EMERGENT®

CLINICAL STUDY PROTOCOL

A Phase 3 Safety, Immunogenicity, and Lot-Consistency Trial of the VLP-Based Chikungunya Vaccine PXVX0317 in Healthy Adults and Adolescents

EBSI-CV-317-004

Version 5.0

20 Mar 2023

ClinicalTrials.gov ID: NCT05072080

Sponsor:

Emergent Travel Health Inc.

[redacted]

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DOCUMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.0	24Aug2020	Initial version of protocol	N/A
2.0	06Jul2021	Added Day 15 Visit Added hierarchical testing to statistical section	Inclusion of Day 15 Visit to have a more robust immunogenicity assessment Hierarchical testing added to control type I error for FDA primary endpoints and seroresponse secondary endpoint at Days 15, 183, and 8
3.0	10Nov2021	Updated MM Updated Inclusion Criteria Updated labeling section.	Change in study personnel Clarified Inclusion Criteria regarding acceptable contraception for adolescents and adults in same-sex relationships Modified to better align with ICH E6(R2) GCP and Emergent's updated protocol template
4.0	10Feb2022	Updated Exclusion Criteria 11 Added a purpose statement Added HCV RNA testing if HCV antibody is positive Definition of medically attended visits was clarified.	Edited from 30 days prior to Screening to Day 1 as it was incorrect in previous versions of the protocol To align with ICH E6(R2) GCP Clarification per memo to Investigators sent 10Jan2022 Clarified that routine scheduled study visits were not considered medically attended visits.
5.0	See effective date	Study objectives and endpoints – coprimary objectives and immunogenicity endpoints have been updated and key secondary endpoints with associated success criteria were added. [redacted] Added EMA definition of seroresponse rate as the presumptive seroprotection rate. Added success criterion of lower bound of the two-sided 95% CI on the seroresponse rate difference [redacted] at Day 15. Removed the drug product composition table. Added the use of paper diaries Updated "CHIKV-luc assay anti-CHIKV SNA NT_{80}" to "human SNA assay"	Simplification for global use of the protocol. To align with FDA and EMA feedback received 26Jul2022 and 25Jan2023, respectively. To align with company position to refer to the IB rather than share product composition in the protocol. To use as a back-up to the e-diary if the e-diary is not available. Per response to FDA IR dated 17Mar2022 Regarding Assay Validation

		Updated “Alhydrogel” to aluminum hydroxide”	Reports and Non-Human Primate Passive Transfer Study Updated terminology.

SPONSOR SIGNATORY

Signatory: *See electronic signature at end of document.* [redacted]

Date (DDMMMYYYY)

KEY STUDY CONTACT INFORMATION

Sponsor's Medical Monitor (MM): [redacted]

Immediately Reportable Adverse Events: Emergent Global Pharmacovigilance [redacted]

For all other contact information refer to study contact list.

INVESTIGATOR SIGNATORY

Compliance Statement: This study is to be conducted in accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and regulatory requirements, as applicable.

EBSI-CV-317-004, Version 5.0:	A Phase 3 Safety, Immunogenicity, and Lot-Consistency Trial of the VLP-Based Chikungunya Vaccine PXVX0317 in Healthy Adults and Adolescents
Clinical Site(s):	**Institution Name:** [redacted] **Institution Address:**

My signature below verifies that I have read and agree to this protocol. I am aware of my responsibilities as an investigator under the current ICH GCP guidelines, the Declaration of Helsinki, United States of America (US) Food and Drug Administration (FDA) Code of Federal Regulations (CFRs) and state and local regulations and applicable laws and regulations of the country of the study site for which I am responsible. I agree to conduct the study according to these regulations.

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

I agree to conduct in person and/or to supervise staff assigned to specific study responsibilities. I will ensure that all staff who assist me in the conduct of the study have access to the protocol and all pertinent information. I will ensure that all assigned staff are trained and qualified and are fully informed of their responsibilities regarding the conduct of the study.

I agree to abide by the terms of the confidentiality disclosure agreement and/or contract with the sponsor and/or its representatives.

Site Principal Investigator: [redacted]

Principal Investigator Name (print)	Title (print)
[redacted]	
Principal Investigator Signature	Date (DDMMMYYYY)

PROTOCOL SYNOPSIS

Name of Sponsor/Company: Emergent Travel Health Inc.	
Name of Investigational Product: PXVX0317	
Name of Active Ingredient(s): Chikungunya virus virus-like particle (CHIKV VLP)	
Protocol Number: EBSI-CV-317-004	
Title of Study: A Phase 3 Safety, Immunogenicity, and Lot-Consistency Trial of the VLP-Based Chikungunya Vaccine PXVX0317 in Healthy Adults and Adolescents	
Study Centers: Multicenter, up to 50 sites in the US	
Study Duration for One Participant: 7 months **Estimated Study Duration:** ~12 months	**Phase of Development:** 3

Coprimary Objectives:

- To evaluate the safety of PXVX0317 in healthy adult and adolescent participants 12 to <65 years of age.
- To compare the anti-CHIKV serum neutralizing antibody (SNA) response to PXVX0317 and placebo at Day 22, as measured by geometric mean titer (GMT) and clinically relevant difference in seroresponse rate (PXVX0317 minus placebo).

  Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA [redacted] See Section 6.1 for assay description.
- To demonstrate the consistency of the anti-CHIKV SNA response across three consecutively manufactured lots of PXVX0317 at Day 22 as measured by GMT.

Secondary Objectives:

- To compare the anti-CHIKV SNA response to PXVX0317 and placebo at Day 15, Day 183, and Day 8, as measured by GMT and seroresponse rate.
- To compare the anti-CHIKV SNA response to PXVX0317 and placebo in participants 12 to <18 years of age, participants 18 to <46 years of age, and participants 46 to <65 years of age as measured by GMT and seroresponse rate.

Exploratory Objective:

- To evaluate anti-CHIKV SNA response across three consecutively manufactured lots of PXVX0317 as measured by seroresponse rate for each lot at Day 22 in participants 18 to <46 years of age.

Methodology/Study Design:

This is a phase 3, randomized, placebo-controlled, double-blind, parallel-group design with four treatment groups. Participants will be randomized in a 2:2:2:1 ratio within each age stratum (12 to <18, 18 to <46, and 46 to <65) to receive one of three consecutively manufactured lots of PXVX0317 or placebo. With 3150 participants enrolled, the treatment group totals are estimated as follows:

- Group 1- PXVX0317 lot A: n=900
- Group 2- PXVX0317 lot B: n=900
- Group 3- PXVX0317 lot C: n=900
- Group 4- Placebo: n=450

A nested lot consistency substudy will be performed in adult participants 18 to <46 years of age in the PXVX0317 treatment groups (lot A, lot B, and lot C).

Procedures and Assessments:

Study procedures and assessments will occur at: Screening (Visit 1), to occur no more than 30 days prior to Day 1 of investigational product (IP) administration; Day 1 (Visit 2, baseline, randomization, and administration of IP); Day 8 (Visit 3); Day 15 (Visit 4); Day 22 (Visit 5); Day 29 (Visit 6, via telephone contact); Day 92 (Visit 7, via telephone contact); Day 183 (Visit 8, End of Study) or; Early Discontinuation Visit. The per participant estimated total study duration is 212 days.

Solicited adverse events (AEs collected from IP administration on Day 1 through Day 8) will consist of local (injection site pain, redness, and swelling) and systemic (fever, chills, fatigue, headache, myalgia, arthralgia, and nausea) reactions. Unsolicited AEs will be collected from Day 1 through Day 29. Serious adverse events (SAEs), adverse events of special interest (AESI), and medically attended adverse events (MAAE) will be collected from Day 1 through to the Day 183 End of Study Visit. An independent Safety Monitoring Committee (SMC) will review aggregated, blinded safety data after the 300^{th} enrolled participant passes Day 29, and again after the last enrolled participant passes Day 29.

The induction of the humoral immune response (immunogenicity) by PXVX0317 vaccine, specifically anti-CHIKV SNA titers, will be measured by a bioluminescence assay developed by the sponsor that uses a modified version of CHIKV containing a reporter gene that expresses a luciferase protein (CHIKV-luc) in infected Vero cells *in vitro*. Reduction of luciferase activity (ie, reduced bioluminescence) occurs in infected cultures of cells following treatment of CHIKV-luc with test serum containing anti-CHIKV SNA. The quantitation of reporter gene expression, a correlate of the level of virus infection of cells, is determined by detection of luciferase using a microplate luminometer. The anti-CHIKV SNA titer 80 (NT_{80}) is the reciprocal of the serum dilution that provides 80% protection of Vero cells from CHIKV-luc infection or an 80% reduction of luciferase activity compared to virus only control.

The levels of anti-CHIKV SNA will be determined for time points up to 182 days after dosing (Day 183 End of Study Visit).

Number of Participants (Planned): At least 3150

Study Population: Healthy adult and adolescent participants 12 to <65 years of age

Criteria for Study Participation

Inclusion Criteria:

Participants must meet **all** the following criteria to be enrolled:

1. Able and willing to provide informed consent (and assent, as applicable) voluntarily signed by participant (and guardian, as applicable).
2. Males or females, 12 to <65 years of age.
3. Generally healthy, in the opinion of the investigator, based on medical history, physical examination, and screening laboratory assessments.
4. Women who are **either**:
    i. Not of childbearing potential (CBP): pre-menarche, surgically sterile (at least six weeks post bilateral tubal ligation, bilateral oophorectomy or hysterectomy), or postmenopausal (defined as a history of ≥12 consecutive months without menses prior to randomization in the absence of other pathologic or physiologic causes, following cessation of exogenous sex-hormonal treatment).

    or:

    ii. Meeting **all** the below criteria:
    - Negative serum pregnancy test at Screening Visit
    - Negative urine pregnancy test immediately prior to dosing at Day 1
    - Use one of these acceptable methods of contraception (if women of CBP) for the duration of participation:
        - Hormonal contraceptives (eg, implants, pills, patches) initiated ≥30 days prior to dosing
        - Intrauterine device (IUD) inserted ≥30 days prior to dosing
        - Double barrier type of birth control (male condom with female diaphragm, male condom with cervical cap)
        - Abstinence is acceptable **only for adolescents** (12 to <18 years old) who are not sexually active.

Note: See Section 3.3.7 for this study's list of acceptable method of contraception.

Note: Contraception requirements do not apply for participants in exclusively same-sex relationships and these participants should have no plans to become pregnant by any other means for the duration of the study.

Exclusion Criteria:

Participants who meet **any** of the following criteria **cannot** be enrolled:

1. Currently pregnant, breastfeeding, or planning to become pregnant during the study.
2. Body Mass Index (BMI) ≥35 kg/m^2.
3. Positive laboratory evidence of current infection with human immunodeficiency virus (HIV-1, HIV-2), hepatitis C virus (HCV) or hepatitis B virus (HBV).
4. History of severe allergic reaction or anaphylaxis to any component of the IP.
5. History of any known congenital or acquired immunodeficiency that could impact response to vaccination (eg, leukemia, lymphoma, generalized malignancy, functional or anatomic asplenia, alcoholic cirrhosis).
6. Prior receipt or anticipated use of systemic immunomodulatory or immunosuppressive medications from six months prior to screening through Day 22. **Note:** For systemic corticosteroids, use at a dose or equivalent dose of 20 mg of prednisone daily for 14 days or more within three months of screening through Day 22 is exclusionary. The use of inhaled, intranasal, topical, ocular, or intraocular steroids is allowed.
7. Receipt or anticipated receipt of blood or blood-derived products from 90 days prior to screening through Day 22.
8. Acute disease within the last 14 days (participants with an acute mild febrile illness can be considered for a deferral of vaccination two weeks after the illness has resolved and treatment has been completed).
9. Clinically significant cardiac, pulmonary, rheumatologic, or other chronic disease, in the opinion of the investigator. This may include chronic illness requiring hospitalization in the last 30 days prior to screening.
10. Enrollment in an interventional study and/or receipt of another investigational product from 30 days prior to screening through the duration of study participation.
11. Receipt or anticipated receipt of any vaccine from 30 days prior to Day 1 through Day 22.
12. Evidence of substance abuse that, in the opinion of the investigator, could adversely impact the participant's participation or the conduct of the study.

13. Prior receipt of an investigational CHIKV vaccine/product.
14. Any other medical condition that, in the opinion of the investigator, could adversely impact the participant's participation or the conduct of the study.

Investigational Product, Dosage, and Mode of Administration:

PXVX0317 vaccine is comprised of CHIKV VLP 40 µg, aluminum hydroxide (Alhydrogel®) 2% adjuvant, and formulation buffer supplied as a single dose of 0.8 mL in a pre-filled syringe administered via intramuscular (IM) injection in the deltoid muscle.

Reference Therapy, Dosage and Mode of Administration:

Placebo is comprised of formulation buffer supplied as a single dose of 0.8 mL in a pre-filled syringe administered via IM injection in the deltoid muscle.

Criteria for Evaluation

Primary Endpoints:

Safety Endpoints:

- Incidence of solicited AEs through Day 8 for PXVX0317 and placebo.
- Incidence of unsolicited AEs through Day 29 for PXVX0317 and placebo.
- Incidence of AESI, MAAEs, and SAEs, through Day 183 for PXVX0317 and placebo.

Coprimary Immunogenicity Endpoints:

- Difference in anti-CHIKV SNA seroresponse rate (PXVX0317 minus placebo) and associated 95% confidence interval (CI) at Day 22.

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA [redacted] See Section 6.1 for assay description. See Section 8.6 for immunogenicity analysis details and Section 8.6.2.5 for success criteria and multiplicity controls.

- Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for PXVX0317 and placebo.
- Anti-CHIKV SNA GMT ratio between all three pairs of PXVX0317 lots (A:B, A:C, B:C) (adults 18 to <46 years of age) at Day 22.

Secondary Endpoints:

- **Key Secondary Endpoints**: Difference in anti-CHIKV SNA seroresponse rate (PXVX0317 minus placebo) with associated 95% CIs at Day 15, Day 183, and Day 8, in that order (see Section 8.6.2.5).

- Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at Day 8, Day 15, and Day 183.
- Geometric mean fold increase (GMFI) from Day 1 to subsequent collection time points.
- Number and percentage of participants with an anti-CHIKV SNA titer ≥15 and 4-fold rise over baseline.

Exploratory Endpoints:

- Anti-CHIKV SNA seroresponse rate difference with associated 95% CIs for each pair of PXVX0317 lots (A minus B, A minus C, B minus C) (adults 18 to <46 years of age) at Day 22.

Statistical Methods

Sample Size Considerations:

The primary statistical comparison between the PXVX0317 and placebo treatment groups is based on difference between treatment groups in seroresponse rate (proportion of participants with CHIKV-luc assay anti-CHIKV SNA NT_{80} [human SNA assay] [redacted] at Day 22.

Based on the data from the phase 2 study (protocol PXVX-CV-317-001), the seroresponse rate for PXVX0317 vaccine is expected to be approximately [redacted] vs <5% for the placebo participants. With an assumed 10% rate of non-evaluable participants, the power to show superiority over placebo with 2430 PXVX0317 vaccine and 405 placebo evaluable participants is >99.9% for the combined age groups. The power is 99% for each of the smaller 12 to <18 and 46 to <65 age groups with samples sizes of 210 vs 35 evaluable participants (PXVX0317: placebo).

The difference in seroresponse rate between PXVX0317 and placebo groups that is considered clinically relevant is [redacted]. With 2430 PXVX0317-treated participants and a target seroresponse rate of [redacted] vs a rate of 0% for placebo, the width of a two-sided 95% CI would be ±1.2%. The difference in seroresponse rate must be above [redacted] for the lower bound of the 95% CI for the difference to be [redacted].

