EMERGENT®

CLINICAL STUDY PROTOCOL

A Phase 3 Safety and Immunogenicity Trial of the VLP-Based Chikungunya Virus Vaccine PXVX0317 in Adults ≥65 Years of Age

EBSI-CV-317-005

Version 6.0

31 Mar 2023

ClinicalTrials.gov ID: NCT05349617

Sponsor:

Emergent Travel Health Inc.

[redacted]

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DOCUMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.0	26Jul2021	n/a	Initial protocol
2.0	19Aug2021	Added clinical relevance endpoint (lower bound of the two-sided 95% CI for the seroresponse rate in adults ≥65 years of age must be ≥60%).	Omitted from initial protocol inadvertently.
3.0	17Sep2021	Changed primary immunogenicity objective to demonstrate superiority to placebo from inferiority to EBSI-CV-317-004. Added preliminary group unblinded immunogenicity analysis.	Change in PXVX0317 program strategy and allowing for earlier regulatory interactions prior to final database lock.
4.0	25Jan2022	Removed intraarticular steroids from exclusion criteria 7 Added labelling information in section 5.1.3 Updated Adverse Event collection to begin at Day 1 Rearranged Immunogenicity endpoints Reasons for allowing rescreening were expanded	Intraarticular steroids were erroneously mentioned as allowed in earlier versions of the protocol. Section 5.1.3 was modified to better align with ICH E6(R2) GCP and Emergent's updated protocol template. Adverse event collection was updated per FDA request that any event that begins prior to first vaccine administration be reported as medical history. Immunogenicity endpoints were reprioritized per EMA Scientific Advice. Rescreening was aligned with EBSI-CV-317-004 protocol.
5.0	27Jul2022	Added exploratory objective and endpoint to evaluate the ability of CHIKV antibodies to PXVX0317 to neutralize different CHIKV genotypes. Clarified inclusion criterion for participants who test positive for COVID-19. Clarified definition of MAAE.	Addition of exploratory objective and endpoint to the protocol per EMA Scientific Advice. Reasons for delay of vaccination were updated to clarify that vaccination of potential participants with evidence of active SARS-CoV-2 virus infection should be delayed (or not enrolled) to reduce the risk of confounding safety and immune response data. MAAE definition was clarified to ensure consistent reporting of events, as intended for this clinical study.
6.0	See effective date	Study objectives and endpoints have been updated and reorganized and GMT analysis was removed from hierarchical testing. [redacted] and EMA definition of seroresponse rate as the presumptive seroprotection rate.	Simplification for global use of the protocol and to align with FDA and EMA feedback received 26Jul2022 and 25Jan2023, respectively.

		Administrative changes such as capitalizations, acronyms, and section organization. Removed Day 183 from exploratory endpoint.	To align with company authoring guidelines for regulatory submissions, provide consistency across CHIK documents, and for clarity. To align with the Day 22 timepoint used in primary endpoints.

SPONSOR SIGNATORY

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

[redacted]
Date (dd/mmm/yyyy)

VP, Clinical Development

Emergent BioSolutions Inc.

KEY STUDY CONTACT INFORMATION

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

Immediately Reportable Adverse Events: Emergent Global Pharmacovigilance [redacted]

For all other contact information please 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-317-005, version 6.0:	A Phase 3 Safety and Immunogenicity Trial of the VLP-Based Chikungunya Virus Vaccine PXVX0317 in Adults ≥65 Years of Age

[redacted]

My signature below verifies that I have read and agree to this protocol. I am aware of my responsibilities as a Principal Investigator (PI) 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) or appropriate local regulations and applicable laws and regulations of the country of the investigational 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 (dd/mmm/yyyy)

PROTOCOL SYNOPSIS

<table>
<tr><td colspan="2">Name of Sponsor/Company: Emergent Travel Health Inc.</td></tr>
<tr><td colspan="2">Name of Investigational Product: PXVX0317</td></tr>
<tr><td colspan="2">Name of Active Ingredient(s): Chikungunya virus virus-like particle (CHIKV VLP)</td></tr>
<tr><td colspan="2">Protocol No.: EBSI-CV-317-005</td></tr>
<tr><td colspan="2">Title of Study: A Phase 3 Safety and Immunogenicity Trial of the VLP-Based Chikungunya Vaccine PXVX0317 in Adults ≥65 Years of Age</td></tr>
<tr><td colspan="2">Study Centers: Multicenter, up to 10 sites in the US</td></tr>
<tr><td>Study Duration for Each Participant: 7 months
Estimated Study Duration: ~12 months
Estimated Enrollment Period: ~6 months
Anticipated Start Date: Q2 2022
Estimated End Date: Q2 2023</td><td>Phase of Development: 3</td></tr>
<tr><td colspan="2">Coprimary Objectives:
• 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) in adults ≥65 years of age.
Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA [redacted].
• To evaluate the safety of PXVX0317 in adults ≥65 years of age.
Secondary Objectives:
• To compare the anti-CHIKV SNA response to PXVX0317 and placebo at Day 15 and Day 183, as measured by GMT and seroresponse rate.
• To compare the anti-CHIKV SNA response to PXVX0317 and placebo in participants ≥65 to <75 and ≥75 years of age as measured by GMT and seroresponse rate.
Exploratory Objective:
• To evaluate the ability of PXVX0317 vaccine-induced CHIKV antibodies to neutralize various CHIKV genotypes.</td></tr>
<tr><td colspan="2">Methodology/Study Design:</td></tr>
</table>

