP/mcg Alhydrogel.

Number of Study Participants

The study population will be composed of at least 420 healthy US adults who meet the eligibility criteria listed in Section 3.4.

3.2 Estimated Study Duration

For Groups 1-9, the study consists of a 30-day screening period, a treatment and observation period from Day 1 through Day 57 and:

- For Groups 2, 3, 5, 6, and 7: a follow-up period through Day 365.
- For Groups 1 and 8: a follow-up period through Day 760 without receiving a boost dose at Day 547.
- For Group 4: a follow-up period through Day 760 after receiving a boost dose of 40/300 mcg at Day 547.
- For Group 9: a follow-up period through Day 182.

For Group 10, the study consists of a 30-day screening period, a treatment and observation period from Day 1 through Day 22 and safety follow-up through Day 29.

The maximum possible study duration for an individual subject is 790 days.

3.3 Study Population

3.3.1 Inclusion Criteria

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

1. Male or female
2. Age 18 to 45 years old (inclusive)
3. Using an acceptable method of contraception (if female of childbearing potential).
4. Able and willing to provide informed consent for study participation.

3.3.2 Exclusion Criteria

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

1. Current acute febrile illness.
2. Clinically significant cardiac, respiratory, or rheumatologic disease, in the opinion of the Investigator.
3. Pregnant or breast-feeding.
4. Laboratory evidence of infection with Hepatitis B/C or HIV.

5. History of chikungunya virus infection.
6. Travel to a WHO-designated chikungunya-endemic region within 30 days prior to Day 1.
7. History of allergic reaction to any component of CHIKV-VLP vaccine, Diluent, or Alhydrogel®.
8. Inability to discontinue systemic immunomodulatory or immunosuppressive medications 30 days prior to Day 1.
9. Received or plans to receive any licensed vaccine from 30 days prior to Day 1 through Day 57.
10. Received or plans to receive an investigational agent from 30 days prior to Day 1 through the duration of study participation.
11. Any other condition that, in the opinion of the Investigator, creates an unacceptable risk to the subject.
12. Any other condition that, in the opinion of the Investigator, may interfere with the conduct of the study or the validity of the data.
13. Any other condition that, in the opinion of the Investigator, creates an unacceptable safety risk for apheresis (Group 9 and 10 subjects only)
14. Restricted venous access that would prevent the collection of PBMCs, plasma and lymphocytes necessary for participation (Group 9 and 10 subjects only).
15. Weight < 110 pounds (Group 9 and 10 subjects only)

4 STUDY VACCINE

4.1 Investigational Vaccine (PXVX0317)

PXVX0317 is the Sponsor's research name for Chikungunya Virus (E1, E2 and capsid Proteins, HEK 293 cells) Virus-Like Particle Vaccine (CHIKV VLP vaccine). PXVX0317 is a field-formulated vaccine with three components: CHIKV-VLP, Diluent as required for each dose level, and Alhydrogel® 2% for adjuvanted formulations. These three components are combined in prespecified volumes to achieve the formulations and doses indicated in this protocol.

4.1.1 CHIKV-VLP

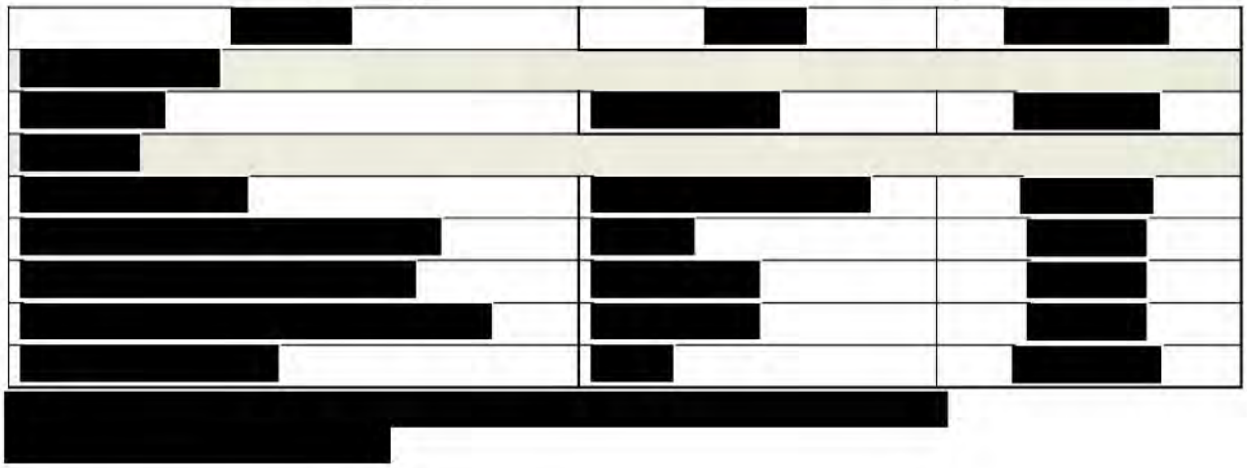
CHIKV-VLP is a virus-like particle (VLP) comprised of three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997: Capsid/core (C, 35 kDa), Envelope 1 (E1, 55 kDa), and Envelope 2 (E2, 50 kDa). CHIKV-VLP is produced by transient transfection of human embryonic kidney (HEK) 293 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-dose 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 must be stored in a qualified, temperature-controlled freezer at ≤ –70°C. The temperature should be monitored by checking and recording current, maximum/minimum temperature readings inside the vaccine storage unit once each working day.

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

Table 2: [redacted]



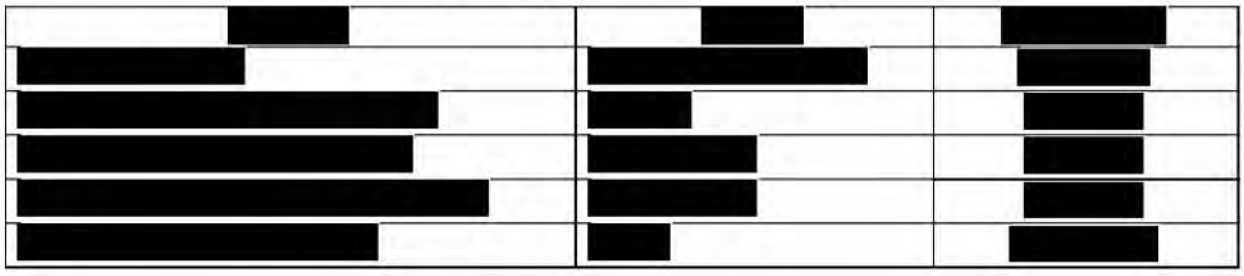
4.1.2 Diluent

The diluent is a sterile aqueous solution with the same excipient composition as the CHIKV-VLP drug product. The diluent is filled into 3 mL single-dose glass vials with a 1.2 mL fill volume. The glass vial is sealed with a rubber stopper and an aluminum seal with a green flip-off cap. The diluent will also be used as the placebo.

The diluent is stored at 15 to 30°C.

The composition of the diluent (Lot Number 1-FIN-2985) is shown in Table 3.

Table 3: [redacted]



4.1.3 Alhydrogel 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® 2% (CRODA Denmark A/S). It meets the requirements of the European Pharmacopoeia monograph for aluminum hydroxide, hydrated for adsorption and has a pH of 6-7. 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.

4.1.4 Other Supplies

The following supplies are supplied by the Sponsor for formulation and administration of study vaccine:

1. 3/10 mL BD Lo-Dose™ U-100 insulin syringe with 29 Gauge x 1/2 (BD Cat # 324702)
2. 1 mL BD Luer-Lok™ Syringe sterile, single use polycarbonate (BD Cat # 309628)
3. 25 Gauge BD™ Needle 1 in. single use, sterile (BD Cat # 305125)
4. 25 Gauge BD™ Needle 1 1/2 in. single use, sterile (BD Cat # 305127)

Plasmapheresis and leukapheresis kits will be supplied by the Community Blood Center of Greater Kansas City.