The second consideration for sizing the study is ensuring adequate power for the lot consistency equivalence intervals testing immunogenicity at Day 22, as measured by human SNA assay GMT ratios between all three pairs of consecutively manufactured PXVX0317 lots (A:B, A:C, B:C) in the 18 to <46 years of age stratum for the immunogenicity evaluable population (IEP). For all three pairwise GMT ratios, the two-sided 95% CI must fall within [0.667, 1.5] for the lots to be considered equivalent.

On the $\log_{10}$ scale, two one-sided equivalence tests have 95% power to reject the null hypothesis that the difference in mean titers between lots is below -0.176 or above 0.176 in favor of an alternative hypothesis of equivalence when the expected difference in $\log_{10}$

means is 0, the common standard deviation (SD) is 0.455, each test is at the 2.5% level and the sample size in each lot is 207. The SD estimate is based on the upper bound of an 80% CI on the SD on the $\log_{10}$ scale observed in the human SNA assay on samples collected 4 weeks after participants were treated with a single dose of PXVX0317. To be conservative, at least 1050 evaluable participants (300 participants administered PXVX0317 from each lot and 150 participants administered placebos) need to be enrolled into the 18 to <46 age group to provide adequate power for the lot consistency objective.

The final consideration for the sample size of the study is the size of the prelicensure safety database. With 2700 participants receiving any lot of PXVX0317, this study is 93.3% likely to detect at least one AE with a true frequency of ≥0.1% (ie, "uncommon").

Analysis

Safety:

All safety analyses will be based on the safety population. The three PXVX0317 lots will be combined in the tabulations and each of the safety analyses will be summarized across all ages and repeated for three (12 to <18, 18 to <46, and 46 to <65) participant age groups. Incidences of local (ie, injection site pain, redness, and swelling) and systemic reactions (ie, fever, chills, fatigue, headache, myalgia, arthralgia, and nausea) occurring from IP administration on Day 1 through Day 8 will be summarized by maximum severity and by treatment group. Unsolicited AEs will also be summarized by maximum severity and treatment group according to Medical Dictionary of Regulatory Activities (MedDRA) by system organ class (SOC) and preferred terms (PT).

Superiority to Placebo at Day 22:

The proportion of participants in each treatment group (PXVX0317 and placebo) with seroresponse at titer [redacted] will be computed, along with the two-sided 95% CI. This analysis will be primarily based the IEP and repeated for the modified intent-to-treat (mITT) population as a measure of the robustness of the findings. The significance of the treatment group difference will be assessed using a chi-square test, based on the response across all age groups combined, with an alpha=0.05. The significance of the difference in seroresponse rates between treatment groups for each age cohort will also be determined.

The difference in seroresponse rates between the two groups will be calculated, along with the 95% CI for the difference based on the Newcombe hybrid score method. The lower bound of the two-sided 95% CI on the difference in seroresponse rates between PXVX0317 and placebo must be [redacted] to satisfy the primary objective to demonstrate a clinically relevant seroresponse. Differences in seroresponse rates between the two groups will be tested similarly at Days 15, 183, and 8, in that order (see Section 8.6.2.5).

Day 22 GMTs will be compared between PXVX0317 and placebo treatment groups and will be analyzed via a linear model based on a two-sided alpha=0.05. The primary model is an analysis of variance (ANOVA), with logarithmically transformed anti-CHIKV SNA titers ($\log_{10}$) as the dependent variable and treatment group and study site as the fixed

effects in the model. The adjusted least square means and their 95% CIs calculated based on the ANOVA will be back transformed and reported as the group GMT values.

Lot Consistency:

For all three pairs of lots (A:B, A:C, and B:C), the two-sided 95% CI for the ratio of anti-CHIKV SNA GMTs at Day 22 in PXVX0317-treated participants ages 18 to <46 will be computed. The 95% CI must be contained within the [0.667; 1.5] interval for the lots to be considered equivalent. This analysis will be based on the IEP. The analyses will also be run for the mITT population as a measure of the robustness of the results.

SCHEDULE OF EVENTS

	Screening Visit 1	Day 1 Visit 2	Day 8 Visit 3	Day 15 Visit 4	Day 22 Visit 5	Day 29 Visit 6 (Phone)	Day 92 Visit 7 (Phone)	Day 183[13] Visit 8
Window (days)	**Within 30 days of Day 1**		**-1/+3**	**-1/+3**	**-1/+3**	**±1**	**±3**	**-14/+7**
Informed Consent/Assent	X							
Eligibility Criteria	X	*X						
Medical History	X[1]	*X[2]						
Demography	X							
Physical Exam	X[3]	*X[4]	X[4]	X[4]	X[4]			X[4]
Viral Marker testing (HIV-1/2, anti-HCV[5], HBsAg)	X							
Urine Toxicology[6]	X							
Vital Signs[7]	X	*X[8]						
Pregnancy Test (female of CBP)	X[9]	*X[10]						
FSH Levels (post-menopausal female)	X							
Randomization		*X						
Administration of Investigational Product		X						
Acute Observation		X						
Solicited AEs		X	X					
Unsolicited AEs		X	X	X	X	X		
SAEs		X	X	X	X	X	X	X
AESI		X	X	X	X	X	X	X
MAAE		X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X[11]	X[11]
Anti-CHIKV SNA Sample		*X	X	X	X			X
e-Diary Training and Distribution of Devices[12]		X						
e-Diary Collection and Review			X					

*To be done prior to IP administration.

FSH = follicle stimulating hormone

[1]To include presence of joint pain.

[2]To be updated if necessary.

[3]Complete physical exam.

[4]Directed physical exam, as needed.

[5]If HCV antibody is positive HCV ribonucleic acid (RNA) testing can be performed.

[6]Urine toxicology, per investigator's discretion.

[7]To include height and body weight (for BMI calculation).

[8]To be taken before and after IP administration.

[9]Serum pregnancy test.

[10]Urine pregnancy test.

[11]Concomitant medications associated with SAE/AESI/MAAE only.

[12]Smart phone (e-diary), digital thermometer, and ruler.

[13]Early Discontinuation/Withdrawal Visit occurring within 7 days postvaccination, from 7 to 21 days postvaccination, or ≥22 days postvaccination the Visit 3, Visit 5, or Visit 8 schedule will be followed respectively.

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APPENDIX

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LIST OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event
AESI	Adverse Event of Special Interest
ANOVA	Analysis of Variance
BMI	Body Mass Index
C	Capsid
CBP	Childbearing Potential
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHIKV	Chikungunya Virus
CI	Confidence Interval
DMP	Data Management Plan
E	Envelope
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMA	European Medicines Agency
FDA	US Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HEK	Human Embryonic Kidney
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IC	Inhibitory Concentration
ICF	Informed Consent Form
ICH	International Council on Harmonization
IEP	Immunogenicity Evaluable Population
IgG	Immunoglobulin G
IM	Intramuscular
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board

IUD	Intrauterine Device
LLOQ	Lower Limit of Quantitation
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary of Regulatory Activities
mITT	Modified Intent-to-Treat Population
MM	Medical Monitor
NHP	Nonhuman primate
NIH	National Institutes of Health
nsP	Nonstructural Proteins
NT	Neutralization Titer
PFU	Plaque Forming Unit
PT	Preferred Term
PV	Pharmacovigilance
RNA	Ribonucleic Acid
RT-qPCR	Reverse Transcriptase Quantitative Polymerase Chain Reaction
RTSM	Randomization and Trial Supply Management
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2 (COVID-19)
SD	Standard Deviation
SMC	Safety Monitoring Committee
SNA	Serum Neutralizing Antibody
SOC	System Organ Class
SR	Seroresponse Rate
SUSAR	Serious and Unexpected Suspected Adverse Reactions
VLP	Virus-Like Particle
VRC	Vaccine Research Center
US	United States of America
WHO	World Health Organization
w/w	Weight per weight

1 BACKGROUND INFORMATION

1.1 Name and Description of Investigational Product

PXVX0317 is a CHIKV VLP vaccine. The vaccine is comprised of CHIKV VLP adsorbed on aluminum hydroxide 2% adjuvant (2% (w/w) aqueous suspension of aluminum hydroxide) and stabilized with formulation buffer, supplied as a single dose of 0.8 mL in a pre-filled syringe to be administered IM in the deltoid. For additional product information, see Section 5.1 and the PXVX0317 Investigator's Brochure (IB).

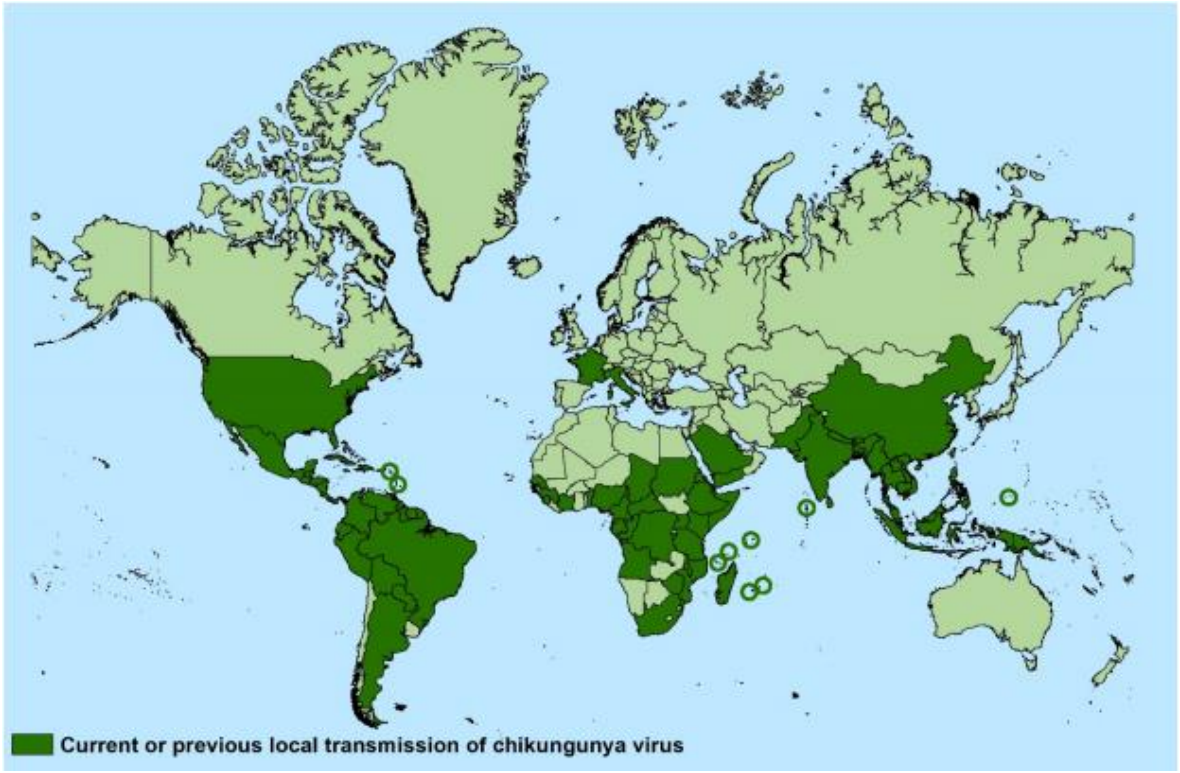
The target indication for PXVX0317 vaccine is for active immunization to prevent disease caused by chikungunya virus.

1.2 Chikungunya Virus and Disease Background

Chikungunya virus 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 nonstructural proteins (nsP1, nsP2, nsP3, and nsP4) required for replication of the virus.

Since the first case reports of CHIKV in a 1952-1953 outbreak in Tanzania (1), this disease has been endemic in Africa and parts of Asia with transmission to humans occurring through *Aedes aegypti* and more recently via *Aedes albopictus* mosquitoes (2). Beginning in 2014, CHIKV disease cases were reported among US travelers returning from affected areas in the Americas and local transmission was identified in Florida, Puerto Rico, and the US Virgin Islands. According to the US Centers for Disease Control and Prevention (CDC), approximately 117 countries or territories have documented cases of CHIKV infection excluding those countries where only imported cases have been documented (Figure 1 adapted from (3)). 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 (4).

Figure 1 Chikungunya Virus Global Burden



Following an incubation period of two to 12 days, acute clinical manifestations include high fever, rash, gastrointestinal complications, headache, muscle pain, nausea, fatigue, myalgia, and joint pain (5-7). The most classic symptom of CHIKV is a debilitating polyarthralgia that is present in greater than 90% of cases (8). This acute phase resolves within several weeks, but joint pain and arthritis may persist for months or years in over 25% to 40% of infected individuals (9).

There are currently no approved vaccines to prevent CHIKV infection or disease. However, protection against subsequent infection has been shown to correlate with the presence of CHIKV serum antibodies that neutralize the virus *in vitro* (10, 11).

1.3 Justification for Use of Investigational PXVX0317 Vaccine

The National Institutes of Health (NIH) Vaccine Research Center (VRC) initiated the development of the CHIKV VLP vaccine, designated VRC-CHKVLP059-00-VP. The VRC completed phase 1 (VRC 311) (12, 13) and phase 2 (VRC 704) (14, 15) clinical studies (14, 15). PaxVax Inc. then manufactured the vaccine as PXVX0317, conducted a mouse immunogenicity study showing comparability to the VRC-CHKVLP059-00-VP vaccine, and proceeded to a phase 2 study (PXVX-CV-317-001) (16). PaxVax Inc. was acquired by Emergent BioSolutions, Inc. in October 2018, and has been renamed Emergent Travel Health

Inc. (Emergent) as the sponsor of two phase 2 studies (PXVX-CV-317-001 (16), EBSI-CV-317-002 (17), and this phase 3 study (EBSI-CV-317-004).

1.3.1 Summary of Animal Studies

1.3.2 Summary of Findings from Clinical Studies

VRC 311 (NIH)

The safety and immunogenicity of VRC-CHKVLP059-00-VP were evaluated under BB-IND 14907 in VRC 311, a phase 1 open-label, dose-escalation study (12, 13). Healthy adult participants 18 to 50 years of age were assigned to sequential dose level groups to receive IM injections of 10 µg, 20 µg, or 40 µg (without adjuvant) on Weeks 0, 4, and 20, with follow-up for 44 weeks after enrollment. The primary endpoints were safety and tolerability of the vaccine. Secondary endpoints were CHIKV-specific immune responses assessed by neutralizing antibody assay and enzyme-linked immunosorbent assay (12, 13).

All injections were well tolerated, with no SAEs 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 (12, 13).

Neutralizing antibodies were detected in all dose groups after the second vaccination. The GMT of the half maximum inhibitory concentration (IC_{50}) was 2688 in the 10 µg group, 1775 in the 20 µg group, and 7246 in the 40 µg group, and a significant boost occurred after the third vaccination in all dose groups (10 µg group $p=0.0197$, 20 µg group $p<0.0001$, and 40 µg group $p<0.0001$). Four weeks after the third vaccination, the GMT of the IC_{50} was 8745 for the 10 µg group, 4525 for the 20 µg group, and 5390 for the 40 µg group (12, 13). These findings were confirmed by both a plaque reduction assay and the sponsor's luciferase-based assay (human SNA assay) (18), confirming both the immunogenicity of the VLP and the suitability of human SNA assay for future studies (see Section 6.1 for assay details).

VRC 704 (NIH)

The NIH's VRC 704 was a phase 2 study conducted at multiple CHIKV-endemic sites in the Caribbean (14, 15). The study was a double-blind, placebo-controlled study with 200 participants (planned) receiving 20 µg of CHIKV VLP and 200 participants receiving placebo in a two-dose series at Weeks 0 and 4. The study was initiated in 2016 and completed in 2018. Approximately 20% of participants demonstrated detectable CHIKV neutralizing antibodies at baseline using the focus reduction neutralization test reported as EC_{50} values. EC_{50} is the dilution of sera that inhibits 50% infection in viral neutralization assay. CHIKV VLP appeared safe and was well tolerated in participants who were followed through Week 72, with no related SAEs or other safety concerns (14, 15). CHIKV VLP appeared highly immunogenic, with a GMT of 2004.5 and 99.5% of recipients having neutralizing antibodies at Week 8. A boosting effect of SNA after administration of CHIKV VLP was also observed in participants with baseline CHIKV neutralizing antibodies (14, 15).

Specimens from VRC 704 were also analyzed ad hoc by the sponsor, using the human SNA assay. A subgroup analysis was performed on participants without baseline CHIKV neutralizing activity. Using a more stringent 80% neutralization cut-off (NT_{80}), the GMT was 123 at Week 4 and 1701 at Week 8. After Week 8, antibody levels declined by about 1 log

but remained elevated above baseline, with GMTs of 213 at Week 24, 115 at Week 48, and 100 at Week 72, indicating that long-term protection can potentially be achieved without the need for booster dose(s). These results demonstrated that CHIKV VLP was well tolerated and immunogenic in adults in CHIKV-endemic areas, including those with serologic evidence of previous CHIKV exposure. The most frequently reported local AE was pain/tenderness at the injection site reported as mild by 58 of 197 (29%) vaccine recipients who received at least one study injection and as moderate by 3/197 vaccine recipients (2.0%). The most frequently reported systemic AEs were mild or moderate headache reported by 54 of 197 (27.4%) vaccine recipients, malaise (53/197, 26.9%), and myalgia (46/197, 23.4%) (14, 15). Placebo recipients reported these systemic reactogenicity symptoms at similar frequencies. One vaccine recipient (0.5%) experienced a headache graded as severe following the second vaccination. A total of 16 SAEs in 15 (3.8%) participants were reported, all were assessed as unrelated to the IP (ie, CHIKV VLP or placebo). All potentially related AEs resolved without clinical sequelae (14, 15).

Taken together, the findings from VRC 311 and VRC 704 suggest that VRC-CHKVLP059-00-VP is well-tolerated and immunogenic in both CHIKV-exposed and CHIKV-naïve adults.

PXVX-CV-317-001 (Emergent)

The sponsor's phase 2 clinical study (PXVX-CV-317-001) conducted in the US compared multiple dose and dosing regimens of CHIKV VLP in healthy adults age 18 to <46 years (16). The dosages of CHIKV VLP ranged from 6 µg to 40 µg, adjuvanted. These doses were below or approximately equivalent to those used in the NIH's VRC 311 (12, 13) and VRC 704 (14, 15) clinical studies using the non-adjuvanted CHIKV VLP (VRC-CHKVLP059-00-VP) vaccine. PXVX0317 vaccine was immunogenic across all dose groups as measured by human SNA assay. Immunogenicity data reported as 80% antibody NT_{80} values supported the benefit of adjuvant was evident after one dose but not two doses. There was a clear dose-response relationship in GMT. Participants receiving 20 µg on the standard schedule (Days 1 and 29), either unadjuvanted (as the NIH reference dose) or adjuvanted, had the highest Day 57 GMT at 1946 and 1884 respectively, similar to those of CHIKV VLP recipients in the VRC 704 study (14, 15). Participants receiving the single 40 µg dose demonstrated only slightly lower GMT levels (1712 at Day 57, i.e., 28 days after vaccination); all other dose groups demonstrated a GMT range of 914 to 1613 at Day 57. Seroconversion rates (titer ≥15) showed that with a single dose administered, up to 98% of study participants produced a neutralizing antibody response by Day 8. Further, the immune response was shown to be persistent through the 12-month visit, including in the one dose 40 µg CHIKV VLP regimen. There was a clear dose-response relationship in GMT, with the Group 8 40 µg CHIKV VLP + 300 µg alum adjuvant single dose resulting in 86% seroresponse (titers ≥40) 7 days postvaccination as well as the highest GMTs at Day 182. Seroresponse was well maintained at 760 days in Group 8 participants (16).

1.3.3 Seroresponse Rate

In historical studies, a human SNA assay threshold titer of ≥40 was used, however after discussions with regulatory agencies, anti-CHIKV SNA titer [redacted] (seroresponse rate, also considered the presumptive seroprotection rate) will be used in the present and future studies.

1.3.4 Rationale for Dosage and Route of Administration

The regimen of PXVX0317 selected for this study is 40 µg of CHIKV VLP with alum adjuvant administered in a single IM dose on Day 1. The alum dose of 300 µg is within the range of doses of alum adjuvants used in many licensed vaccines, including VLP-based vaccines. This dose also creates a concentration ratio to PXVX0317 that achieves high (~90%) levels of adsorption, thought to enhance both immunogenicity and short-term stability. The IM route of administration is consistent with that in previous clinical studies (VRC 311 (12, 13), VRC 704 (14, 15), PXVX-CV-317-001 (16), and EBSI-CV-317-002). This regimen was selected by an interim analysis of the PXVX-CV-317-001 study data. All regimens tested were given to groups of 50 to 53 healthy adults and all resulted in 100% seroconversion by Day 57. The group receiving a single dose of 40 µg plus alum showed the highest anti-CHIKV SNA GMT at Day 182 and Day 365.

The PXVX0317 vaccine (40 µg CHIKV VLP + 300 µg alum adjuvant) single dose was selected for further development by the sponsor in this phase 3 clinical study (EBSI-CV-317-004).

1.4 Justification for Inclusion of Adolescents 12 to <18 Years of Age

This study will enroll adolescents 12 to <18 years of age. The accumulated evidence to date supports the safety and immunogenicity of PXVX0317 and inclusion of adolescents in this study. To date, 713 adults have received at least one dose of the vaccine candidate (PXVX0317 or VRC-CHKVLP059-00-VP). No vaccine-related SAEs or deaths have occurred. No significant imbalances in unsolicited AEs have been observed that raise concern for a safety signal associated with immunization. Based on results from completed phase 1 and 2 studies (VRC 311 (12, 13), VRC 704 (14, 15)), and the phase 2 clinical study (PXVX-CV-317-001) (16), PXVX0317 vaccine is expected to induce robust SNA titers postvaccination. The 40 µg single, adjuvanted dose resulting in 96% seroresponse (human SNA assay titers ≥40) at Day 365 and SNA GMT of 1712 at Day 57 and 466 at Day 365. Data from the completed double-blind, randomized, placebo-controlled, multi-center phase 2 study (VRC 704) conducted with an unadjuvanted formulation (two doses of 20 µg on Days 0 and 28) in 400 healthy adults living in a CHIKV-endemic area (Caribbean) indicated that the product elicited neutralizing antibodies in 99.5% of vaccine recipients and that the neutralizing antibody response persisted for at least 18 months (14, 15).

The US adolescents enrolled in this study have the prospect to directly benefit from vaccination against CHIKV following exposure to CHIKV due to: i) travel to CHIKV-

endemic areas or areas where CHIKV epidemics are actively occurring; ii) local autochthonous transmission in the US and; iii) the occurrence of a CHIKV epidemic in the US. Although CHIKV has been known to cause periodic epidemics in tropical regions, in 2013 CHIKV was identified in the Western Hemisphere in the Caribbean (19). With the global expansion of CHIKV and the frequency of travel, the potential for exposure to CHIKV has increased. Local transmission of CHIKV has been reported in approximately 117 countries or territories worldwide, including the US (20). Each year, millions of travelers visit countries where CHIKV outbreaks are ongoing (21, 22). In 2014 alone, 2799 cases of CHIKV disease occurred in US travelers returning from affected areas (22). From 2014 to 2017, approximately 10% of 3941 travel-acquired CHIKV cases were in individuals under 20 years of age (7.5% were 10 to 19 years of age and 2% were <10 years of age). Of those 10 to 19 years of age, just under 20% were hospitalized as a result of their illness (23). Locally transmitted cases have been reported in the US since 2013 (22).

With expanded distribution of the *Ae. aegypti* and *Ae. albopictus* mosquito-vectors in the US and the increase in travel, epidemiologic models indicate that the US remains susceptible to epidemics (24). Although locally transmitted cases are currently reported to occur in small numbers, as more CHIKV-infected travelers come into the US, the likelihood that local CHIKV transmission could occur is increased (25).

CHIKV epidemics, when they occur, are characterized by decades of inactivity interspersed with sudden outbreaks. The available methods to accurately predict when emerging CHIKV epidemics will occur are limited (26). In this regard, susceptible adolescents living in the US and susceptible adolescents living in an area where a CHIKV epidemic has occurred in the past share a similar prospect for direct benefit of vaccination.

2 STUDY OBJECTIVES AND PURPOSE

2.1 Purpose

The purpose of this phase 3, randomized, double-blind, placebo-controlled study is to evaluate the safety and immunogenicity to PXVX0317 in adult and adolescent participants 12 to <65 years of age.

2.2 Coprimary Objectives

- To evaluate the safety of PXVX0317 in healthy adult and adolescent participants 12 to <65 years of age.
- To compare the anti-CHIKV SNA response to PXVX0317 and placebo at Day 22, as measured by GMT and clinically relevant difference in seroresponse rate (PXVX0317 minus placebo).

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA titer [redacted] See Section 6.1 for assay description.

- To demonstrate the consistency of the anti-CHIKV SNA response across three consecutively manufactured lots of PXVX0317 at Day 22 as measured by GMT.

2.3 Secondary Objectives

- To compare the anti-CHIKV SNA response to PXVX0317 and placebo at Day 15, Day 183, and Day 8, as measured by GMT and seroresponse rate.
- To compare the anti-CHIKV SNA response to PXVX0317 and placebo in participants 12 to <18 years of age, participants 18 to <46 years of age, and participants 46 to <65 years of age as measured by GMT and seroresponse rate.

2.4 Exploratory Objective

- To evaluate anti-CHIKV SNA response across three consecutively manufactured lots of PXVX0317 as measured by seroresponse rate for each lot at Day 22 in participants 18 to <46 years of age.

3 STUDY DESIGN

3.1 Study Description

This is a phase 3 randomized, placebo-controlled, double-blind, parallel-group design with four treatment groups. Participants will be randomized in a 2:2:2:1 ratio within each of the three age strata (ages 12 to <18, 18 to <46, and 46 to <65) to receive one of three consecutively manufactured lots of PXVX0317 or placebo. With 3150 participants enrolled, the treatment group totals are estimated as follows:

- Group 1- PXVX0317 lot A: n=900
- Group 2- PXVX0317 lot B: n=900
- Group 3- PXVX0317 lot C: n=900
- Group 4- Placebo: n=450

A nested lot consistency sub study will be performed in adult participants 18 to <46 years of age in the PXVX0317 treatment groups (lot A, lot B, and lot C).

3.1.1 Study Centers

This multicenter study will be conducted in the US, using up to 50 sites.

3.1.2 Number of Participants (Planned)

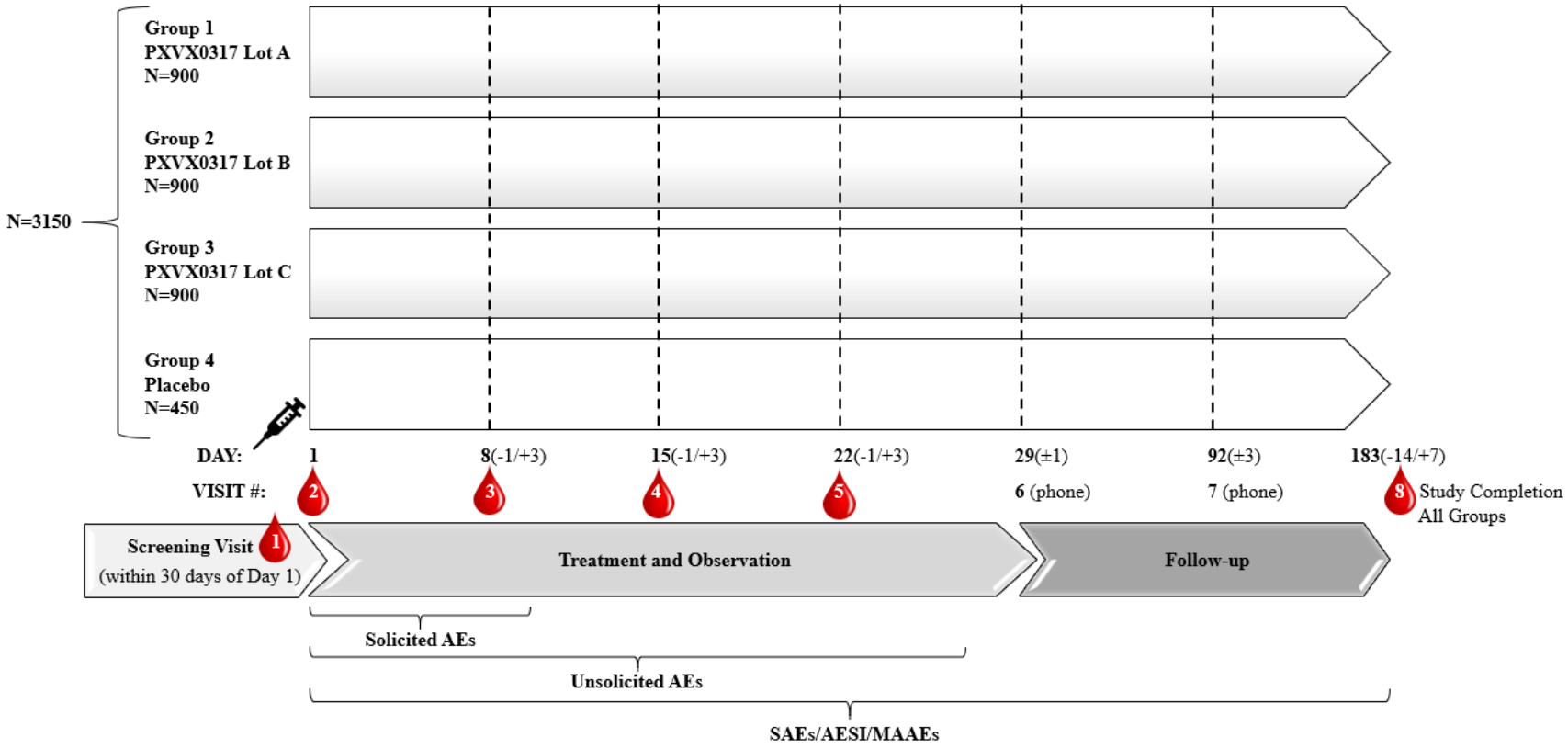
At least 3150 enrolled healthy US adolescent and adult participants ages 12 to <65, stratified in three age groups: 12 to <18, 18 to <46, and 46 to <65 years of age. Enrollment will target at least 245 participants (210 PXVX0317 participants and 35 placebo participants) into the youngest and oldest age groups (12 to <18 and 46 to <65 years of age, respectively). The middle age group must include at least 1050 participants to provide adequate power for the lot consistency analysis.

3.1.3 Estimated Study Duration

The per participant estimated total study duration is 212 days. The screening window will be no greater than 30 days prior to Day 1 (randomization and administration of IP). The immune response to PXVX0317 will be measured by anti-CHIKV SNA up to Day 183 End of Study Visit.

3.2 Schematic Diagram of Study Design

Figure 2 EBSI-CV-317-004 Schematic Diagram of Study Design



3.3 Description of Study Assessments

The underlying subsections (Section 3.3.1 through Section 3.3.12) describe the planned study procedures and assessments. For the per visit timing of these procedures and assessments refer to Section 5.2 and the study Schedule of Events. Information on participant consent (and assent, as applicable) is described in Section 11.1.

3.3.1 Review of Eligibility Criteria

Review of inclusion and exclusion criteria should be completed, and participant eligibility confirmed prior to planned vaccination. Refer to Section 4.1 and Section 4.2 for criteria.