4.1.5 Formulations and Doses of PXVX0317

The unadjuvanted formulation of PXVX0317 contains CHIKV-VLP and Diluent. The only dose of this formulation used in this protocol is 20 mcg (unadjuvanted). The Alhydrogel-adjuvanted formulations of PXVX0317 contain CHIKV-VLP, Diluent, and Alhydrogel adjuvant.

Four doses are used in this protocol: 6 mcg, 10 mcg, 20 mcg, or 40 mcg CHIKV-VLP with 300 mcg Alhydrogel.

4.1.6 Study Vaccine Preparation

To prepare the 20 mcg (unadjuvanted) dose:

1. 0.8 mL Diluent is added to one vial (0.8 mL) of CHIKV-VLP, which is now the Dose Vial and contains 1.6 mL.
2. 0.8 mL is withdrawn from the Dose Vial into a syringe.

To prepare the 6 mcg / 300 mcg dose:

1. 0.1 mL is withdrawn and discarded from a Diluent vial (1.2 mL), which is now the Dose Vial and contains 1.1 mL.
2. 0.2 mL CHIKV-VLP is added to the Diluent Vial (Dose vial), which now contains 1.3 mL.
3. 0.05 mL Alhydrogel is added to the Dose Vial and mixed by swirling.
4. The Dose Vial is held at room temperature for 15 minutes.
5. 0.8 mL is withdrawn from the Dose Vial into a syringe.

To prepare the 10 mcg / 300 mcg dose:

1. 0.4 mL CHIKV-VLP is added to a Diluent vial (1.2 mL), which is now the Dose Vial and contains 1.6 mL.
2. 0.06 mL Alhydrogel is added to the Dose Vial and mixed by swirling.
3. The Dose Vial is held at room temperature for 15 minutes.
4. 0.8 mL is withdrawn from the Dose Vial into a syringe.

To prepare the 20 mcg / 300 mcg dose:

1. 0.8 mL Diluent is added to a CHIKV-VLP vial (0.8 mL), which is now the Dose Vial and contains 1.6 mL.
2. 0.06 mL Alhydrogel is added to the Dose Vial and mixed by swirling.
3. The Dose Vial is held at room temperature for 15 minutes.
4. 0.8 mL is withdrawn from the Dose Vial into a syringe.

To prepare the 40 mcg / 300 mcg dose:

1. 0.03 mL Alhydrogel is added to a first CHIKV-VLP vial (0.8 mL) and mixed by swirling (First dose vial).
2. 0.03 mL Alhydrogel is added to a second CHIKV-VLP vial (0.8 mL) and mixed by swirling (Second dose vial).
3. Both vials are held at room temperature for 15 minutes.
4. 0.4 mL of CHIKV-VLP is withdrawn from the first dose vial into a syringe, syringe plunger is pulled to 0.8 mL mark and then CHIKV-VLP injected into the second dose vial. A total of 0.8 mL is then withdrawn from the second dose vial into syringe.

4.2 Control Vaccine (Diluent)

The control vaccine for this protocol is the Diluent component of PXVX0317.

To prepare the Diluent dose:

1. 0.8 mL of Diluent is withdrawn from one Diluent vial into a syringe.

All doses are prepared into Sponsor-provided syringes using sterile technique and all doses must be administered no later than 1 hour post thaw of CHIKV-VLP or 1 hour post placebo preparation.

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

5 STUDY PROCEDURES

5.1 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 consent form (ICF). For further details on informed consent, refer to Section 10.3.

5.2 Screening

Screening procedures are listed in the Schedule of Events in Appendix A1 for Groups 2, 3, 5, 6, and 7, in Appendix A2 for Groups 1, 4 and 8, in Appendix B1 for Group 9, and Appendix B2 for Group 10.

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: CV317001-two digit site number-three digit identification number, e.g., CV317001-01-001. Subject numbers will also be sequential since screen failed subjects will be entered into the Medrio 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 Medrio EDC disposition eCRF.

Re-screening:

Subjects who meet exclusion criterion 1 (current 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 through 10 (prohibited travel or medications within 30 days prior to Day 1) may be re-screened after the appropriate duration has passed. There may be situations in which an eligible subject is not able to be randomized and treated within 30 days of their screening period. This will involve undergoing all screening procedures again, including re-consenting and use of the same subject ID number. Subjects may be re-screened one time only. Re-screening is not otherwise permitted.

5.3 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.

5.4 Physical Examination

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

The examination should include:

- Height
- Body weight
- Vital signs
- General appearance
- Eyes-ears-nose-throat
- Head-neck
- Lungs-chest
- Heart
- Abdomen
- Musculoskeletal
- Lymph nodes
- Skin
- Extremities
- Neurological

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

5.5 Vital Signs

Vital signs collected from subjects will include blood pressure, heart rate, respiratory 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. Repeat measurements on abnormal vital parameters are not allowed for inclusion into the study. After Day 1, abnormal vital signs can be repeated for confirmation of clinical significance.

5.6 Laboratory Tests

At an initial screening visit, blood samples will be collected for serum testing for HBV, HCV and HIV.

Neutralizing antibody will be assessed by a luciferase-based anti-CHIKV neutralization assay at Day 1 (pre-vaccination) and at later time points.

Antibody will also be assessed by a licensed anti-CHIKV ELISA at Day 1 (pre-vaccination) and Day 57 for Groups 1-9 only.

Exploratory testing of cellular responses will be performed on cells from PBMC collections at Days 1, 29, 57, and 182 and leukapheresis at Day 182 for Group 9 subjects and Days 1, 8, 15, and 22 from Group 10 subjects. Exploratory testing of antibody responses will be performed on plasma collected at Day 57 for Group 9 subjects and Day 22 from Group 10 subjects.

The schedule of sample collection Groups 2, 3, 5, 6, and 7 is shown in Appendix A1, for Groups 1, 4 and 8 in Appendix A2, for Group 9 in Appendix B1 and Group 10 in Appendix B2. 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.

5.7 Pregnancy Testing and Contraception

Female subjects of childbearing potential will undergo a urine pregnancy test at screening, prior to each injection and prior to the plasmapheresis and leukapheresis procedures (Group 9 only) and prior to plasmapheresis procedure (Group 10 only). The subject must have a negative pregnancy test on Day 1, prior to study vaccine administration, as well as prior to undergoing plasmapheresis at Day 57 and leukapheresis at Day 182 (Group 9 only) and plasmapheresis procedure at Day 22 (Groups 10 only). The Investigator must report any pregnancies as described in Section 7.7.1. Female subjects of childbearing potential must also use an acceptable method of contraception from prior to Day 1 through Day 365 for Groups 2, 3, 5, 6, and 7; prior to Day 1 through Day 760 for Groups 1, 4 and 8,prior to Day 1 through Day 182 for Group 9 and prior to Day 1 and Day 22 for Group 10. Acceptable methods include highly effective forms of contraception such as 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 prior to Day 1 (e.g. hormonal contraception) to be considered fully effective.

5.8 Randomization

Subject eligibility will be confirmed and documented by the Investigator immediately prior to randomization of each subject.

Blinded study staff will indicate on a Randomization eCRF within the EDC system that they want to generate a randomization number for the subject. When they indicate yes, a randomization number will be generated from the EDC randomization module. The randomization number is separate from, and does not replace, the subject identification number which is assigned at Screening. Blinded study staff will provide the randomization number to the Unblinded Pharmacist who will match the randomization number to its corresponding treatment allocation from the randomization schedule and dispense the appropriate treatment to either the blinded study staff or unblinded study staff for administration.

Subjects will be considered enrolled once a randomization number has been assigned within the EDC system. The study will be conducted as a double-blind study through the last Day 365 visit. Neither subjects, nor clinical site personnel (except for the unblinded staff), including the principal Investigator, nor the Sponsor will know subjects' individual treatment assignments until all subjects have completed their participation in the study through the last Day 365 visit and the database has been cleaned and frozen.