3.3.2 Medical History and Demography

Medical history information will be collected from participants at the Screening Visit and confirmed at the Day 1 Visit (at baseline ie, prior to IP administration) and will include (but not be limited to) demographic information (date of birth, race, ethnicity, and sex of participant), current and past medical conditions (including presence of joint pain), prior and concomitant medications (see Section 5.3) taken within 30 days of Screening Visit (or within 90 days, for blood products).

3.3.3 Physical Examination

A complete physical examination will be performed on participants during the Screening Visit. The examination should include general appearance, eyes-ears-nose-throat, head-neck, lungs-chest, heart, abdomen, musculoskeletal, lymph nodes, skin, extremities, and neurological assessment.

A targeted physical examination may be performed on participants at additional time points if indicated by AE, MAAE, SAE, or AESI reports.

3.3.4 Vital Signs

Vital signs collected after 5 minutes of rest and prior to blood work without distractions from participants 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 electronic case report form (eCRF) for inclusion of the participant into the study. Repeat measurements on abnormal vital parameters are allowed twice for confirmation of eligibility to receive the vaccination.

Measurement of body weight and height for BMI calculations will be obtained at the Screening Visit.

3.3.5 Laboratory Tests

At the Screening Visit, blood samples will be collected for serum testing for Hepatitis B surface antigen (HBsAg), HCV antibody (if HCV antibody is positive HCV RNA testing can be performed), HIV-1/HIV-2, serum pregnancy test for women of CBP, and for FSH testing for postmenopausal women (for additional information on FSH assessment see Section 3.3.7). Urine toxicology screening may be done per investigator's (or designee's) discretion at the Screening Visit. Any request to repeat testing should be discussed with the MM.

For additional information on sample collection, processing, storage, and shipment refer to the study Laboratory Manual.

3.3.6 Immunogenicity Sample

Anti-CHIKV SNA titers will be assessed using a validated human SNA assay that measures neutralization titer of 80% (NT_{80}). Blood (serum) samples will be taken on Day 1 (prior to IP administration), Day 8, Day 15, Day 22, and the Day 183 End of Study Visit (or at Early Discontinuation/Withdrawal). For information on the immunogenicity assessment see Section 6.1.

For additional information on sample collection, processing, storage, and shipment refer to the study Laboratory Manual.

3.3.7 Pregnancy Testing and Contraception

Women of CBP will undergo a serum pregnancy test at the Screening Visit, and a urine pregnancy test prior to IP administration on Day 1. The participant must have a negative urine pregnancy test on Day 1, prior to administration of IP.

Women of CBP must also use an acceptable method of contraception from prior to Day 1 through Day 183 End of Study Visit. Acceptable methods include highly effective forms of contraception: hormonal contraceptives (eg, implants, pills, patches) containing combined estrogen and progestogen, or progestogen-only initiated ≥30 days prior to dosing **or** IUD inserted ≥30 days prior to dosing **or** use of double barrier type of birth control (male condom with female diaphragm, male condom with cervical cap). Abstinence is acceptable only for adolescents (12 to <18 years of age) who are not sexually active.

Note: Contraception requirements do not apply for participants in exclusively same-sex relationships and these participants should have no plans to become pregnant by any other means for the duration of the study.

The investigator must confirm that contraception methods (eg, hormonal contraceptive or intrauterine device) were initiated ≥30 days prior to Day 1 to be considered fully effective.

For female participants who are postmenopausal, documented FSH level of ≥40 mIU/mL must be obtained. If the FSH is <40 mIU/mL, the participant must agree to use an acceptable form of contraception (see above).

The investigator must report any pregnancies as described in Section 7.3.1.

3.3.8 Investigational Product Administration

Investigational product single dose is 0.8 mL in volume and administered by IM injection into the deltoid with a pre-filled syringe attached to 25 gauge 1" (or 1.5") needle, using universal precautions and sterile technique in accordance with General Best Practice Guidelines for Immunization: Best Practices Guidance of the Advisory Committee on Immunization Practices (ACIP) (27) per Table 1. Injections will be administered by a staff member delegated by the investigator.

Table 1 Needle Length of Intramuscular Injections for Children (by Age) and Adults Aged ≥19 Years (By Sex and Weight)

Sex, Weight	Needle length	Injection Site
Men and women, <60 kg (<130 lbs)	1 inch (25 mm)	Deltoid muscle of arm
Men and women, 60-70 kg (130-152 lbs)	1 inch (25 mm)	
Men, 70-118 kg (152-260 lbs)	1-1.5 inches (25-38 mm)	
Women, 70-90 kg (152-200 lbs)		
Men, >118 kg (260 lbs)	1.5 inches (38 mm)	
Women, >90 kg (200 lbs)		
Children, 12 to <18 years of age	5/8[1]-1 inch (16-25 mm)	

Source: Adapted from Table 6-2, General Best Practice Guidelines for Immunization: Best Practices Guidance of the Advisory Committee on Immunization Practices (ACIP) (27).
[1]If skin is stretched tightly and subcutaneous tissues are not bunched.

Investigational product is to be administered only under the direct supervision of the investigator or a qualified subinvestigator identified on the FDA Form 1572. Under no circumstances will the investigator allow PXVX0317 vaccine to be used other than as specified in the protocol.

For further details on the IP refer to Section 5.1.

3.3.9 Acute Observation After Investigational Product Administration

The participant will be monitored by study staff for signs of an acute adverse reaction for 30 min after injection. Vital signs will be obtained 30 min to 1 hr after injection.

3.3.10 Solicited Adverse Events

Solicited AEs will be collected from IP administration until Day 8. Solicited AEs for this study are local events of pain, redness, and swelling at the injection site and systemic events of oral temperature ≥38.0°C (≥100.4°F), chills, fatigue, headache, myalgia, arthralgia, and nausea (Section 7.1.2 and Section 7.1.2.1).

Participants will be trained to complete an e-diary to observe, measure, and record these solicited AEs. In the occurrence of issues with the e-diary, a paper diary (subject memory aid) may be implemented as a back up. To measure oral temperature, a digital thermometer will be provided to the participant to measure their temperature each day and record them in their e-diary. To record injection site local reactions, a ruler will be provided to the participant to measure and record the diameter of redness and swelling at the largest point of the reaction each day. Adolescent participants will be instructed to complete the e-diary. Parents/guardians may provide assistance to adolescents to measure and record temperature and injection site reactions.

Study staff will review the signs and symptoms recorded in the e-diary. The investigator will then assess all solicited AEs for severity (Section 7.1.7) and the action taken, and causality (Section 7.1.8). The results of the investigator's assessment will be recorded as a separate source document and will be entered on the solicited AE eCRF. Symptoms continuing beyond the solicited AE collection period will be collected and recorded on the AE eCRF.

Details on definition, evaluation, reporting periods and documentation are outlined in Section 7.

3.3.11 Unsolicited Adverse Events

Unsolicited AEs (AEs not listed in the e-diary) will be collected from Day 1 through 28 days postvaccination (Day 29) (Section 7.1.3).

Details on definition, evaluation, reporting periods and documentation are outlined in Section 7.

3.3.12 Serious Adverse Events and Adverse Events of Special Interest

Serious adverse events, AESI, and MAAEs will be collected for all participants from Day 1 through Day 183 End of Study Visit (Section 7.1.4 and Section 7.1.5).

Details on definition, evaluation, reporting periods and documentation are outlined in Section 7.

3.4 Measures Taken to Minimize/Avoid Bias

3.4.1 Treatment Assignment/Randomization

Participant eligibility will be confirmed and documented by the investigator immediately prior to randomization of each participant. Study staff will indicate on a randomization eCRF within the electronic data capture (EDC) system that they want to generate a randomization number for the participant. When they agree to proceed, a randomization number will be generated from the EDC randomization module but hidden from study staff. The EDC randomization module will match the randomization number to an IP kit available at the site

and inform the study staff of the assigned kit. Study staff will use the appropriate kit for administration to the participant.

Participants 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 Day 183 End of Study Visit. Neither participants, nor clinical site personnel, including the investigators, nor the sponsor will know participants' individual treatment assignments until all participants have completed their participation in the study through the Day 183 End of Study Visit and the database has been cleaned and locked.

3.4.2 Masking/Blinding Procedures

Study participants, the investigator, and study site personnel will remain blinded to all randomization assignments throughout the study. The sponsor's MM and personnel, who are in regular contact with the study site and/or involved with documentation associated with the study will remain blinded to all participant randomization assignments.

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

- Use of a standardized pre-filled syringe and injection volume for all injections.
- All PXVX0317 and placebo syringes have a semitransparent barrel cover to mask any difference in appearance between placebo and PXVX0317.
- No sponsor personnel will have access to the randomization schedule. No site personnel will have access to treatment assignments. The Randomization and Trial Supply Management (RTSM) system allows for unblinding in an emergency (see Section 7.7).
- Assays will be run in a blinded manner. The assay titer results will not be delivered to the sponsor data management and analysis team until after database lock, as they are potentially unblinding.
- Should any participant or staff member become inadvertently unblinded, the investigator will promptly (within 24 hours of their awareness of the error) disclose the event to the sponsor's MM in a blinded fashion (disclosing only participant 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.

3.5 End of Study

An individual participant is considered to have completed study participation after completion of the Day 183 (-14/+7) Visit 8 and any required safety follow-up.

4 SELECTION AND WITHDRAWAL OF PARTICIPANTS

4.1 Inclusion Criteria

Participants must meet **all** the following criteria to be enrolled:

1. Able and willing to provide informed consent (and assent, as applicable) voluntarily signed by participant (and guardian, as applicable).
2. Males or females, 12 to <65 years of age.
3. Generally healthy, in the opinion of the investigator, based on medical history, physical examination, and screening laboratory assessments.
4. Women who are **either**:
    i. Not of CBP: pre-menarche, surgically sterile (at least six weeks post bilateral tubal ligation, bilateral oophorectomy, or hysterectomy), or postmenopausal (defined as a history of ≥12 consecutive months without menses prior to randomization in the absence of other pathologic or physiologic causes, following cessation of exogenous sex-hormonal treatment).

    or:

    ii. Meeting **all** the below criteria:
    - Negative serum pregnancy test at Screening Visit
    - Negative urine pregnancy test immediately prior to dosing at Day 1
    - Use one of these acceptable methods of contraception (if women of CBP) for the duration of participation:
        - Hormonal contraceptives (eg, implants, pills, patches) initiated ≥30 days prior to dosing
        - Intrauterine device inserted ≥30 days prior to dosing
        - Double barrier type of birth control (male condom with female diaphragm, male condom with cervical cap)
        - Abstinence is acceptable only for adolescents (12 to <18 years old) who are not sexually active.

Note: See Section 3.3.7 for this study's list of acceptable method of contraception.

Note: Contraception requirements do not apply for participants in exclusively same-sex relationships and these participants should have no plans to become pregnant by any other means for the duration of the study.

4.2 Exclusion Criteria

Participants who meet **any** of the following criteria **cannot** be enrolled:

1. Currently pregnant, breastfeeding, or planning to become pregnant during the study.
2. Body mass index ≥35 kg/m^2.
3. Positive laboratory evidence of current infection with HIV-1/HIV-2, HCV, or HBV.
4. History of severe allergic reaction or anaphylaxis to any component of the IP.
5. History of any known congenital or acquired immunodeficiency that could impact response to vaccination (eg, leukemia, lymphoma, generalized malignancy, functional or anatomic asplenia, alcoholic cirrhosis).
6. Prior receipt or anticipated use of systemic immunomodulatory or immunosuppressive medications from six months prior to screening through Day 22. **Note:** For systemic corticosteroid use at a dose or equivalent dose of 20 mg of prednisone daily for 14 days or more within three months of screening through Day 22 is exclusionary. The use of inhaled, intranasal, topical, ocular, or intraocular steroids is allowed.
7. Receipt or anticipated receipt of blood or blood-derived products from 90 days prior to screening through Day 22.
8. Acute disease within the last 14 days (participants with an acute mild febrile illness can be considered for a deferral of vaccination two weeks after the illness has resolved and treatment has been completed).
9. Clinically significant cardiac, pulmonary, rheumatologic, or other chronic disease, in the opinion of the investigator. This may include chronic illness requiring hospitalization in the last 30 days prior to screening.
10. Enrollment in an interventional study and/or receipt of another investigational product from 30 days prior to screening through the duration of study participation.
11. Receipt or anticipated receipt of any vaccine from 30 days prior to Day 1 through Day 22.
12. Evidence of substance abuse that, in the opinion of the investigator, could adversely impact the participant's participation or the conduct of the study.
13. Prior receipt of an investigational CHIKV vaccine/product.
14. Any other medical condition that, in the opinion of the investigator, could adversely impact the participant's participation or the conduct of the study.

4.3 Withdrawal of Participants

4.3.1 Participant Consent Withdrawal

All participants can withdraw from participation in this study at any time, for any reason, specified or unspecified, and without penalty. The investigator will ask (but cannot require) such participants to provide the reason(s) for withdrawal of consent and to undergo an Early Withdrawal Visit. An individual is considered to undergo early withdrawal if they stop study participation before Day 183 End of Study Visit.

Safety follow-up for AEs should occur for all participants. For information on safety follow-up for withdrawn participants see Section 4.3.3.

4.3.2 Investigator-Based Participant Withdrawal

The Investigator may withdraw a participant from further participation in the study, at their discretion, if medically necessary or for reasons of noncompliance. The reason for withdrawal of any participant must be clearly documented on the study source documents and the appropriate eCRF. The investigator is encouraged to consult the sponsor prior to the withdrawal of any participant, except in the event of a medical emergency.

The investigator (and/or sponsor) may withdraw a participant from the study for any of, but not limited to, the following reasons:

- Noncompliance with the protocol. If the participant is noncompliant with protocol requirements, the issue should be discussed with the participant and, if not resolved, consideration given to withdrawing the participant.
- Lost to follow-up; requires documentation of at least three unsuccessful attempts to contact participants. Lost to follow-up will be determined after the date of the participant's projected last visit.
- 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 participant.

Safety follow-up for AEs should occur for all participants. For information on safety follow-up for withdrawn participants see Section 4.3.3.

4.3.3 Follow-up for Withdrawn Participants

Outreach will be made to ensure that participants who are withdrawn, or who withdraw from the study, during the active observation or follow-up period will complete all safety and available assessments for the Early Withdrawal Visit as outlined in this protocol. The investigator should inform the participant that these assessments are for their own well-being and, if possible, for study purposes. Additional information regarding ongoing AEs may be provided as a follow-up report.

4.3.4 Documentation of Withdrawal/Discontinuation

Reasons for withdrawal of individual participants from the study prior to final protocol required visit and/or final safety follow-up are to be recorded on the eCRF. The reason for withdrawal from the study will be recorded as one of the following:

- Lost to Follow-up (**Note:** Requires documentation of at least three unsuccessful attempts to contact participants, lost to follow-up will be determined after the date of the participant's projected last visit)
- Adverse event
- Pregnancy
- Protocol deviation (that results in discontinuation)
- Noncompliance to IP
- Physician (ie, investigator) decision
- Other

Safety follow-up for AEs should occur for all participants, for information on safety follow-up for withdrawn participants see Section 4.3.3.

4.3.5 Participant Replacement

Participants who undergo Early Discontinuation or withdrawal of consent after randomization and before receiving treatment may be replaced at the sponsor's discretion. Participants who undergo Early Discontinuation after vaccination will not be replaced.

5 TREATMENT OF PARTICIPANTS

5.1 Investigational Products

5.1.1 PXVX0317 Vaccine

The PXVX0317 vaccine is comprised of CHIKV VLP adsorbed on aluminum hydroxide adjuvant 2%, and formulation buffer supplied as a single dose in a pre-filled glass syringe. CHIKV VLP refers to the VLP component of PXVX0317 produced by transient transfection of human embryonic kidney (HEK293) cells with a deoxyribonucleic acid expression plasmid encoding capsid (C) and envelope (E) 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. Virus-like particles are then concentrated from the cell supernatant purified, and buffer exchanged. The VLPs are then formulated with excipients and aluminum hydroxide adjuvant, mixed, and

filled into 1 mL glass syringes with a 0.8 mL deliverable volume. The glass syringe is sealed with a rubber closure and plastic cap. The rubber closure does not contain natural latex.