The following safeguards will be employed to reduce the risk of inadvertent unblinding:

- Use of a standardized syringe and injection volume for all injections.
- All formulations of PXVX0317 and placebo are identical in appearance.
- No Sponsor personnel other than the designated independent unblinded monitor(s) and third-party biostatistics and programming staff producing the Day 57 interim will have access to the randomization schedule. No site personnel other than the Unblinded Pharmacist will have access to treatment assignments.
- Should any subject or blinded staff member become inadvertently unblinded, the Investigator will promptly (within 24 hours of their awareness of the error) disclose the event to the Medical Monitor (MM) in a blinded fashion (disclosing only subject number, not treatment) so that corrective action can be initiated. The unblinding sequence of events will be documented and retained as source documents. A protocol deviation will be entered in the eCRF.

Group 9 and 10 enrollment will be open label and thus no blinding nor treatment randomization will be required.

5.9 Study Vaccine Administration

For Groups 1 through 8 on Days 1, 15, and 29 (and 547 for Group 4) before study vaccine administration, the medical history, including physical exam (if indicated by updated medical history) and vital signs, and concomitant medications will be collected and updated in the subject file, serum samples will be collected, and females of childbearing potential must have a negative urine pregnancy test. These activities will occur for Group 9 subjects as well, but only on Day 1 and Day 29 and for Group 10 only on Day 1. Immediately prior to study vaccine administration, staff should recheck eligibility to ensure that the subject is still eligible. Once these procedures are performed, study vaccine will be administered. All doses of study vaccine are 0.8 mL in volume and are administered by intramuscular injection with a Sponsor-provided 1mL syringe and 25 gauge 1" (or 1.5", at the Investigator's discretion) needle as described above, using universal

precautions and sterile technique. All injections will be administered into the deltoid muscle. Alternate arms will be used for successive injections for all Groups, e.g. for Groups 1 through 8, the Day 1 injection into the right deltoid, Day 15 into the left, and Day 29 into the right again and for Group 9, the Day 1 injection will be in the right deltoid and the Day 29 injection will be in the left and for Group 10, the Day 1 injection can be in the right or left deltoid. Injections will be done either by a blinded or unblinded staff member delegated by the Investigator.

5.10 Acute Observation

The subject will be monitored by blinded study staff for signs of an acute adverse reaction for 30 minutes after each injection and vital signs will be obtained at least 30 minutes and no longer than 60 minutes after injection.

5.10.1 Observation Following Apheresis

The subjects for Group 9 will be monitored for 30 minutes following the plasmapheresis and leukapheresis procedures and for Group 10 following the plasmapheresis procedure only.

5.11 Solicited Adverse Events

Solicited AEs will be collected for 7 days after each injection, including booster injection. Solicited adverse events for this study are local events of pain, redness, and swelling at the injection site and systemic events of oral temperature $\geq$ 100.4° F, chills, malaise, fatigue, headache, myalgia, joint pain, 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 reaction, a ruler will be provided to the subject to measure the diameter of redness and 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. The memory aid will be collected as a source document. The Investigator will then assess all solicited events for severity and relatedness. Severity will be graded according to the Toxicity Grading Scale (Appendix C). 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 solicited adverse event eCRF.

Symptoms continuing beyond the solicited AE collection period (7 days following each injection), will be collected and recorded on the adverse event eCRF.

5.12 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 7.

5.13 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. From Day 1 through Day 57 visit (for Groups 1-9), and again from Day 547 through Day 575 visit (for Group 4 only), and from Day 1 through Day 29 visit (Group 10) the details of all concomitant medications including those associated with solicited AEs and unsolicited AEs will be collected.

Concomitant medications associated with a SAE will be collected through the end of the study.

5.14 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 57 visit (for Groups 1-9) and from 30 days prior to Day 547 through Day 575 visit (for Group 4 only) and from 30 days prior to Day 1 through Day 22 visit (for Group 10) .
- Licensed vaccines from 30 days prior to Day 1 through Day 57 visit (for Groups 1-9) and from 30 days prior to Day 547 through Day 575 visit (for Group 4 only) and from 30 days prior to Day 1 through Day 22 visit (for Group 10).
- Investigational agents from 30 days prior to Day 1 through the duration of study participation.
- Participants cannot undergo plasmapheresis or leukapheresis if they are on:
    - Antiplatelet agents, Plavix® and/or Ticlid®
    - Anticoagulants, Coumadin, Effient®, and/or Brilinta®

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

5.15 Emergency Unblinding

The Investigator may obtain a treatment assignment for a study subject only in the case of a medical emergency where knowledge of the treatment is necessary for the management of an

adverse event. The Investigator will notify the Medical Monitor (MM) (in a blinded manner) immediately after unblinding, document the reason and circumstances for the unblinding event, and distribute unblinding information only as needed for medical management.

5.16 Protocol Deviations

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). IPDs may require additional documentation as requested by the Sponsor.

5.17 Study Completion: For Individual Subjects

An individual subject is considered to complete study participation after completion of the Day 760 visit for Groups 1, 4 and 8, after completion of Day 365 for Groups 2, 3, 5, 6 and 7 and after completion of Day 182 for Group 9 and after completion of Day 29 for Group 10, as well as completion of any required safety follow-up.

5.18 Early Discontinuation

An individual subject is considered to undergo Early Discontinuation if they stop study participation before the Day 760 visit (for Groups 1, 4, and 8), Day 365 (Groups 2, 3, 5, 6, and 7) and before the Day 182 visit (for Group 9) and before the Day 29 visit (for Group 10).

An enrolled subject may voluntarily withdraw consent for further participation at any time before the Day 760 visit (for Groups 1, 4, and 8), Day 365 (Groups 2, 3, 5, 6, and 7) and before the Day 182 visit (for Group 9) and before the Day 29 visit (for Group 10). 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.

In addition, the Investigator, at his or her discretion, may withdraw a subject from further participation in the study. Criteria for withdrawal by the Investigator include:

- Noncompliance with the protocol
- Pregnancy
- Immediate hypersensitivity reaction associated with a study injection
- Loss to follow-up – requires documentation of at least 3 unsuccessful attempts to contact subjects. Lost to follow-up will be determined after the date of the subject's projected last visit.
- Grade 3 or higher AE assessed as related to a study injection.

- Other reason(s) which, in the opinion of the Investigator, indicates that continued participation in the study is not in the best interest of the subject.

5.19 Subject Replacement

Subjects who undergo Early Discontinuation after randomization and before Day 1 may be replaced at the Sponsor's discretion. Subjects who undergo Early Discontinuation after first vaccine administration will not be replaced, for Groups 1 through 8 participants only. Subjects in Group 9 who discontinue study participation before Day 57 (or who do not undergo Day 57 plasmapheresis for other reasons) may be replaced and Group 10 who discontinue study participation before Day 22 (or who do not undergo Day 22 plasmapheresis for other reasons) may be replaced.

5.20 Study Completion: Overall

The study is planned to be completed after all subjects have completed the Day 760 visit (for Groups 1, 4, and 8 only), Day 365 visit (for Groups 2, 3, 5, 6 and 7) and the Day 182 visit for Group 9 and the Day 29 visit for Group 10 (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.

6 STUDY PROCEDURES BY VISIT

The overall summary of evaluations by visit is given in the Schedule of Assessments for Groups 2, 3, 5, 6, and 7 in Appendix A1, Groups 1, 4, and 8 in Appendix A2, for Group 9 in Appendix B1, and Group 10 in Appendix B2. All visits are relative to the first day of vaccine administration, Day 1. Acceptable time windows for the visit schedule are indicated.

6.1 Scheduled Study Visits

6.1.1 Screening (-30 days to Day 1) – All groups

The following will take place during the visit, which will occur within 30 days prior to Day 1:

- Informed consent
- Demographics
- Review of eligibility criteria
- Medical history
- Physical exam
- Vital signs
- Blood Collection for:
    - HBV, HCV, HIV evaluation
- Urine pregnancy (females of childbearing potential)
- Prior and concomitant medications

6.1.2 Day 1 – All groups

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

- Updated medical history
- Confirmation Inclusion/Exclusion criteria are met
- Directed physical examination, if indicated by updated medical history
- Vital signs
- Blood Collection (pre-dose) for:
    - Anti-CHIKV neutralization
    - Anti-CHIKV ELISA (only for Groups 1-9)
    - PBMC collection– Group 9 and 10 subjects only
- Urine pregnancy test (females of childbearing potential)
- Prior and concomitant medications
- Randomization/Enrollment

All eligible, consented and randomized subjects will be vaccinated at the Day 1 baseline visit.

Study Vaccine Administration

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

- Acute observation for up to 30 minutes
- Vital signs after at least 30 minutes and no later than 60 minutes
- AE (solicited and unsolicited) and SAE evaluation
- Concomitant medications
- Memory aid, ruler and thermometer distribution and training

6.1.3 Day 8 (+3 days) – All groups

The following procedures will take place at this visit:

- Review of the memory aid
- Concomitant medications
- AE (solicited and unsolicited) and SAE evaluation
- Blood collection for Anti-CHIKV neutralization
- PBMC collection– Group 10 subjects only

6.1.4 Day 15 (± 1 day) – Groups 1 through 8 only

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

- Urine pregnancy test (females of child-bearing potential)
- Confirmation of continued eligibility
- Vital signs
- Concomitant medications
- AE (unsolicited) and SAE evaluation
- Blood collection for anti-CHIKV neutralization

Study Vaccine Administration

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

- Acute observation for up to 30 minutes
- Vital signs after at least 30 minutes and no later than 60 minutes
- Issuing of memory aid

6.1.5 Day 15 (± 2 days) – Group 10 only

The following procedures will take place at this visit:

- Concomitant medications
- AE (unsolicited) and SAE evaluation
- Blood collection for Anti-CHIKV neutralization
- PBMC collection

6.1.6 Day 22 (+3 days) – Groups 1 through 8 only

The following procedures will take place at this visit:

- Review of the memory aid
- Concomitant medications
- AE (solicited and unsolicited) and SAE evaluation
- Blood collection for Anti-CHIKV neutralization

6.1.7 Day 22 (+ 5 day) – Group 10 only

The following procedures will take place during the visit:

- Urine pregnancy test (females of child-bearing potential)
- Confirmation of continued eligibility
- Concomitant medications
- AE (unsolicited) and SAE evaluation
- Blood Collection for:
    - Anti-CHIKV neutralization
    - PBMC collection
    - Plasmapheresis procedure performed at the blood bank
        - AE follow-up 30 minutes post plasmapheresis

6.1.8 Day 29 (± 1 day) – Groups 1-9

The following procedures will take place during the visit and ***prior*** to vaccination:

- Urine pregnancy test (females of child-bearing potential)
- Confirmation of continued eligibility
- Vital signs

- Concomitant medications
- AE (unsolicited) and SAE evaluation
- Blood Collection for:
    - Anti-CHIKV neutralization
    - PBMC collection – Group 9 subjects only

Study Vaccine Administration

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

- Acute observation for up to 30 minutes
- Vital signs after at least 30 minutes and no later than 60 minutes
- Issuing of memory aid

6.1.9 Day 29 (-1/+5 days) – Group 10 only (phone call follow-up)

- Concomitant medications
- AE (unsolicited) and SAE evaluation

6.1.10 Day 36 (+3 days) – Groups 1-9

The following procedures will take place at this visit:

- Review of the memory aid
- Concomitant medications
- AE (solicited and unsolicited) and SAE evaluation
- Blood collection for anti-CHIKV neutralization

6.1.11 Day 57 (± 3 days for Groups 1-8) and (-14/+3 days for Group 9)

The following procedures will take place at this visit:

- Concomitant medications
- AE (unsolicited) and SAE evaluation
- Urine pregnancy (for females of childbearing potential in Group 9)
- Confirmation of exclusionary criteria (Group 9 only)
- Blood collection for:
    - Anti-CHIKV neutralization
    - Anti-CHIKV ELISA
    - PBMC collection– Group 9 subjects only
    - Plasmapheresis procedure performed at the blood bank (*Group 9 subjects only)

- *AE follow-up 30 minutes post plasmapheresis

6.1.12 Day 182 (± 7 days) – Groups 1-9

The following procedures will take place at this visit:

- SAE evaluation
- Concomitant medications potentially related to SAEs or prohibited medications
- Urine pregnancy (for females of childbearing potential in Group 9)
- Confirmation of exclusionary criteria (Group 9 only)
- Blood collection for:
    - Anti-CHIKV neutralization
    - PBMC collection– Group 9 subjects only
    - Leukapheresis procedure performed at the blood bank (optional procedure for *Group 9 subjects only)
        - *AE follow-up 30 minutes post leukapheresis

6.1.13 Day 365 (± 7 days) – Groups 1 through 8 only

The following procedures will take place at this visit:

- SAE evaluation
- Concomitant medications potentially related to SAEs or prohibited medications
- Blood collection for anti-CHIKV neutralization

6.1.14 Day 547, Boost Visit (± 7 days) – Group 4 only

The following procedures will take place at this visit:

Prior to booster administration:

- Urine pregnancy test (females of child-bearing potential)
- Confirmation of continued eligibility
- Concomitant medications potentially related to SAEs or prohibited medications
- Vital signs
- Blood collection for anti-CHIKV neutralization

Booster Injection – Group 4 only

After booster administration:

- Acute observation for up to 30 minutes

- Vital signs after at least 30 minutes and no later than 60 minutes
- Issuing of memory aid
- AE (solicited) and SAE evaluation

6.1.15 Day 547, without boost (± 7 days) – Groups 1 and 8 only

The following procedures will take place at this visit:

- SAE evaluation
- Concomitant medications potentially related to SAEs or prohibited medications
- Blood collection for Anti-CHIKV neutralization

6.1.16 Day 554, phone visit (+ 3 days) – Group 4 only

This visit is a follow-up phone call to ensure that the Group 4 subjects have entered information in the memory aid for all solicited events following the Day 547 boost.

6.1.17 Day 575 (± 3 days) – Groups 4 only

The following procedures will take place at this visit:

- Review of the memory aid
- AE (solicited and unsolicited) SAE evaluation
- Concomitant medications
- Blood collection for Anti-CHIKV neutralization

6.1.18 Day 760 (-14/+90 days) – Groups 1, 4 and 8 only

The following procedures will take place at this visit:

- SAE evaluation
- Concomitant medications potentially related to SAEs or prohibited medications
- Blood collection for anti-CHIKV neutralization

6.2 Early Discontinuation Visit

All subjects who discontinue study participation before the Day 760 visit (for Groups 1, 4, and 8), before Day 365 (Groups 2, 3, 5, 6, and 7) and before the Day 182 visit (for Group 9) and before the Day 29 visit (for Group 10) will be requested to undergo an Early Discontinuation visit.

If the visit occurs before Day 57 (Groups 1-9 only) and Day 29 (Group 10 only), the following will be conducted:

- Review AEs and SAEs
- Review Concomitant medications associated with new or ongoing AEs and any SAEs
- Blood Collection for:

- Anti-CHIKV neutralization
- Anti-CHIKV ELISA (Groups 1-9 only)

- Urine pregnancy test for females of child-bearing potential

If the visit occurs within 7 days after study vaccine administration, the following will ***also*** be conducted:

- Review of the memory aid
- AE (solicited) evaluation

If the visit occurs after Day 57 (Groups 1-9 only), the following will be conducted:

- Review SAEs
- Review Concomitant medications associated with new or ongoing SAEs
- Blood Collection for:
  - Anti-CHIKV neutralization

6.3 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.

6.4 Summary of Apheresis Collection Procedures

The Community Blood Center of Greater Kansas City will be the designated apheresis site for subjects participating in Groups 9 and 10. Per the blood bank guidelines, plasmapheresis can be done once every 28 days and the frequency of the leukapheresis is determined by the study physician. The U.S. Food and Drug Administration (FDA) regulations allow cytapheresis donations at a frequency of 24 donations per year, and plasmapheresis donations twice weekly.

6.4.1 Plasmapheresis Procedure

Plasmapheresis will be performed using either the Fresenius Aurora Plasmapheresis system while connected to the DXT relay data management system (preferred option) or the Trima Accel Automated Blood Component Collection System while connected to Vista® Information Systems per their SOPs.