Placebo (formulation buffer) is supplied as a single dose of 0.8 mL in a pre-filled syringe.

See the pharmacy manual and the IB for additional information, including composition of PXVX0317 vaccine.

5.1.2 Placebo Pre-filled Syringe

The placebo is a sterile aqueous solution with the same excipient composition as the drug product without CHIKV VLP and aluminum hydroxide adjuvant. The placebo is filled into 1 mL glass syringes with a 0.8 mL deliverable volume. The placebo is stored at 2.0 to 8.0°C. The glass syringe is sealed with a rubber closure and plastic cap. The rubber closure does not contain natural latex.

See the pharmacy manual for additional information, including composition of the placebo.

5.1.3 Labeling

Investigational product (PXVX0317 vaccine and placebo) will be shipped in carton kits containing labelled pre-filled syringes. Kits and syringes will be labelled with the following information:

- "Caution: New Drug – Limited by Federal Law to Investigational Use Only"
- Investigational product name – PXVX0317 vaccine or placebo (to maintain the blind both names will be included)
- Dose volume and container – single dose of 0.8 mL in a pre-filled syringe
- Route of administration – Intramuscular
- Randomization kit number (in lieu of lot numbers and manufacture date and/or expiry date to maintain the blind)
- Protocol number – EBSI-CV-317-004
- Recommended storage temperature/conditions – stored refrigerated at 2.0 to 8.0°C
- Sponsor – Emergent Travel Health Inc.

5.1.4 Investigational Product Shipment

Investigational product will be shipped to the site at a temperature of 2.0 to 8.0°C. During shipment, the temperature of the IP will be monitored to ensure the required temperature conditions are maintained. The principal investigator (or designee) will be responsible for checking the number of syringes and the condition of the syringes received, and entering this information into the drug accountability records, and reporting the condition of shipment to

the sponsor and drug depot. Investigational product received in good condition will be automatically released for use upon confirmation of receipt as such within the RTSM system; shipments received with any excursions/issues will be placed under quarantine by the site and released for use by the sponsor following investigation. For additional information on IP shipments please refer to the study pharmacy manual.

5.1.5 Storage Conditions

Investigational product (PXVX0317 vaccine and placebo) must be stored refrigerated at 2.0 to 8.0°C in a secured area until used. The temperature in the storage area must be monitored with properly calibrated instruments and recorded on a temperature log. Any excursions must be promptly reported to the sponsor via the RTSM system; product should be quarantined by the site and may be released for use by the sponsor only after investigation and confirmation of continued stability. Please refer to the pharmacy manual for additional details.

5.1.6 Preparation

Investigational product (PXVX0317 vaccine and placebo) will be supplied as a single dose of 0.8 mL in a pre-filled syringe shipped in kits. See Section 3.3.8 for more details.

5.1.7 Drug Accountability

The investigator is responsible for maintaining accurate inventory records of IP. The investigator (or designee) will inventory all IP shipments upon receipt. The investigator must ensure that all drug supplies are kept in a secure location in the site pharmacy in accordance with recommended storage conditions (Section 5.1.5). Inventory and ongoing record of test material supplies will be documented using the electronic drug accountability form provided within the clinical trial material tracking module of the sponsor's RTSM system. These records will be reviewed by representatives of the sponsor and may be reviewed by regulatory agencies.

5.2 Study Procedures and Assessments by Visit

The timing of the study procedures and assessment below are shown in the Schedule of Events and a description of each procedure and assessment is described in Section 3.3.

5.2.1 Screening (Visit 1, within 30 days of Day 1)

Eligible participants will first undergo informed consent counseling. Once informed consent has been obtained, participants will undergo a Screening Visit to ascertain their eligibility in this study within 30 days prior to Day 1. The Screening Visit assessments will include:

- Informed consent (and informed assent, as applicable) (Section 11.1).
- Review of eligibility criteria (Section 3.3.1, Section 4.1, Section 4.2).

- Medical history (including presence of joint pain) (Section 3.3.2).
- Demographics (date of birth, race, ethnicity, and sex of participant) (Section 3.3.2).
- Prior and current medications (Section 3.3.2, Section 5.3).
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature) as well as height and body weight (for BMI calculation) (Section 3.3.4).
- Complete physical exam (general appearance, eyes-ears-nose-throat, head-neck, lungs-chest, heart, abdomen, musculoskeletal, lymph nodes, skin, extremities, and neurological assessment) (Section 3.3.3).
- Blood collection for laboratory viral marker testing (HBsAg, HCV antibody [if HCV antibody is positive, HCV RNA testing can be performed], HIV-1/HIV-2) (Section 3.3.5).
- For women of CBP: blood sample for serum pregnancy test (Section 3.3.5, Section 3.3.7).
- For postmenopausal women: blood sample for FSH levels (Section 3.3.5, Section 3.3.7).
- Urine sample for toxicology (per investigator's [or designee] discretion) (Section 3.3.5).

5.2.1.1 Rescreening

Rescreening may occur given the following circumstances:

- If the participant has an acute febrile illness at the time of their scheduled enrollment, they may be re-screened 14 days after resolution of their acute illness.
- If the participant is asymptomatic but tests positive by reverse transcription polymerase chain reaction (RT-PCR) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) they may be rescreened provided 14 days have elapsed since the participant tested RT-PCR positive for SARS-CoV-2, the participant continues to be asymptomatic, and the participant is otherwise eligible for enrollment.
- Participants who have received licensed vaccines within 30 days of anticipated Day 1 may be rescreened provided no additional doses are anticipated up to Day 22.
- Participants who have received blood products or investigational products from participation in another clinical study may be rescreened after the appropriate duration has passed (90 days for blood products; 30 days for investigational drugs; six months prior to Screening Visit for systemic immunomodulatory or immunosuppressive medications).
- Eligible participants who are not able to be vaccinated with IP within 30 days of their screening period may be rescreened.

Rescreened participants must undergo all screening procedures again, including reconsenting and use of the same participant ID number. Participants may be rescreened one time only. Rescreening is not otherwise permitted.

5.2.2 Day 1 (Baseline, Randomization, and Administration of IP) Visit 2

The following will take place during the visit and prior to IP administration:

- Reverification of eligibility criteria.
- Review medical history.
- Review prior and concomitant medications.
- Vital signs.
- Directed physical exam (as needed, if indicated by medical history).
- For women of CBP: urine pregnancy test.
- Blood collection for anti-CHIKV SNA (Section 3.3.6, Section 6.1).
- Randomization (Section 3.4.1).
- IP administration (Section 3.3.8, Section 5.1).

The following will take place during the visit and after IP administration:

- In-clinic acute observation (Section 3.3.9).
- Vital signs.
- E-diary device, digital thermometer, and ruler distribution, set-up, and training (Section 3.3.10).
- Solicited AE, unsolicited AE, MAAE, SAE, and AESI (Sections 3.3.10 - 3.3.12, Section 7) evaluation.
- Record any medications that were given to participants after IP administration.

5.2.3 Day 8 (-1/+3) Visit 3

- Review e-diary.
- Solicited AE, unsolicited AE, MAAE, SAE, and AESI evaluation.
- Review concomitant medications.
- Blood collection for anti-CHIKV SNA.
- Directed physical exam (as needed).

5.2.4 Day 15 (-1/+3) Visit 4

- Unsolicited AE, MAAE, SAE, and AESI evaluation.
- Review concomitant medications.
- Blood collection for anti-CHIKV SNA.
- Directed physical exam (as needed).

5.2.5 Day 22 (-1/+3) Visit 5

- Unsolicited AE, MAAE, SAE, and AESI evaluation.
- Review concomitant medications.
- Blood collection for anti-CHIKV SNA.
- Directed physical exam (as needed).

5.2.6 Day 29 (±1) Telephone Contact for Visit 6

- Unsolicited AE, MAAE, SAE, and AESI evaluation.
- Review concomitant medications.

5.2.7 Day 92 (±3) Telephone Contact for Visit 7

- SAE, AESI, and MAAE evaluation.
- Review concomitant medications (only if associated with SAE/AESI/MAAE).

5.2.8 Day 183 (-14/+7) Visit 8 End of Study

- SAE, AESI, and MAAE evaluation.
- Review concomitant medications (only if associated with SAE/AESI/MAAE).
- Directed physical exam (as needed).
- Blood collection for anti-CHIKV SNA.

5.2.9 Early Discontinuation/Withdrawal Visit

If the Early Discontinuation/Withdrawal Visit occurs: i) within 7 days after IP administration, the Visit 3 schedule will be followed; ii) from 7 days through 21 days after IP administration, the Visit 5 schedule will be followed and; iii) after Day 22, the Visit 8 schedule will be followed.

5.2.10 Unscheduled Visits

Any study procedure, excluding 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 SAE/AESI/MAAE/AE.

5.3 Prior and Concomitant Medications

At the Screening Visit, the details of prior and concomitant medication usage will be collected (through 30 days prior to screening, or 90 days for blood products, or six months prior to screening for systemic immunomodulatory or immunosuppressive medications). Concomitant medications will be collected at each on site visit or by phone interview (or early discontinuation/withdrawal) for all groups. The details of all concomitant medications including those associated with solicited AEs and unsolicited AEs will be collected. After Day 29 only concomitant medications associated with SAEs, AESI, and MAAEs will be collected through the end of the study.

5.3.1 Required Concomitant Medications

Not applicable.

5.4 Procedures for Monitoring Participant Compliance

All participants will be administered the IP by study staff. Compliance with study protocol and procedures will be monitored on an ongoing basis by study staff.

6 IMMUNOGENICITY ASSESSMENT

6.1 Immunogenicity Analysis

The immunogenicity analysis will be performed using a high-throughput *in vitro* infectivity assay developed by the sponsor for assessing titers of neutralizing antibodies against CHIKV in serum samples. The assay is based on using a modified version CHIKV that expresses luciferase (CHIKV-luc) and measures the reduction of luciferase activity in infected cultures of Vero cells following treatment of virus with test serum. Using the human SNA assay, antibody neutralization titers can be determined via characterization of reductions of luciferase activity in the presence of immune serum. The quantitation of reporter gene expression, a correlate of the level of virus infection of cells, is determined by detection of luciferase in assay wells using a micro-plate luminometer. The CHIKV neutralizing antibody titer 80 (NT_{80}) is the reciprocal of the serum dilution that provides 80% protection of Vero cells from CHIKV-luc infection or an 80% reduction of luciferase activity compared to virus only control.

For the statistical analysis of the anti-CHIKV SNA titers results obtained from sample analysis refer to Section 8.6.

7 SAFETY ASSESSMENTS AND REPORTING

7.1 Definitions

7.1.1 Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Notes:

- *A diagnosis should be captured as an AE term and signs and symptoms should be captured only in the absence of a unifying diagnosis. If a diagnosis is ultimately made, it should replace the previous report based on signs and symptoms.*
- *In the event that there are multiple diagnoses, then all diagnoses should be captured.*
- *The worsening of an existing sign, symptom or disease is also considered to be an AE.*
- *An abnormal laboratory finding deemed by the principal investigator (or designee) as not clinically significant (NCS) will not be captured as an AE, but an abnormal laboratory finding that worsens after dosing with the IP, from not clinically significant to clinically significant (CS), is considered an AE.*
- *Surgical procedures are not AEs. They are the action taken to treat a medical condition. Interventions that were planned prior to study entry for medical conditions that started prior to study entry but did not worsen during the clinical study are not reported as AEs.*
- *Medically attended adverse events: medically attended visits include hospital, emergency room, urgent care clinic, or other visits to or from medical personnel. Routine scheduled study visits will not be considered medically attended visits.*

7.1.2 Solicited Adverse Event

A solicited AE is a protocol-specified AE about which the investigator (or designee) proactively asks the participants during a protocol-specified time period. A reactogenicity event may be considered as a solicited AE per discretion of the investigator (as described below in Section 7.1.2.1).

7.1.2.1 Reactogenicity

Reactogenicity (solicited systemic and local injection site reactions) will be assessed by the participants using e-diary cards. Information will be solicited on the following injection site reactions: pain, redness, and swelling. In addition, information will be solicited for AEs, on the following systemic reactions: temperature increases (oral) ≥38.0°C (≥100.4°F), chills, fatigue, headache, myalgia, arthralgia, and nausea.

If injection site or systemic reactions continue beyond seven days postvaccination, these will be recorded in the EDC as unsolicited AEs. For any type of reactogenicity persisting two weeks or more, the investigator (or designee) will evaluate the reaction at the next scheduled visit and/or determine based on the nature and severity if a more immediate unscheduled follow-up visit is required to fully assess the reaction.

7.1.3 Unsolicited Adverse Event

An unsolicited AE is an AE that is spontaneously reported by the participant or discovered by the investigator.

7.1.4 Adverse Event of Special Interest

Arthralgia will be included as an AESI. The occurrence of new onset or worsening arthralgia that is medically attended will be monitored throughout the study for all participants. Medically attended visits include hospital, emergency room, urgent care clinic, or other visits to or from medical personnel. Routine scheduled study visits will not be considered medically attended visits.

7.1.5 Serious Adverse Event

An SAE is any untoward medical occurrence that: results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions, or is a congenital anomaly or birth defect.

Important medical events which may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Notes:

- *Death is an outcome and not an event. The condition leading to death is the event. If no other information regarding the cause of death is available, the event will be considered "Unspecified Adverse Event".*
- *Overnight stay at hospital/clinic that occurs during a study for social reasons (eg, transportation difficulties, respite care) is not considered to be a hospitalization event.*

7.1.6 Suspected Unexpected Serious Adverse Reaction

Suspected Unexpected Serious Adverse Reaction (SUSAR) is the term used to refer to an AE that occurs in a clinical study participant, which is assessed by the sponsor and or study investigator as being unexpected, serious, and as having a reasonable possibility of a causal relationship (Section 7.1.8) with the IP.

7.1.7 Severity of Adverse Events

The investigator will grade all adverse events for severity. Adverse events listed in the grading scale in Appendix I will be graded according to the criteria in the table. Adverse events not listed in the grading scale will be graded as follows:

- **Mild (Grade 1):** No interference with activity
- **Moderate (Grade 2):** Some interference with activity
- **Severe (Grade 3):** Significant; prevents daily activity
- **Potentially Life-Threatening (Grade 4):** As determined by emergency room visit or hospitalization.

For the study grading scale see Appendix I.

7.1.8 Causality of Adverse Events

The investigator is responsible for the assessment of the causality of an AE and sponsor's MM will also assess SAE causality, independent of the investigator.

The following guidelines are provided for this assessment:

- **Unrelated:** No relationship between the IP and the reported event.
- **Possibly related:** The event follows a reasonable temporal sequence from the time of administration of IP and/or follows a known response pattern to the IP but could also have been produced by other factors.
- **Probably related:** A reasonable temporal sequence of the event with administration of IP exists and based on the known response to the IP, known or previously reported adverse reactions to the IP or similar products, or in the investigator's (or designee) clinical judgment the association of the event with the IP seems likely.

If the relationship between the AE and the IP is determined to be "possible" or "probable", the event will be considered to be "related" to the IP, otherwise it will be considered "not related" for reporting purposes.

7.2 Eliciting Adverse Events

Adverse events reported spontaneously by the participant in response to an open question from the principal investigator (or designee) or revealed by observation (eg, during physical exam) will be recorded by the principal investigator (or designee) on the AE eCRF. Study participants will be provided with a 24-hour telephone number to contact study personnel in case of an untoward reaction.

7.3 Reporting Requirements for Immediately Reportable Events

7.3.1 Principal Investigator's Reporting Requirements

The following events must be reported within 24 hours of awareness by the principal investigator (or designee) to sponsor's Global Pharmacovigilance Department (Global PV), through completion of the AE eCRF within the EDC system, and identifying the event as serious (SAE):

- Any SAE regardless of causal association with the IP.
- Any AESI regardless of causal association with the IP.
- Confirmed pregnancy of female participants.

Should the EDC system for reporting SAEs, AESI, and pregnancy be unavailable, the appropriate form(s) (listed below) will be completed and sent by email to the following address:

Global Pharmacovigilance Department

Emergent BioSolutions Inc.

[redacted]

The paper reported SAEs, AESI, and pregnancy should be entered in EDC once the system is available.

For SAE/AESI, the "Serious Adverse Event/Adverse Event of Special Interest Report Form" will be completed (abbreviated hereafter as "SAE/AESI Report Form"). The SAE/AESI Report Form is **not** the same as the AE eCRF. Supporting documentation that may be uploaded into the EDC to accompany the form(s) can include source documentation or medical records (eg, discharge summary for hospitalizations, lab reports) which support a diagnosis. Participant identifiers (eg, name, address, telephone number, social security number, medical record number, or hospital/laboratory number) must be redacted from the source documentation.

All SAEs that are unexpected (eg, adverse drug reactions) must be reported to the Institutional Review Board (IRB) by the investigator (or designee) as required by ICH GCP E6.

If a participant becomes pregnant during a study, the sponsor will be notified. All pregnancies where conception occurred after first exposure to the IP through the End of Study visit are to be followed to outcome (eg, delivery, spontaneous/elective/therapeutic abortion), including after the study is completed and even if the participant is withdrawn from the study. If a pregnancy results in an abnormal outcome that the reporting health care professional considers might be due to the IP, then the guidelines for expedited reporting of SUSAR should be followed (see Section 7.3.2).

Any pregnancy that occurs during study participation must be reported using the clinical study pregnancy forms ("Pregnancy Notification Form" and "Pregnancy Follow-up Form"). To ensure participant safety, each pregnancy must be reported to the sponsor within 24 hours of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.

Any SAE occurring in association with a pregnancy brought to the investigator's attention during or after the participant has completed the study and considered by the investigator as possibly related to the study treatment, must be promptly reported to the sponsor.

The investigator is responsible to notify their IRB according to their policy.

7.3.2 Sponsor's Reporting Requirements

A SUSAR is a suspected adverse reaction that is both serious and unexpected (Section 7.1.6). As specified in 21 Code of Federal Regulations (CFR) 312.32, SUSARs will be reported by the sponsor of the Investigational New Drug Application (IND) to the FDA and to all participating principal investigators in an IND safety report as soon as possible, no later than 15 calendar days after the sponsor becomes aware of the suspected adverse reaction (21 CFR 312.32(c)(1)).

In addition, any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than seven calendar days after the sponsor's initial receipt of the information (21 CFR 312.32(c)(2)).

7.4 Reporting of Other Information - Unanticipated Problems

As outlined by the Office for Human Research Protection, unanticipated problems must be reported to the IRB according to the requirements of 45 CFR Part 46. Unanticipated

problems are considered to include any incident, experience, or outcome that meets **ALL** the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  - Procedures that are described in the study-related documents, such as the IRB approved research protocol and informed consent document.
  - The characteristics of the participant population being entered into the study.
- Related or possibly related to participation in the study which means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the sample collection.
- Suggests that the study places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

An incidence, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in the study or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of participants or others. Only a small subset of AEs occurring in human participants participating in a clinical study will meet these three criteria for an unanticipated problem. There are other types of incidents, experiences, and outcomes that occur during the conduct of a clinical study that represent unanticipated problems but are not considered AEs. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with AEs. In other cases, unanticipated problems place participants or others at increased risk of harm, but no harm occurs.

The investigator should promptly notify the IRB when an unanticipated problem involving risks to participants or others is identified. Also, the investigator should notify the sponsor of unanticipated problem(s).

7.5 Follow-up of Adverse Events

All SAEs/AESI/MAAEs/AEs will be followed until resolution, stabilization, or up to 30 days after the last study visit.

7.5.1 Follow-up of Nonserious Adverse Events

Nonserious AEs that are identified during the 28-day postvaccination period and still ongoing on the last scheduled visit must be recorded on the AE eCRF with the current status noted. All nonserious events that are ongoing at this time will be recorded as “Not Recovered/Not Resolved” on the AE eCRF. The status of ongoing, previously reported AEs will be subject to active follow-up.

7.5.2 Follow-up of Serious Adverse Events or Adverse Events of Special Interest

This study requires that participants be monitored for SAE/AESI/MAAE up to Day 183 End of Study Visit. From Day 1 (Visit 2), confirmed SAEs, confirmed AESI, and confirmed MAAEs will be recorded on the AE eCRF.

The investigator will provide or arrange appropriate care for participants for whom SAEs, potential AESI or MAAEs are reported. Withdrawal/discontinuation of participants from the study to treat SAEs/AESI/MAAEs are at the discretion of the investigator.

All SAEs/AESI/MAAEs will be followed by the principal investigator (or designee) until one or the other condition is met:

- The event is resolved or stable if condition is expected to remain chronic.
- The participant is referred to a specialist or other physician for treatment and follow-up. The principal investigator (or designee) will follow the participant's condition even if the participant is seen by another physician, to obtain information about the diagnosis and outcome and any treatments and medications administered for the event.

The following will be considered acceptable reasons for discontinuation of follow-up of ongoing SAEs:

- Participant withdraws consent.
- Participant is referred to appropriate long-term medical care.
- Participant is considered lost to follow-up.

It is expected that the clinical site will obtain supporting medical records from appropriate physicians and record and/or upload this information on the SAE Report Form within the AE eCRF. Additional information received related to any SAE must be added to the SAE Report Form within the AE eCRF or forwarded to the Emergent Global PV Department within 24 hours of awareness (Section 7.3.1).

7.6 Safety Data Monitoring

An independent SMC will be constituted under a separate charter. The SMC will review aggregated, blinded safety data after the 300th enrolled participant passes Day 29, and again after the last enrolled participant passes Day 29.

Safety data in this study will also be reviewed on an ongoing basis by the sponsor’s MM as outlined in the study Medical Monitoring Plan.

7.7 Breaking the Blind for Individual Participants

The blind may be broken if the health or safety of a participant is at risk and knowledge of the study arm may be beneficial to the medical management of the participant. The

pharmacist, investigator or other authorized individual may receive the allocation of an individual participant in the event that unblinding is required. In such an event, the sponsor must be notified.

If the investigator determines that knowledge of a participant's treatment assignment is urgently needed to guide treatment or ensure the participant's safety, the investigator may perform emergency unblinding via the RTSM system. The investigator may not delegate this responsibility. The investigator must attempt to notify the sponsor's MM prior to unblinding and must notify the MM within 24 hours after unblinding at the latest.

If a participant's study treatment assignment is unblinded for safety reasons, or if a participant becomes accidentally unblinded for any reason, the participant will be requested to remain in the study for safety follow-up.

The procedures to be followed in the event of an emergency unblinded for a medical emergency are outlined in the study pharmacy manual.

8 STATISTICAL CONSIDERATIONS

8.1 Sample Size Rationale

8.1.1 Sample Size and Power for Immunogenicity

The primary statistical comparison between the PXVX0317 and placebo treatment groups is based on the difference between treatment groups in seroresponse rate (proportion of participants with anti-CHIKV SNA NT_{80} [redacted] as measured by the human SNA assay) at Day 22, which will be tested using a chi-square test with a two-sided alpha=0.05 in the IEP across all age groups combined. The analysis will be repeated for the mITT as a measure of the robustness of the result.

Based on the data from the phase 2 study (protocol PXVX-CV-317-001) (16, 28), the seroresponse rate for PXVX0317 vaccine is expected to be approximately [redacted] vs <5% for the placebo participants at Day 22. With an assumed 10% rate of nonevaluable participants, the power to show superiority over placebo with 2430 PXVX0317 vaccine and 405 placebo-evaluable participants is >99.9% for the combined ages groups. The power is 99% for each of the smaller 12 to <18 and 46 to <65 age groups with samples sizes of 189 vs 32 evaluable participants (PXVX0317: placebo).

The difference in seroresponse rate between PXVX0317 and placebo groups that is considered clinically relevant is [redacted] With 2430 PXVX0317-treated participants and a target seroresponse rate of [redacted] vs a rate of 0% for placebo, the width of a two-sided 95% CI would be ±1.2%. The difference in seroresponse rate must be above [redacted] for the lower bound of the 95% CI for the difference to be [redacted].

8.1.2 Sample Size and Power for Lot Consistency

The second consideration for sizing the study is ensuring adequate power for the lot consistency equivalence intervals testing immunogenicity at Day 22, as measured by human SNA assay anti-CHIKV SNA GMT ratios between all three pairs of consecutively manufactured PXVX0317 lots (A:B, A:C, B:C) in the 18 to <46 years of age stratum for the IEP. Pairwise testing of lots will be performed without multiplicity adjustment, and for each GMT ratio the two-sided 95% confidence interval must fall within the equivalence interval of [0.667, 1.5] for the lots to be considered equivalent.

On the $\log_{10}$ scale, two one-sided equivalence tests have 95% power to reject the null hypothesis that the difference in mean titers between lots is below -0.176 or above 0.176 in favor of an alternative hypothesis of equivalence when the expected difference in $\log_{10}$ means is 0, the common SD is 0.455, each test is at the 2.5% level and the sample size in each lot is 207. The SD estimate was based on the upper bound of an 80% CI on the SD on the $\log_{10}$ scale observed in the human SNA assay on samples collected 4 weeks after participants were treated with a single dose of PXVX0317. To be conservative, at least 1050 evaluable participants (300 participants in each lot along with 150 placebo participants) need to be enrolled into the 18 to <46 age group to provide adequate power for the lot consistency objective.

8.1.3 Sample Size and Power for Adverse Events

The final consideration for the sample size of the study is the size of the prelicensure safety database. With 2700 participants receiving any lot of PXVX0317, this study is 93.3% likely to detect at least one AE with a true frequency of ≥0.1% (ie, “uncommon”).

8.2 Analysis Populations

Analysis will be based on the following study populations:

Randomized Population: All screened participants who provide informed consent and provide demographic and other screening measurements and are randomized.

Exposed Population: All participants in the randomized population who receive IP.

Safety Population: All participants 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. Participants will be analyzed as treated (ie, according to the treatment a participant received, rather than the treatment to which the participant may have been randomized).

Modified Intent-to-Treat Population: All randomized participants who are vaccinated and have at least one post-injection anti-CHIKV SNA NT_{80} result. Participants are counted in the group to which they were randomized.

Immunogenicity Evaluable Population:

The IEP includes all participants in the mITT population who:

- Provide evaluable serum sample results for the relevant postvaccination time points, and within the required time frames:
  - Day 22: Day 19 through Day 27, inclusive.
- Have no measurable anti-CHIKV SNA at Day 1 (baseline).
- Have no major protocol deviation or other reason to be excluded as defined prior to unblinding.

Tables will be displayed by treatment group (PXVX0317 and placebo) columns with lots pooled with the exception of the lot consistency analysis, where the separate lots will be displayed.

8.3 Study Endpoints

8.3.1 Primary Endpoints

Safety Endpoints:

- Incidence of solicited AEs through Day 8 for PXVX0317 and placebo.
- Incidence of unsolicited AEs through Day 29 for PXVX0317 and placebo.
- Incidence of AESI, MAAEs and SAEs through Day 183 for PXVX0317 and placebo.

Coprimary Immunogenicity Endpoints:

- Difference in anti-CHIKV SNA seroresponse rate (PXVX0317 minus placebo) and associated 95% CI at Day 22.

  Success criterion: Lower bound of the two-sided 95% CI on the difference in seroresponse rates between PXVX0317 and placebo groups [redacted] (equivalent to a difference >0 using a two-sided chi-square test).

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA titer [redacted] See Section 6.1 for assay description. See Section 8.6 for immunogenicity analysis details and Section 8.6.2.5 for success criteria and multiplicity controls.

- Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for PXVX0317 and placebo.

  Success criterion: Significant difference in the IEP across all age groups combined using an ANOVA model with logarithmically transformed anti-CHIKV SNA titers (log_{10}) as the dependent variable and treatment group and study site as the fixed effects with a two-sided significance level of 0.05.

- Anti-CHIKV SNA GMT ratio between all three pairs of PXVX0317 lots (A:B, A:C, B:C) (adults 18 to <46 years of age) at Day 22.

  Success criterion: Pairwise GMT ratios (A:B, A:C, B:C) each with a two-sided 95% CI within [0.667; 1.5] for the IEP in the 18 to <46 years of years of age stratum using an ANOVA model with log_{10}-transformed anti-CHIKV SNA titers as the dependent variable and vaccine lot and study site as the fixed effects to derive the GMT ratios.

8.3.2 Secondary Endpoints

- **Key secondary endpoints**: Difference in anti-CHIKV SNA seroresponse rate (PXVX0317 minus placebo) with associated 95% CI at Day 15, Day 183, and Day 8, in that order (see Section 8.6.2.5).

  Success criterion: At Day 15 only, lower bound of the two-sided 95% CI on the difference in seroresponse rates between PXVX0317 and placebo groups [redacted] (equivalent to a difference >0 using a two-sided chi-square test).
- Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at Day 8, Day 15, and Day 183.
- Geometric mean fold increase from Day 1 to subsequent collection time points.
- Number and percentage of participants with an anti-CHIKV SNA titer ≥15 and 4-fold rise over baseline.

8.3.3 Exploratory Endpoint

- Anti-CHIKV SNA seroresponse rate difference with associated 95% CIs for each pair of PXVX0317 lots (A minus B, A minus C, B minus C) (adults 18 to <46 years of age) at Day 22.

8.4 Handling Missing Data

Participants with missing immunogenicity data at Days 8, 15, 22, or 183 will be excluded from the corresponding analysis; missing immunogenicity data will not be imputed. Human SNA assay values below the lower limit of quantitation (LLOQ) will be replaced by LLOQ/2 in the immunogenicity analyses. Imputation rules for partial or missing dates will be described in the study statistical analysis plan (SAP).

8.5 Analysis of Disposition, Demographic and Baseline Characteristics

Disposition will be summarized for all randomized participants. Demographic and baseline characteristics including age, sex, race, ethnicity, BMI, will be tabulated by treatment group for the randomized, safety, mITT, and IEP populations. Continuous endpoints will be

summarized by descriptive statistics; categorical endpoints will be summarized by the number of participants, frequency counts, and percentages.

Medical history will be coded to SOC and PT using the MedDRA dictionary and summarized by treatment group for the randomized population. Protocol deviations will be categorized as important or not important and evaluated for exclusion of data from analyses. They will be presented by treatment group for the randomized population.

8.6 Immunogenicity Analysis

Summary statistics for immunogenicity results by scheduled visit for each treatment group will be provided for the IEP population, unless otherwise specified. In the analysis of the human SNA assay data as a continuous variable, anti-CHIKV SNA NT_{80} values will be logarithmically transformed (base10), and the GMTs and associated 95% CI for each treatment group will be computed by exponentiating the corresponding log-transformed means and two-sided 95% CIs. Results will be presented for all age groups combined as well as separately. Geometric mean fold increase will also be displayed by postvaccination scheduled visit for each treatment group.

Proportions of participants with human SNA assay titer [redacted] and secondary response rates at other titers (eg, 15 and 4-fold rise over baseline) will be reported with associated two-sided 95% Wilson method CIs by scheduled visit for each treatment group.

Reverse cumulative distribution plots of human SNA assay vs proportion of participants in each treatment group will be generated by scheduled visit. Geometric mean titer will also be plotted over time for each treatment group.