Subjects will be advised to consume a well-balanced meal and a 12-ounce glass of a decaffeinated beverage prior to the procedure. The amount of plasma collected may range from 600-800 mLs (depending on donor weight) and the amount of red blood cell loss will be approximately 10-15 mLs. Subject identity will be confirmed before the procedure begins. Prior to venipuncture, the

designated phlebotomist will prepare each subject's arm for phlebotomy by sterilizing the designated insertion site on the subject's arm and by ensuring adequate venous access. Each subject should expect plasma drawing to take approximately 1-2 hours. Subjects will be monitored for any reactions or discomfort during and after the procedure. Subject tolerance to the anticoagulant must be monitored as well.

6.4.2 Leukapheresis Procedure

Leukapheresis will be performed using the Spectra Optia Apheresis System per their SOP.

Subjects will be advised to consume a well-balanced meal and a 12-ounce glass of a decaffeinated beverage prior to the procedure, as the volume of plasma removed may range from 100-600 mL. Subject identity will be confirmed before the procedure begins. Prior to venipuncture, the designated phlebotomist will prepare each subject's arm for phlebotomy by sterilizing the designated insertion site on the subject's arm and by ensuring adequate venous access. Each subject should expect lymphocyte drawing to take approximately 1-4 hours. The amount of lymphocytes collected will be approximately 250 mLs (depending on gender, height, weight, etc.). Subjects will be monitored for any reactions or discomfort during and after the procedure. Subject tolerance to the anticoagulant must be monitored as well.

7 SAFETY

7.1 Definitions

7.1.1 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)*
    - *Any complications that occur specifically from the apheresis procedures will be captured and recorded as AEs
- 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 elective surgery or social admissions)

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.

7.1.2 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 oral temperature ≥ 100.4° F, chills, malaise, fatigue, headache, myalgia, joint pain, and nausea.

7.1.3 Unsolicited Adverse Event

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

7.1.4 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 observation or 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 7.9.

7.2 Severity Grading

The Investigator will grade all adverse events for severity. Adverse events listed in the Toxicity Grading Scale in Appendix C 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) – ER visit or hospitalization

7.3 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.

7.4 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.

In the event of a non-fatal SAE, subjects will be instructed to contact the Investigator (or designee) immediately. 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.

7.5 Reporting of Adverse Events

7.5.1 Reporting Periods

The four reporting periods for solicited AEs begin immediately after each injection and continue for 7 days after each injection, including booster injection. The two reporting periods for unsolicited AEs are: immediately after study vaccine administration on Day 1 and continues through Day 57; and immediately after booster injection on Day 547 and continues through Day 575. AEs that correspond to solicited AE terms but occur outside of (or continue past) the solicited AE collection periods are also collected through the unsolicited AE reporting periods. The reporting period for SAEs begins at the time of informed consent and continues for the duration of study participation.

The summary of the reporting periods for Groups 2, 3, 5, 6, and 7 is described in Figure 3 and in Figure 4 for Groups 1, 4, and 8,for Group 9 in Figure 5 and Group 10 in Figure 6.

Figure 3: Safety Events Reporting Periods (Groups 2, 3, 5, 6, and 7)

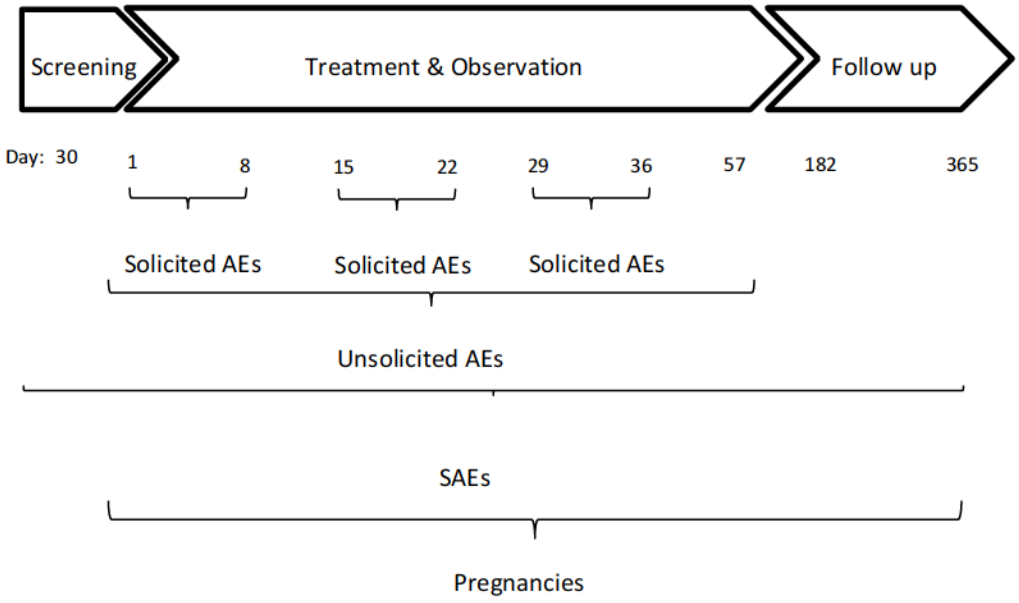
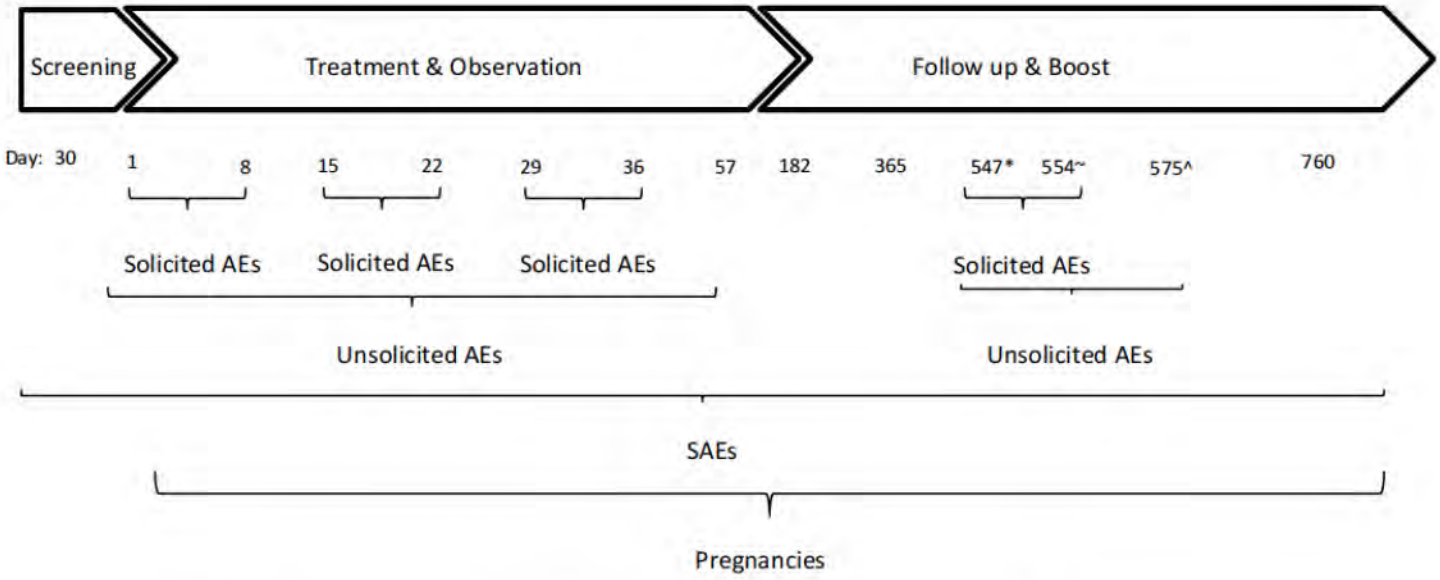


Figure 4: Safety Events Reporting Periods (Groups 1, 4, and 8 only)

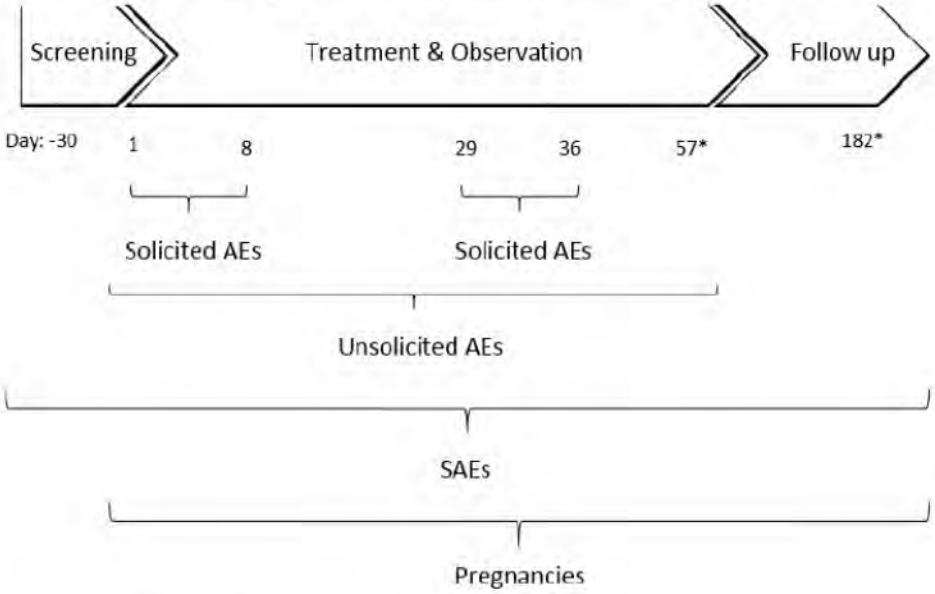


* Only Group 4 will receive the boost at Day 547 along with the memory aid.

~ Follow-up phone call for Group 4 only

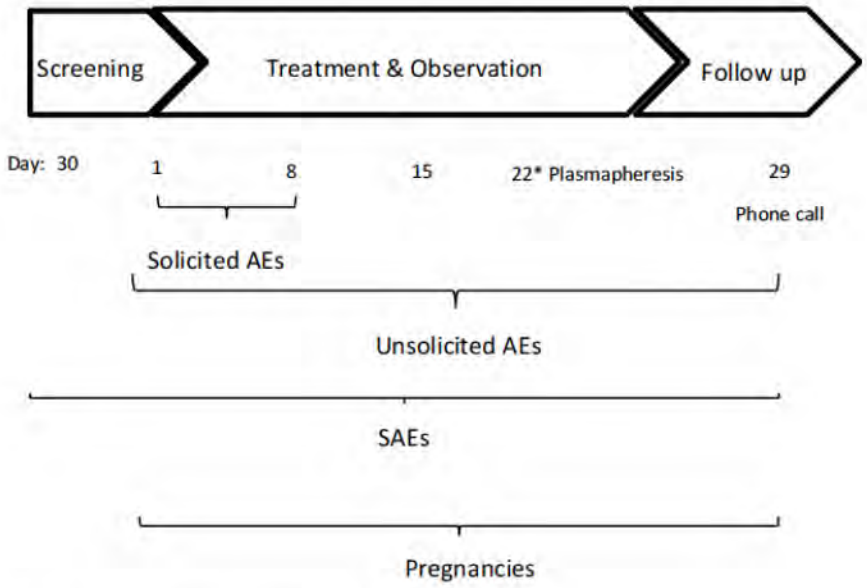
^ Day 575 is for Group 4 only (collection and recording of solicited AEs)

Figure 5: Safety Events Reporting Periods (Group 9 only)



*Specifies apheresis procedure-related AEs.

Figure 6: Safety Events Reporting Periods (Group 10 only)



*Specifies apheresis procedure-related AEs.

7.5.2 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
- Seriousness (Yes/No) and if Yes, Seriousness criteria met
- Relationship to study vaccine
- Action taken in response to the AE
- Action taken with Study Drug

7.6 SAE Reporting

The Investigator or designee must report all SAEs to the Medical Monitor or designee 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.

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 MM will notify the Investigator of SAEs that meet criteria for expedited reporting to regulatory authorities. The Investigator is responsible for notifying the applicable IRB of these events and adhering to any other applicable local reporting requirements.

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

It is also the responsibility of the MM to make the determination of which SAEs are "serious and unexpected suspected adverse reactions" (SUSARs), 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.

All SUSARs, as determined by the IND Sponsor, will be reported to FDA and will be provided to all participating Investigators by the IND Sponsor.

For further information regarding SAE reporting, please refer to the PXVX-CV-317-001 Safety Management Plan (SMP).

7.7 Other Events Requiring Immediate Reporting

7.7.1 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.

7.7.2 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 IPD.

7.7.3 Early Discontinuation for Safety Reasons

The Investigator or designee must report any early discontinuation for safety reasons to the MM or designee within 24 hours of discontinuation. Additional information regarding ongoing adverse events may be provided as a follow-up report.

7.7.4 Emergency Unblinding for Safety Reasons

The Investigator or designee must report emergency unblinding to the MM or designee within 24 hours of unblinding. Additional information regarding the unblinding, excluding the actual treatment assignment, may be provided as a follow-up report.

For further information regarding all safety reporting procedures, please refer to the PXVX-CV-317-001 Safety Management Plan (SMP).

7.7.5 Safety Events Related to Apheresis

AEs related to apheresis (plasmapheresis and leukapheresis) procedures will be collected at the Day 57 and Day 182 visits for Group 9 and (plasmapheresis) at Day 22 for Group 10. SAEs and AEs that result in changes to the apheresis procedure(s) (such as early termination of collection) must be reported within 24 hours of awareness to the MM.

7.8 Medical Monitor

The Medical Monitor (MM) will provide safety oversight for the study. The MM will review blinded study data and assess causality of SAEs on an ongoing basis.

Name: [redacted]
Address: [redacted]

7.9 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 PXVX-CV-317-001 Safety Management Plan (SMP).

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.

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.

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

8.3 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. Subject identity information will be maintained for 15 years unless a longer period is required by applicable law or regulation.

8.4 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.

8.5 Laboratory Data

This study will employ electronic transfers of external 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.

8.6 Audit Compliance

The Investigator must permit the Sponsor and/or designee, regulatory agencies, and/or the IRB 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

For the comparison of percentage of subjects with four-fold increase and a sample size of 50 subjects per group, there is at least 80% power, at alpha=0.05, to detect a standard response of 70% in treatment group 1 vs. 95% in any of the other treatment groups. The formulation and schedule used in group 1 (20 mcg VLP unadjuvanted at Day 1 and Day 29) is the regimen that resulted in 100% of subjects with seroconversion at 8 weeks post-first vaccination in the NIH phase 1 study VRC 311.

Based on these prior data and the likely universal response in this trial's Reference Group, it is unlikely that the proportion-based analyses will be able to discriminate between treatment groups. For this reason, the treatment group GMTs will also be used for dose selection.

In the titer-based comparisons, with a sample size of 50 subjects per group assuming a standard deviation (SD) estimate of 0.525 (log10 scale) there is 80% power, at alpha=0.05, to detect a two-fold difference between any pair of GMTs. The SD estimate is based on the SD observed in the Emergent Travel Health luciferase assay on samples collected 4 weeks after subjects were treated with two doses, 4 weeks apart, in VRC 311. Power is calculated based on the normal approximation and a two-sample t-test.

The size of the open-label group (Group 9) is based on practical, rather than statistical, considerations. Twenty subjects will provide adequate plasma to future non-clinical purposes without needlessly exposing additional subjects to the more invasive procedures.

The size of the open-label group (Group 10) is based on practical, rather than statistical, considerations. Ten subjects will provide adequate plasma to future non-clinical purposes without needlessly exposing additional subjects to the more invasive procedures.

9.2 Treatment Period

The treatment and observation period begins at the time of first study vaccine administration and extends through the Day 57 visit for Group 1-9 and extends through Day 22 for Group 10. The follow-up period spans the time following the Day 57 visit through Day 182 for Group 9, through Day 365 for subjects in Groups 2, 3, 4, 5, 6, and 7, through Day 760 for Groups 1 and 8, and then includes a booster period from Day 547 to Day 760 for Group 4. Group 10 is only followed through Day 29.