8.6.1 Coprimary Immunogenicity Analysis

The familywise error rate will be fixed at a two-sided alpha of 0.05 by the requirement that all coprimary endpoint hypotheses must be met for a successful outcome: Day 22 seroresponse (titer of [redacted] Day 22 GMT, and lot consistency pairwise GMT ratios, each discussed below (see Section 8.6.2.5). Note that success on the Day 22 lot consistency endpoint is only required for FDA.

8.6.1.1 Day 22 Seroresponse Rate Analysis

The superiority of the immune response to PXVX0317 vaccine over that to placebo will be demonstrated at Day 22 by comparing seroresponse rates between the two treatment groups (the proportion of participants with a human SNA assay [redacted] considered the presumptive seroprotection rate). The difference in seroresponse rates between the PXVX0317 and placebo groups will be calculated, along with the 95% CI for the difference based on the Newcombe hybrid score method. The lower bound of the two-sided 95% CI on the difference in seroresponse rates between PXVX0317 and placebo groups must be [redacted]. Additionally, the null hypothesis of no difference between seroresponse rate proportions will be tested

using a chi-square test with alpha=0.05. No multiplicity adjustment will be employed (see Section 8.6.2.5), and no covariate adjustment will be performed.

The primary comparison will be in the IEP across all age groups combined. Tests will be repeated in the mITT population as a measure of robustness, along with tests for each population in the separate age strata. No multiplicity adjustment will be made for the analysis of the separate age strata as the primary population will be the combined age groups.

8.6.1.2 Day 22 Geometric Mean Titer Analysis

In addition, Day 22 GMTs will be compared between PXVX0317 and placebo treatment groups and will be analyzed via a linear model based on an alpha=0.05. The primary model is an ANOVA, with logarithmically transformed anti-CHIKV SNA titers (log_{10}) as the dependent variable and treatment group and study site as the fixed effects in the model. The adjusted least square means and their 95% CIs calculated based on the ANOVA will be back transformed and reported as the group GMT values. All tests will be carried out at a two-sided significance level of 0.05 and no adjustment for multiplicity will be applied (see Section 8.6.2.5).

The primary comparison will be in the IEP across all age groups combined. Tests will be repeated in the mITT population as a measure of robustness, along with tests for each population in the separate age strata. No multiplicity adjustment will be made for the analysis of the separate age strata as the primary population will be the combined age groups.

8.6.1.3 Day 22 Lot Consistency Analysis

The consistency of the immune response across three consecutively manufactured lots of PXVX0317 vaccine will be confirmed at Day 22. Immunogenicity as measured by human SNA assay anti-CHIKV SNA GMTs will be compared using ratios of GMTs between all three pairs of PXVX0317 lots (A:B, A:C, B:C) for the IEP in the 18 to <46 years of age stratum, and the analysis will be repeated for the mITT population as a measure of the robustness of the results.

The GMTs associated with the primary lot consistency objective will also be calculated via a linear model for the anti-CHIKV SNA titers collected at Day 22. The primary model is an ANOVA, with log_{10}-transformed anti-CHIKV SNA titers as the dependent variable and vaccine lot and study site as the fixed effects in the model. The adjusted least square means and their 95% CIs calculated based on the ANOVA will be back transformed and reported as the vaccine lot GMT values. The ratio of GMTs for the comparison of the three pairs of PXVX0317 lots (A:B, B:C, A:C) will be derived from this model.

All tests will be carried out at a two-sided significance level of 0.05 and no adjustment for multiplicity will be applied (see Section 8.6.2.5). If for all pairs of vaccine lots, the two-sided 95% CI of the GMT ratio is within [0.667; 1.5] then lot-to-lot consistency will be demonstrated.

The seroresponse rates for each vaccine lot at Day 22 also will be assessed, but as an exploratory endpoint, as outlined in Section 8.3.3.

8.6.2 Secondary Immunogenicity Analysis

8.6.2.1 Key Secondary Immunogenicity Analyses: Seroresponse Rate and Seroresponse Rate Difference at Days 15, 183, and 8, in that Order

Seroresponse rates and seroresponse rate differences (PXVX0317 minus placebo) with associated 95% CIs based on antibody titers measured at Days 15, 183, and 8 will be analyzed as described above for Day 22 (see Section 8.6.2.5 for multiplicity controls). For each time point, the null hypothesis of no difference between seroresponse rate proportions in the PXVX0317 vs placebo group will be tested using a chi-square test with alpha=0.05. For Day 15 only, the lower bound of the two-sided 95% CI on the difference in seroresponse rates between PXVX0317 and placebo will be compared to [redacted] for assessment of clinical significance.

8.6.2.2 Geometric Mean Titer at Days 8, 15, and 183

For the comparison of PXVX0317 to placebo, GMTs based on antibody titers measured at Days 8, 15, and 183 will be analyzed as described above for Day 22. Geometric mean fold increases for increase over Day 1 titer will be analysed as described for GMTs for each postvaccination time point.

8.6.2.3 Seroconversion at Other Titers

As described above, secondary response rates at other titers (eg, 15 and 4-fold rise over baseline) will be reported with associated two-sided 95% Wilson method CIs by scheduled visit for each treatment group.

8.6.2.4 Immunogenicity Analysis in Adolescents and Older Adults

As noted above, the immunogenicity analyses will include separate evaluations of seroresponse rate and GMT for each age group in addition to the overall study participant population.

8.6.2.5 Multiplicity Controls

8.6.2.5.1 Coprimary Immunogenicity Endpoints

The familywise error rate will be fixed at a two-sided alpha of 0.05 by the requirement that all coprimary immunogenicity endpoints must be met for a successful outcome:

1. Day 22 seroresponse in the IEP across all age groups combined, including superiority to placebo and the success criterion of the lower bound of the two-sided Newcombe

hybrid score method 95% CI on the difference in seroresponse rates between PXVX0317 and placebo [redacted].

2. Day 22 GMTs significantly different between PXVX0317 and placebo in the IEP across all age groups combined using an ANOVA model with logarithmically transformed anti-CHIKV SNA titers (log_{10}) as the dependent variable and treatment group and study site as the fixed effects with a two-sided significance level of 0.05.
3. Day 22 lot consistency pairwise GMT ratios (A:B, A:C, B:C) each with a two-sided 95% CI within [0.667; 1.5] for the IEP in the 18 to <46 years of years of age stratum using an ANOVA model with log_{10}-transformed anti-CHIKV SNA titers as the dependent variable and vaccine lot and study site as the fixed effects to derive the GMT ratios. Note that success on the Day 22 lot consistency endpoint is only required for FDA.

8.6.2.5.2 Key Secondary Immunogenicity Endpoints

A prespecified hierarchical approach will be employed for the key secondary immunogenicity endpoint hypothesis testing to preserve the type I error rate without the need for further multiplicity adjustment. If the null hypotheses are rejected for all coprimary endpoints, only then will the key secondary endpoints be formally tested in sequential order. If a nonsignificant test is reached formal testing will stop, and the remaining endpoints will be reported for information only. The seroresponse rates and rate differences at Days 15, 183, and 8 will be tested sequentially in order, each for the IEP across all age groups combined, as follows:

1. Day 15 seroresponse superior to placebo and the success criterion of the lower bound of the two-sided Newcombe hybrid score method 95% CI on the difference in seroresponse rates between PXVX0317 and placebo [redacted].
2. Day 183 seroresponse superior to placebo.
3. Day 8 seroresponse superior to placebo.

After testing the coprimary immunogenicity endpoints and, if all met, the hierarchical key secondary immunogenicity endpoints, no other formal hypothesis testing will be carried out. The remaining secondary immunogenicity endpoints including GMTs, GMFI, other titers, the exploratory endpoint, and all safety endpoints will be evaluated and reported for information only, thus no further multiplicity adjustment is needed.

8.7 Safety Analysis

The safety of PXVX0317 in healthy adult and adolescent participants 12 to <65 years of age will be evaluated using solicited AEs occurring from IP administration on Day 1 until Day 8 and unsolicited AEs through Day 29, AESI, MAAEs, and SAEs though Day 183 End of Study Visit, and vital signs. Solicited AEs include local (i.e., pain, redness, and swelling) and systemic reactions (i.e., fever, chills, fatigue, headache, myalgia, arthralgia, and nausea).

8.7.1 Exposure

The frequencies and percentages of participants treated will be summarized by treatment group for the randomized population.

All safety analyses will be based on the safety population.

8.7.2 Adverse Events

Adverse events will be coded to SOC and PT using the MedDRA dictionary. Solicited AEs, unsolicited AEs, AESI, MAAEs, and SAEs will be summarized separately by treatment group and maximum severity for the safety population for all age strata combined as well as for separate age groups.

8.7.2.1 Solicited Adverse Events

With the exception of redness and swelling, all solicited AEs will be summarized according to severity grading scales defined in Section 7.1.7 from "mild" to "potentially life-threatening."

Solicited AEs will be recorded daily until 7 days post-injection (Day 8) using an e-diary. The analyses of solicited AEs will be performed by maximum severity and by treatment group. In addition, solicited AEs ongoing after seven days post-injection (Day 8) will also be recorded as unsolicited AEs.

Frequencies and percentages of participants experiencing each solicited AE will be presented by maximum severity. Summary tables showing the occurrence of any local or any 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 = 25-50 mm
- Grade 2/moderate = 51-100 mm
- Grade 3/severe = >100 mm
- Grade 4/potentially life threatening = necrosis or exfoliative dermatitis

Refer to Appendix I.

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

The following summaries of solicited events will be performed:

1. Solicited events by day post-injection for each event and for any event.
2. Time of first onset of solicited AEs, for each event and any event.

3. Solicited AEs by maximum event severity, for each event and for any event.
4. Duration of solicited AEs, for each event and any event.

Solicited AEs, occurrence of at least one event by category (local, systemic), will also be included.

Only participants with at least one observation (i.e., any nonmissing values but excluding "Not done/unknown") for the solicited AEs will be summarized.

8.7.2.2 Unsolicited Adverse Events

All the unsolicited AEs occurring during the proscribed collection period in 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 PT using the MedDRA dictionary. The unsolicited AEs will then be grouped by MedDRA PT into frequency tables according to SOC. All reported AEs, as well as AEs judged by the investigator as at least possibly related to IP, will be summarized by treatment group, according to SOC and PT. When an unsolicited AE occurs more than once for a participant, 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 participant has given informed consent, but before vaccination).

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

- Any unsolicited AE
- Related ("possibly" or "probably" related) unsolicited AEs
- Serious adverse events
- Related ("possibly" or "probably" related) SAEs
- Adverse events of special interest
- Medically-attended AEs
- Related MAAEs
- Unsolicited AEs leading to withdrawal
- Any AE leading to death

Listings of all AEs will be provided by participant.

Combined solicited and unsolicited AEs: Solicited AEs continuing beyond 7 days after vaccination will be coded by MedDRA and combined with the unsolicited AEs. A summary of participants with all combined solicited and unsolicited AEs, by SOC and PT, will also be provided.

8.7.3 Clinical Laboratory Data

No clinical safety laboratory data will be collected in this study.

8.7.4 Physical Examinations

Physical examinations by body system include a complete examination at screening and directed examinations at Days 1, 8, 15, 22, and 183. Physical examination data will be listed.

8.7.5 Vital Signs

Vital signs including temperature, blood pressure, respiratory rate, and heart rate at screening and pre- and postvaccination on Day 1 will be summarized by treatment group.

8.7.6 Prior and Concomitant Medications

Prior and concomitant medications will be coded to preferred drug name using the World Health Organization DRUG dictionary and summarized by treatment group for the safety population.

8.7.7 Other Safety Variables

None.

8.7.8 Subgroup Analysis

Except for lot consistency analyses, PXVX0317 lots will be pooled as the PXVX0317 treatment group. Summaries will pool all age groups and will be repeated separately for each age group. Analyses of the primary immunogenicity and safety endpoints will also be summarized by sex, race, and ethnicity along with treatment group.

8.7.9 Interim Analysis

No formal interim analysis is planned. The SMC (Section 7.6) will review aggregated, blinded safety data after the 300th enrolled participant passes Day 29, and again after the last enrolled participant passes Day 29.

9 DATA HANDLING AND RECORD KEEPING

9.1 Source Documents and Access

Source data are all information, original records of clinical findings, and observations in a clinical study necessary for the reconstruction and evaluation of the study. The source documentation requirements described below apply to all source documentation and study

records in any form, including those maintained in the institution's electronic health record system, if applicable.

The investigator/institution will maintain adequate and accurate source documents and study records that include all pertinent information related to participants' participation in the study, including details but not limited to signed and dated notes on consenting, eligibility, medical history, study assessments, IP administration, AEs, concomitant medications, participant follow-up information and other relevant observations.

Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry and should be explained if necessary (eg, via an audit trail).

The investigator/institution shall permit study-related monitoring, audits, IRB review, and regulatory inspection(s), providing direct access to source data/documents.

Records from the study that identify the participant will be confidential except that they may be given to and inspected by sponsor (or designee), the IRB, the FDA, EMA, and other government agencies as appropriate and will not otherwise be released except as required by law. All information provided to the investigator by the sponsor is to be considered confidential unless otherwise stated.

9.2 Data Management

A validated, EDC system will be used during the study. Data management activities to be performed for the study will be described in detail in the Data Management Plan (DMP) documents.

9.3 Data Collection and Discrepancy Management

Data collected during the study will be recorded in the eCRFs designed for this study. Investigational sites will have the responsibility for capturing and maintaining accurate eCRF data, records, and relevant source documentation, as well as conforming to procedures established by the sponsor around system access/security and ensuring a data audit/edit trail for data corrections. All source documents will be verified by the study monitor for accuracy. Information from external sources such as laboratory data, images, etc. as defined in this protocol will be collected and maintained outside the EDC and reconciled with the eCRF data periodically (as applicable). As data are entered into the eCRF, automated edit checks will validate data. Additionally, manual reviews will be performed for data discrepancy by the monitor and queries will be generated into the EDC system. After clinical sites respond to queries and data corrections are made and reviewed by the monitor, the investigator will review and electronically sign the eCRF for each participant. The sponsor will review data for accuracy, completeness, and consistency during the conduct of the study and prior to database lock.

For further information on eCRFs, refer to the CRF Completion Guidelines. Details on data handling will be described in the DMP.

9.4 Laboratory Data

No external laboratory data transfers will be done. Sites will manually enter results for HBsAg/HCV antibody/HIV-1/HIV-2, HCV RNA (when applicable), and pregnancy testing into applicable eCRFs. Anti-CHIKV SNA titer results will be handled by the sponsor.

9.5 File Management at the Investigational Site

The investigator will ensure that the study site file is maintained in accordance with the ICH GCP guidelines and as required by applicable local regulations. The investigator/institution will take measures to prevent accidental or premature destruction of these documents.

9.6 Records Retention at the Investigational Site

Per ICH GCP guidelines, study documents will be retained for one of the following periods:

- A period of at least two years after the date of the last approval of a marketing application in an ICH region until there are no pending or contemplated marketing applications.
- A period of at least two years after the sponsor has notified the regulatory authority(ies) that clinical investigation with this product is discontinued.

The investigator must not dispose of any records relevant to this study without either (1) written permission from the sponsor or (2) provision of an opportunity for the sponsor to collect such records. The investigator will be responsible to maintain adequate and accurate electronic or hard copy source documents of all observations and data generated during this study, including any data queries received from the sponsor (or designee). Such documentation is subject to inspection by the sponsor (or designee) and relevant regulatory agencies. If the investigator withdraws from the study (eg, due to relocation or retirement), all study-related records will be transferred to a mutually agreed upon designee within a sponsor-specified timeframe. Notice of such transfer will be given to the sponsor in writing.