9.3 Treatment Groups

Subjects will be randomized equally into 8 treatment groups of varying doses (and number of doses), adjuvanted vs. nonadjuvanted, and vaccination interval of either 2 or 4 weeks for active

vaccine, as displayed in Table 4. An additional ninth group will serve as an open-label group to further evaluate the selected formulation and dosing schedule of PXVX0317.

Table 4: Treatment Groups

Treatment Group	N	Dose (VLP in mcg / Alhydrogel in mcg)			
		Day 1	Day 15	Day 29	Day 547
1	50	20 / unadjuvanted	Placebo	20 / unadjuvanted	N/A
2	50	6 / 300	Placebo	6 / 300	N/A
3	50	10 / 300	Placebo	10 / 300	N/A
4	50	20 / 300	Placebo	20 / 300	40 / 300
5	50	Placebo	6 / 300	6 / 300	N/A
6	50	Placebo	10 / 300	10 / 300	N/A
7	50	Placebo	20 / 300	20 / 300	N/A
8	50	Placebo	Placebo	40 / 300	N/A
9	20	20 / 300	N/A	20 / 300	N/A
10	10	40/300	N/A	N/A	N/A
Total	430				

9.4 Populations for Analysis

Randomized population: All screened subjects who provide informed consent and provide demographic and other baseline screening measurements, are randomized (Groups 1 through 8) or are in open-label Groups 9 and 10, and assigned a study subject ID.

Exposed population: All subjects in the Randomized Population who receive at least one 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. Subjects will be analyzed as treated (i.e., according to the vaccine regimen a subject received, rather than the vaccine regimen to which the subject may have been randomized).

Immunogenicity evaluable population (IEP): For this phase 2 study, the immunogenicity population will be as treated (i.e., according to the vaccine regimen a subject receives, which may be different from the vaccine regimen to which the subject is randomized in the case of treatment errors).

The IEP includes all subjects in the Exposed Population who:

- Have no major protocol deviation or other reason to be excluded as defined prior to unblinding or analysis.
- Received all three scheduled vaccinations through Day 29

- 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 (Groups 1-8 only):
    - Baseline: Day 1 or within 30 days before first study vaccine administration
    - Day 57: Day 54 through Day 62, inclusive

Analysis of demographic and baseline characteristics: The demographic and baseline characteristics will be summarized according to treatment 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 treatment group and overall.

The frequencies and percentages of subjects by sex, race, and ethnicity will be presented by treatment group and overall. Demographic data will be tabulated for the Randomized, 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 vaccinations, will be summarized by treatment group for the Randomized 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 7.2 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 be also be recorded as unsolicited AEs.

Frequencies and percentages of subjects experiencing each solicited AE will be presented by maximum severity. Summary tables showing the occurrence of any local or systemic solicited AE overall and at each time point will also be presented.

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 = 0-24 mm.
- Grade 1/mild >24-50 mm.
- Grade 2/moderate >50-100 mm.
- Grade 3/severe >100 mm.

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 injection, for each event and for any event.
2. Time of first onset of solicited adverse events, after each injection and after any injection, for each event and any event.
3. Solicited adverse events by maximum event severity, after each injection and after any injection, for each event and for any event.
4. Duration of solicited adverse events, after each injection, for each event and any event.
5. Solicited adverse events, occurrence of at least one event by category (local, systemic), after each injection and after any 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 treatment 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 treatment 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 any 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

GMT/GMR:

The GMTs and GMRs associated with the primary objective will be analyzed via linear model. The primary model is an analysis of variance (ANOVA), with logarithmically-transformed anti-CHIKV titers ($\log_{10}$) as the dependent variable and treatment group and study site as the fixed effects in the model. The results included in the main tables of this report will be based on this basic ANOVA model. As a secondary analysis, in order to remove the effect of baseline concentration on the GMTs, an analysis of covariance (ANCOVA) will also be performed. The ANCOVA includes treatment group and study site as the fixed effects and $\log_{10}$ baseline anti-CHIKV titer as the covariate in the model. The rationale for including baseline values as a covariate is that prior data have shown vaccine response is correlated with baseline antibody levels.

The least square means and their 95% CIs calculated based on the ANOVA and ANCOVA will be back transformed and reported as the group GMT values (adjusted for the mean baseline in the case of the ANCOVA).

Comparisons between relevant groups will be based on the estimated adjusted GMTs measured at Day 57 and mean square error calculated from the basic ANOVA model using contrast statements.

The analysis of GMR relative to Day 1 will also be computed using the ANOVA model. The analysis of the boost GMR will be relative to Day 547.

Specifically, testing will be done of the null hypothesis:

H_0: $\mu_i = \mu_j$

against the alternative hypothesis of inequality. Here μ_i and μ_j denote the population means for any two of the treatment groups. The main comparison of interest will be the two dose 20 mcg CHIKV-VLP (unadjuvanted) group versus any of the adjuvanted groups. However, other pair-wise comparisons will also be of interest. All tests will be carried out at a 2-sided significance level of 0.05 and no adjustment for multiplicity will be applied, since the goal will be to rank different formulations rather than to establish inferential values.

The GMTs and GMRs (relative to Day 1 or 547, as applicable) based on antibody titers measured at all other protocol-specified time points will be analyzed as described above for Day 57. The difference in persistence of the antibody response induced by different treatment groups after first vaccination at each time point will be visually assessed.

Threshold analyses:

The percentage of subjects achieving specified thresholds (e.g., ≥ 10, 100, 500, 1000, four-fold rise over baseline), and associated Wilson 95% CI, will be calculated for the Day 57 visit data for each treatment group. Differences between pair-wise treatment groups will be determined via Fisher's Exact test.

Dose selection criteria:

The primary assessment will be based on antibody titers measured at Day 57 after the first injection. GMTs for any 2 treatment groups will be considered different if the test is significant (0.05 significance level), and if the percentage of subjects in the dominant groups that were above a clinically-relevant titer level was no less than 75%. While the clinically-relevant level is not yet established, the specific thresholds analyses are expected to bracket the potentially protective levels.

The reverse cumulative distribution of antibody titers for all treatment groups will be produced to aid in the selection of the future dose group(s) to use in the clinical program.

For groups that appear indistinguishable, selection of a formulation will be based on a combined body of evidence obtained from the GMT values, percentage of responders, safety profiles and additional summary variables if deemed necessary.

Exploratory analyses based on the results from the PBMC collection and apheresis procedures will be descriptive only (mean, median, SD, min/max for continuous variables and frequency counts and percentages for categorical variables).

9.7 Interim Analyses

There will be a safety and immunogenicity interim analysis for the selection of formulation(s) and schedule based on the data collected through Day 57. The results will be reported by treatment group preserving the double-blind status on the subject level. After the interim analysis, one existing or new group (Group 9) will be considered for a plasmapheresis and leukapheresis.

For further information, please refer to the PXVX-CV-317-001 Statistical Analysis Plan (SAP).

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 local regulatory requirements and laws.

10.1.1 Future Use of Stored Specimens and Data

Samples collected from the study may be used for future research to learn more about the CHIK-VLP vaccine.

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 an IRB appropriate to each study site. 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. At the Investigator's discretion, the site may develop a separate ICF for Step 1 Screening as described in Section 5.2.

All site-specific ICFs must be approved by the Sponsor or designee and the IRB and must be in compliance with ICH GCP, local regulatory requirements, 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.

10.4 COVID-19 related considerations

Emergent will monitor the situation related to the COVID-19 pandemic to ensure that potential risks to study participants and staff are mitigated. The following strategies will be implemented:

- The conduct of the study will be in accordance with state and local travel limitations/restrictions.
- Study staff at the plasma center will take appropriate precautions to protect study participants.
- Safety assessments will be performed by phone call when appropriate.
- If travel restrictions or COVID-19 related illness impact the conduct of the study, specific measures will be taken to mitigate risk to study staff and participants and monitor protocol deviations due to COVID-19 illness and/or COVID-19 control measures.

10.5 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.

10.6 Compensation for Injury

The Sponsor will adhere to local regulations and guidelines regarding clinical trial compensation to subjects whose health is adversely affected by taking part in the study. The applicable policy for compensation for injury will be described in the master ICF template.

10.7 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.

10.8 Public Disclosure and Publication Policy

All publication rights are delineated in the Clinical Study Agreement.

10.9 Amendments

The protocol may be amended only by the Sponsor.

The IRB must generally be informed of all amendments prior to implementation. In addition, the Investigator must obtain IRB approval for any amendments likely to affect the safety of study participants prior to implementation.

The Sponsor may implement an amendment prior to IRB notification or approval **only** in order to eliminate an apparent, immediate hazard to study participants. In that event, the Sponsor will notify the IRB in writing within 7 calendar days after the implementation.

Amendments, including descriptions and rationales, will be documented in a Summary of Changes.

APPENDIX A1: SCHEDULE OF EVENTS (GROUPS 2, 3, 5, 6, AND 7)

Visit:	Treatment and observation								Follow-up		
	Screen	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 57	Day 182	Day 365	Early D/C
Window:	-30d	0	+3d	±1d	+3d	±1d	+3d	±3d	±7d	±7d	n/a
Informed Consent	X										
Medical History	X	X									
Focused Physical Exam	X	X[a]									
HBV, HCV, HIV	X										
Inclusion/Exclusion Criteria	X	X[f]		X[f]		X[f]					
Anti-CHIKV ELISA		X[f]						X			X[b]
Vital Signs[c]	X	X[c]		X[c]		X[c]					
Pregnancy Test[d]	X	X		X		X					X[b]
Randomization		X									
Study Vaccine Administration		**X**		**X**		**X**					
Acute Observation (30min)		X		X		X					
Issue Memory Aid		X		X		X					
Review Memory Aid			X		X		X				X[g]
Adverse Event Evaluation		X	X	X	X	X	X	X	X[e]	X[e]	X
Prior/Con Med Evaluation	X	X	X	X	X	X	X	X	X[e]	X[e]	X
Anti-CHIKV Neutralization		X[f]	X	X[f]	X	X[f]	X	X	X	X	X

[a] Only if indicated by updated Medical History.

[b] If only before Day 57.

[c] Vitals to be taken prior and after each study vaccine administration.

[d] Urine pregnancy test at Screening and prior to each study vaccine administration.

[e] Only SAEs and associated con meds after Day 57 and prohibited medications

[f] Done pre-vaccination

[g] If the visit occurs within 7 days after study vaccine administration

APPENDIX A2: SCHEDULE OF EVENTS (GROUPS 1, 4, AND 8)

Visit:	Treatment and observation										Follow-up and Boost					Early D/C
	Screen	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 57	Day 182	Day 365	Day 547 Boost (Group 4 only *)	Day 547 No Boost (Groups 1 & 8)	Day 554 Phone (Group 4 only *)	Day 575 (Group 4 only *)	Day 760	
Window:	-30d	0	+3d	±1d	+3d	±1d	+3d	±3d	±7d	±7d	±7d	±7d	+3d	±3d	-14/+90	n/a
Informed Consent	X															
Medical History	X	X														
Focused Physical Exam	X	X[a]														
HBV, HCV, HIV	X															
Inclusion/Exclusion Criteria	X	X[f]		X[f]		X[f]					X[f]					
Anti-CHIKV ELISA		X[f]						X								X[b]
Vital Signs[c]	X	X[c]		X[c]		X[c]					X[c]					
Pregnancy Test[d]	X	X		X		X					X					X[b]
Randomization		X														
Study Vaccine Administration		**X**		**X**		**X**					**X**					
Acute Observation (30min)		X		X		X					X					
Issue Memory Aid		X		X		X					X					
Review Memory Aid			X		X		X						X[h]	X		X[g]
Adverse Event Evaluation		X	X	X	X	X	X	X	X[e]	X[e]	X[i]	X[e]		X[i]	X[e]	X
Prior/Con Med Evaluation	X	X	X	X	X	X	X	X	X[e]	X[e]	X[i]	X[e]		X[i]	X[e]	X
Anti-CHIKV Neutralization		X[f]	X	X[f]	X	X[f]	X	X	X	X	X[f]	X		X	X	X

[a] Only if indicated by updated Medical History.

[b] If only before Day 57.

[c] Vitals to be taken prior and after each study vaccine administration.

[d] Urine pregnancy test at Screening and prior to each study vaccine administration.

[e] Only SAEs and associated con meds after Day 57 and prohibited medications for Groups 1 and 8

[i] Adverse Event and Con Med collection and evaluation from Day 547 to Day 575 for Group 4 only

[f] Done pre-vaccination; on Day 547 only for Group 4

[g] If the visit occurs within 7 days after study vaccine administration

[h] Phone follow-up for Group 4 only to ensure memory aid has been completed

*Group 4 only

APPENDIX B1: SCHEDULE OF EVENTS (GROUP 9 ONLY)

Visit:	Treatment and observation							
	Screen	Day 1	Day 8	Day 29	Day 36	Day 57	Day 182	Early D/C
Window:	-30d	0	+3d	±1d	+3d	-14/+3	±7d	n/a
Informed Consent	X							
Medical History	X	X						
Focused Physical Exam	X	X[a]						
HBV, HCV, HIV	X							
Inclusion/Exclusion Criteria	X	X[f]		X[f]		X	X	
Anti-CHIKV ELISA		X[f]				X		X[b]
Vital Signs[c]	X	X[c]		X[c]				
Pregnancy Test[d]	X	X		X		X	X	X[b]
Treatment Assigned		X						
Study Vaccine Administration		**X**		**X**				
Acute Observation (30min)		X		X				
Issue Memory Aid		X		X				
Review Memory Aid			X		X			X[g]
Adverse Event Evaluation		X	X	X	X	X	X[e]	X
Prior/Con Med Evaluation	X	X	X	X	X	X	X[e]	X
Anti-CHIKV Neutralization		X[f]	X	X[f]	X	X	X	X
PBMC Collection (B and T cells)		X[f]		X[f]		X	X	
Plasmapheresis						X[eh]		
Leukapheresis							X[eh]	

[a] Only if indicated by updated Medical History.

[b] If only before Day 57.

[c] Vitals to be taken prior and after each study vaccine administration.

[d] Urine pregnancy test at Screening, prior to each study vaccine administration and prior to plasmapheresis and leukapheresis.

[e] Only SAEs and associated con meds after Day 57 and prohibited medications

[f] Done pre-vaccination

[g] If the visit occurs within 7 days after study vaccine administration

[h] Adverse events occurring 30 mins post plasmapheresis at Day 57 and leukapheresis procedure at Day 182 will be collected.

APPENDIX B2: SCHEDULE OF EVENTS (GROUP 10 ONLY)

Visit:	Treatment and observation					Follow-up	
	Screen	Day 1	Day 8	Day 15	Day 22	Day 29 (phone call)	Early D/C
Window:	-30d	0	+3d	±2d	+5d	-1/+5	n/a
Informed Consent	X						
Medical History	X	X					
Focused Physical Exam	X	X[a]					
HBV, HCV, HIV	X						
Inclusion/Exclusion Criteria	X	X[e]			X[i]		
Vital Signs[c]	X	X[c]					
Pregnancy Test [d]	X	X			X		X[b]
Treatment Assigned		X					
Study Vaccine Administration		**X**					
Acute Observation (30min)		X			X		
Issue Memory Aid		X					
Review Memory Aid			X				X[f]
Adverse Event Evaluation		X	X	X	X[g]	X	X
Prior/Con Med Evaluation	X	X	X	X	X	X	X
Anti-CHIKV Neutralization		X[e]	X	X	X		X
PBMC Collection (B and T cells)		X[e]	X	X	X		
Plasmapheresis					X		

[a] Only if indicated by updated Medical History.

[b] If only before Day 22.

[c] Vitals to be taken prior and after each study vaccine administration.

[d] Urine pregnancy test at Screening, prior to study vaccine administration and prior to plasmapheresis.

[e] Done pre-vaccination

[f] If the visit occurs within 7 days after study vaccine administration

[g] Adverse events occurring 30 mins post plasmapheresis at Day 22 will be collected.

[i] Confirmation of continued eligibility prior to plasmapheresis procedure

APPENDIX C: 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 C: 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 Travel Health 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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