9.7 Deviations from the Protocol

The principal investigator agrees to conduct the clinical study in compliance with the protocol agreed to by the sponsor and approved by the site's IRB.

A protocol deviation is defined as a site personnel or a study participant's departure from the protocol requirements, whether inadvertent or planned.

The investigator or site staff may not deviate from the protocol, except, in rare circumstances, as necessary to eliminate immediate hazards to study participants. In such event, both the sponsor and IRB will be immediately notified.

The occurrence of protocol deviations will be routinely monitored for, by evaluation of investigator compliance with the protocol, GCP, and regulatory requirements. The sponsor will review all protocol deviations on an ongoing basis and will be responsible for determining if the deviation should be categorized as an important protocol deviation. Important protocol deviations may require additional documentation as requested by the sponsor.

Continued protocol deviations despite re-education of investigational site personnel, or persistent protocol deviations that are reportable to regulatory agencies may result in discontinued shipment of IP and termination of further enrollment at the investigational site, or termination of the investigational site from the study.

10 QUALITY CONTROL AND ASSURANCE

10.1 Monitoring

The assigned clinical study monitor will verify eCRFs entries 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 ICH GCP guidelines and local and federal regulations applicable to the conduct of the clinical study. The investigator must make source documentation accessible to the study monitor as needed to verify the information in the eCRFs. The investigator agrees to meet with the study monitor at regular intervals to discuss study progress and ensure that any problems detected in the course of data monitoring are resolved appropriately.

10.2 Auditing

The sponsor's Quality Assurance Department (or designee) may conduct investigational site audits before study initiation, during the study, or after study completion, as documented in the Clinical Quality Oversight Plan. Audits will include, but are not limited to, review of drug supply, presence of required documents, informed consent process, and comparison of eCRFs with source documents. The investigator agrees to participate in site audits and assist in the prompt resolution of any issues found during audits.

Regulatory authorities or the IRB may inspect the investigational site during or after the study. The investigator will cooperate with such inspections and will contact the sponsor immediately if such an inspection occurs.

In the event the investigator is contacted by a regulatory agency in relation to this study, the investigator and investigational site staff must be available to respond to reasonable requests and inspection queries made during the inspection process. The investigator must provide the

sponsor with copies of all correspondence that may affect the review of the current study (eg, Form FDA 483, inspectional observations, warning letters). The sponsor will provide any needed assistance in responding to regulatory inspections.

11 ETHICS AND RESPONSIBILITY

This study must be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and in compliance with the protocol, current ICH GCP guidelines, national regulatory authorities, and all other applicable local laws and regulatory requirements. Each investigational site will seek approval by an IRB according to regional requirements. The IRB will evaluate the ethical, scientific, and medical appropriateness of the study. Further, in collecting and handling participant data and completing eCRFs, the investigator and investigational site staff will take measures to ensure adequate care in protecting participant privacy. To this end, a participant identification number will be used to identify each participant.

11.1 Informed Consent/Assent

The investigator must obtain written informed consent from study participants prior to starting any study-related activities. For adolescent participants 12 to <18 years of age, the investigator must obtain both written informed consent from study participant's parent or legal guardian and assent from the adolescent participant prior to starting any study-related activities. Emancipated or mature minors (defined by local laws) may be capable of giving autonomous consent. Adolescents reaching 18 years of age during the study must provide written informed consent as adults at the next study visit. All prospective participants (and/or legal guardian or legally authorized representative) must sign and date an IRB-approved ICF or assent form.

11.2 Institutional Review Board

Before the start of the study, the Investigator's Brochure (IB), the protocol, proposed informed consent form(s), participant compensation (if any), sponsor-approved study materials and advertisements, and any other written information to be provided to the participant, will be submitted to a properly constituted IRB for review. The sponsor must receive a copy of the written approval from the IRB for all of the above applicable documents prior to recruitment of participants into the study and shipment of IP.

The IRB must provide written approval for all amendments to any of the above documents prior to implementation of these amendments at the investigational site.

The names (or title, if IRB procedures prohibit publishing of names) and associated backgrounds of the members of IRB (to assist in assuring that the board membership is properly constituted and operates according to 21 CFR part 56) will be given to the sponsor prior to the start of the study along with a signed and dated statement stating that the protocol

and ICF and, where applicable, any other document listed above, have been approved by them.

All correspondence between the investigator and the IRB will be available for review by the sponsor (or designee), and the applicable regulatory authority(ies).

11.3 Compensation for Injury

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

11.4 Participant Confidentiality

The investigator must ensure the anonymity of each participant is maintained at all times. Participants should only be identified by their participant study ID number on the CRF or on any other study documents provided to the sponsor (or designee). Biospecimens should only be identified by sample numbers/codes as specified in the Laboratory Manual. Any documents that identify the participant should be kept in strict confidence by the study site.

Based on ICH GCP guidelines and regulatory requirements, the principal investigator is required to allow authorized personnel of the sponsor (or designee), the IRB, and members of the appropriate regulatory authority(ies) to review participant's files that are related to EBSI-CV-317-004. Participants must be informed that their records may be reviewed by the sponsor (or designee), the IRB, and the appropriate regulatory authority(ies) through direct access to the participant's original medical records.

11.5 Future Use of Stored Samples

Any remnant (leftover) blood samples collected for the anti-CHIKV SNA analysis will be retained for future testing. Specimens will be identified by sample numbers/codes, thereby maintaining blinding while in storage. Participants will be asked to consent to the future use of these samples as part of the informed consent process.

Samples may be retained for at least two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least two years have elapsed since the formal discontinuation of clinical development.

12 ADMINISTRATIVE AND LEGAL REQUIREMENTS

12.1 Sponsorship

This clinical study is sponsored by Emergent Travel Health Inc. [redacted]. The manufacturer of PXVX0317 is Emergent BioSolutions Inc.

12.2 Protocol Amendments

Protocol amendments will only be made by the sponsor. In general, any change to the protocol must be made in the form of a formal amendment to the protocol and must be approved in writing by the principal investigator, the sponsor, and the IRB prior to implementation. The investigator must receive written IRB approval for all protocol amendments prior to implementing protocol amendments at the study site. The investigator must send a copy of any IRB correspondence and all approval/disapproval letters from the IRB to the sponsor.

A protocol change intended to eliminate an apparent immediate hazard to participants will be implemented immediately, followed by IRB notification within five working days.

The sponsor will submit protocol amendments to the applicable regulatory authority(ies).

12.3 Clinical Study Registration

For purposes of clinical study registration including reporting to ClinicalTrials.gov, the sponsor is the responsible party and will provide information regarding this study in accordance with applicable regulations.

12.4 Publication Policy

Following the completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the sponsor will be responsible for these activities and may work with the investigator(s) to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted and other related issues. The sponsor has final approval authority over all such issues.

Any proposed publication will be subject to review conditions and timelines agreed between the sponsor and the site investigator and detailed in the agreements with these parties prior to the start of the study. The sponsor will also post the results of the clinical study on ClinicalTrial.gov in a period no greater than 12 months from the completion of the study, defined as the time the final participant was examined or received an intervention for purposes of final collection of data for the primary outcome.

Data are the property of the sponsor and cannot be published without prior authorization from the sponsor, but data and publication thereof will not be unduly withheld.

All publication rights are delineated in the clinical trial agreement.

12.5 Terminating the Study

The sponsor and/or the principal investigator may elect to terminate the study early as defined by the clinical trial agreement. The sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Any decision to voluntarily suspend or terminate a clinical study will be carefully reviewed and fully justified. The sponsor will notify the FDA and the IRB of any suspension or termination and provide the justification. The principal investigator must notify the IRB in writing of the study's completion or early termination. The sponsor must receive a copy of the notification letter from the IRB indicating receipt of the completion or early termination letter.

12.6 Terminating the Study at an Individual Site

A study site may be terminated from the study at the discretion of the principal investigator, the sponsor, or IRB. The sponsor may decide to replace a terminated site.

13 REFERENCES

1. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond). 1956;54(2):177-91.
2. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol. 2007;88(Pt 9):2363-77.
3. CDC. Geographic Distribution: countries and territories where chikungunya cases have been reported (as of October 30, 2020) 2020 [Available from: https://www.cdc.gov/chikungunya/geo/.
4. Contopoulos-Ioannidis D, Newman-Lindsay S, Chow C, LaBeaud AD. Mother-to-child transmission of Chikungunya virus: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12(6):e0006510.
5. Borgherini G, Poubeau P, Staikowsky F, Lory M, Le Moullec N, Becquart JP, et al. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. Clin Infect Dis. 2007;44(11):1401-7.
6. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis. 2007;7(5):319-27.

7. Taubitz W, Cramer JP, Kapaun A, Pfeffer M, Drosten C, Dobler G, et al. Chikungunya fever in travelers: clinical presentation and course. Clin Infect Dis. 2007;45(1):e1-4.
8. Schilte C, Staikowsky F, Couderc T, Madec Y, Carpentier F, Kassab S, et al. Chikungunya virus-associated long-term arthralgia: a 36-month prospective longitudinal study. PLoS Negl Trop Dis. 2013;7(3):e2137.
9. Amaral JK, Bilsborrow JB, Schoen RT. Brief report: the disability of chronic chikungunya arthritis. Clin Rheumatol. 2019;38(7):2011-4.
10. EBSI. PXVX-EPI-CV-001 Pediatric dengue cohort study in Nicaragua 2019.
11. Yoon IK, Alera MT, Lago CB, Tac-An IA, Villa D, Fernandez S, et al. High rate of subclinical chikungunya virus infection and association of neutralizing antibody with protection in a prospective cohort in the Philippines. PLoS Negl Trop Dis. 2015;9(5):e0003764.
12. Chang LJ, Dowd KA, Mendoza FH, Saunders JG, Sitar S, Plummer SH, et al. VRC 311: safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: a phase 1 dose-escalation trial. Lancet. 2014;384(9959):2046-52.
13. Ledgerwood J. VRC 311: a phase 1 open label, dose-escalation clinical trial to evaluate the safety and immunogenicity of a virus-like particle (VLP) chikungunya vaccine, VRC-CHKVLP059-00-VP, in healthy adults. ClinicalTrials.gov: National Institutes of Health Clinical Center (CC) (National Institute of Allergy and Infectious Diseases (NIAID) 2016 [Available from: https://clinicaltrials.gov/ct2/show/record/NCT01489358].
14. Chen G, Ledgerwood, J. VRC 704: phase 2 randomized, placebo-controlled trial to evaluate the safety and immunogenicity of a chikungunya virus-like particle vaccine, VRC-CHKVLP059-00-VP, in healthy adults. ClinicalTrials.gov: ClinicalTrials.gov Identifier: NCT02562482; 2019 [Available from: https://clinicaltrials.gov/ct2/show/record/NCT02562482?term=VRC+704&rank=1.
15. Chen GL, Coates EE, Plummer SH, Carter CA, Berkowitz N, Conan-Cibotti M, et al. VRC 704: effect of a chikungunya virus-like particle vaccine on safety and tolerability outcomes: a randomized clinical trial. JAMA. 2020;323(14):1369-77.
16. Bennett S. PXVX-CV-317-001 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). ClinicalTrials.gov2018 [Available from: https://clinicaltrials.gov/ct2/show/NCT03483961.

17. Bennett S. EBSI-CV-317-002 A phase 2 open-label study to assess the safety and immunogenicity of an alum-adjuvanted chikungunya virus-like particle vaccine (PXVX0317) in prior recipients of other alphavirus vaccines (WRAIR). ClinicalTrials.gov: United States National Library of Medicine 2020 [A phase 2 open-label study to assess the safety and immunogenicity of an alum-adjuvanted chikungunya virus-like particle vaccine (PXVX0317) in prior recipients of other alphavirus vaccines versus alphavirus naïve controls.]. Available from: https://www.clinicaltrials.gov/ct2/show/NCT03992872.
18. Mendy J. Poster: Plaque reduction luciferase-based assay (CHIKV-luc). 2016.
19. Morens DM, Fauci AS. Chikungunya at the door--deja vu all over again? N Engl J Med. 2014;371(10):885-7.
20. CDC. Chikungunya Geographic Distribution [Available from: https://www.cdc.gov/chikungunya/geo/index.html.
21. CDC. Chikungunya Nowcast for the Americas: Centers for Disease Control and Prevention; 2020 [Available from: https://www.cdc.gov/chikungunya/modeling/index.html.
22. CDC. Chikungunya virus in the United States 2013-2019: Centers for Disease Control and Prevention 2020 [Available from: https://www.cdc.gov/chikungunya/geo/united-states.html.
23. Lindsey NP, Prince HE, Kosoy O, Laven J, Messenger S, Staples JE, et al. Chikungunya virus infections among travelers-United States, 2010-2013. Am J Trop Med Hyg. 2015;92(1):82-7.
24. Ruiz-Moreno D, Vargas IS, Olson KE, Harrington LC. Modeling dynamic introduction of chikungunya virus in the United States. PLoS Negl Trop Dis. 2012;6(11):e1918.
25. Evans D, Meires, JL. Chikungunya Virus: a rising health risk in the United States and how nurse practitioners can help address and reduce the risk. The Journal for Nurse Practitioners. 2016;12(5):289-98.
26. Del Valle SY, McMahon BH, Asher J, Hatchett R, Lega JC, Brown HE, et al. Summary results of the 2014-2015 DARPA Chikungunya challenge. BMC Infect Dis. 2018;18(1):245.
27. CDC. Vaccine Recommendations and Guidelines of the ACIP: Centers for Disease Control and Prevention; 2020 [TABLE 6-2. Needle length and injection site of IM injections for children aged ≤18 years (by age) and adults aged ≥9 years (by sex and

weight)]. Available from: https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/administration.html.

28. EBSI. Emergent Travel Health Inc. Data on File. 2020.

APPENDIX I TOXICITY GRADING SCALE TABLES

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness*	25 – 50 mm***	51 – 100 mm***	>100 mm***	Necrosis or exfoliative dermatitis
Induration/Swelling**	25 – 50 mm*** and does not interfere with activity	51 – 100 mm*** or interferes with activity	>100 mm*** or prevents daily activity	Necrosis

*In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

**Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

***Revised by the sponsor

Vital Signs*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C)** (°F)**	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	>40 >104
Tachycardia – beats per minute	101 – 115	116 – 130	>130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute***	50 – 54	45 – 49	<45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mmHg	141 – 150	151 – 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mmHg	91 – 95	96 – 100	>100	ER visit or hospitalization for malignant hypertension

Vital Signs*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hypotension (systolic) – mmHg	85 – 89	80 – 84	<80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	>25	Intubation

*Participant should be at rest for all vital sign measurements.

**Oral temperature; no recent hot or cold beverages or smoking.

***When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy participant populations, for example, conditioned athletes.

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or <400 g/24 hours	4 – 5 stools or 400 – 800 g/24 hours	6 or more watery stools or >800 g/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Systemic Illness	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Serum*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	<125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	>150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	>5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	<3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	<45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	>31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	>2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	<7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	>12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	<0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	<1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 ULN	3.1 – 10 x ULN	>10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	<2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	<5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	>10 x ULN
Liver Function Tests – ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 - 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	>1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	>3.0 x ULN
Cholesterol	201 – 210	211 – 225	>226	--

Serum*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	>5.0 x ULN

*The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the participant had a new seizure associated with the low sodium value.

***"ULN" is the upper limit of the normal range.

Hematology*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	<8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	>5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	<8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	>5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25,000	>25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	<1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	<250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	<500
Eosinophils - cell/mm^3	650 – 1,500	1,501 – 5,000	>5,000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	<25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	>1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	>1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	>600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	<100 or associated with gross bleeding or disseminated

Hematology*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
				intravascular coagulation (DIC)

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

**"ULN" is the upper limit of the normal range.

Urine*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 – 10	11 – 50	>50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Document Approvals
Approved Date: 20 Mar 2023

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