This is a phase 3, randomized, double-blind, placebo-controlled, parallel-group study with two treatment groups. Participants will be randomized in a 1:1 ratio to receive either a single intramuscular (IM) dose of PXVX0317 or placebo at Day 1.

The target population is adults ≥65 years of age. Participants will be stratified by age subgroups (65 to <75 and ≥75 years of age), with a target of 25% enrollment of participants ≥75 years of age. With 400 participants enrolled, the treatment group totals are estimated as follows:

- Group 1- PXVX0317: n=200
- Group 2- Placebo: n=200

An independent Safety Monitoring Committee (SMC) will provide safety oversight. The SMC will review aggregated, blinded safety data after the first 50 participants have completed seven days of safety follow-up. The remaining participants will be enrolled following the safety review by the SMC and based on the sponsor's consideration of the SMC's recommendation.

Number of Participants (Planned): 400

Study Population: Adults ≥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 voluntarily signed by participant. Must verbalize understanding of the general procedures of, and reason for the study.
2. Males or females, ≥65 years of age.
3. Able to complete all scheduled visits and comply with all study procedures.
4. Women who are not of childbearing potential (CBP): 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 post menopausal sex-hormonal treatment).
5. Participants must be in stable health in the opinion of the investigator for at least 30 days prior to screening (eg, no hospital admission for acute illness in the last 30 days prior to screening).

Exclusion Criteria:

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

1. Participation or planned participation in an investigational clinical trial (eg, vaccine, drug, medical device, or medical procedure) within 30 days of Day 1 and for the duration of the study. **Note:** Participation in an observational trial or follow-up phase of a trial may be allowed; however, these instances should be discussed with the sponsor's MM prior to enrollment.
2. Prior receipt of any CHIKV vaccine.
3. Positive laboratory evidence of current infection with human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis B virus (HBV).
4. Body Mass Index (BMI) ≥35 kg/m^2.
5. History of any known or suspected allergy or history of anaphylaxis to any component of the investigational product (IP).
6. History of any known congenital or acquired immunodeficiency or immunosuppressive condition that could impact response to vaccination (eg, leukemia, lymphoma, malignancy, functional or anatomic asplenia, alcoholic cirrhosis). **Note:** History of basal cell and squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix considered cured would not be exclusionary. History of a malignancy considered cured from over five years from the date of screening with minimal risk of recurrence is not exclusionary.
7. Prior or anticipated use of systemic immunomodulatory or immunosuppressive medications from six months prior to screening through Day 22. **Note:** Systemic corticosteroid use at a dose or equivalent dose of 20 mg of prednisone daily for 14 days or more within 90 days of screening through Day 22 is exclusionary. The use of inhaled, intranasal, topical, or ocular steroids is allowed.
8. Bleeding disorder or receipt of anticoagulants in the 21 days prior to screening, contraindicating IM vaccination, as judged by the investigator.
9. Moderate or severe acute illness with or without fever (oral temperature ≥100.4°F [≥38.0°C]).
10. Receipt or anticipated receipt of immunoglobulin from 180 days prior to screening through Day 22.
11. Medical condition (such as dementia) that, in the opinion of the investigator, could adversely impact the participant's participation in or conduct of the study.
12. Evidence of substance abuse that, in the opinion of the investigator, could adversely impact the participant's participation in or conduct of the study.
13. Identified as an investigator or employee of an investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (ie, parent, spouse) of the investigator or employee with direct involvement in the proposed study.

14. Receipt or anticipated receipt of any vaccine from 30 days prior to Day 1 through Day 22.
15. Receipt or anticipated receipt of blood or blood-derived products from 90 days prior to screening through Day 22.
16. Any planned elective surgery that may interfere with study participation or conduct.
17. Any other medical condition that, in the opinion of the investigator, could adversely impact the participant's participation in or conduct of the study.

Reasons for Delay of Study Vaccination:

i. Any fever (oral temperature ≥100.4°F [≥38.0°C]) within 24 hours of planned study vaccination.

ii. Any condition that may interfere with assessment of reactogenicity and/or other safety assessments.

iii. Signs and/or symptoms of an acute infectious illness.

iv. Participants should not be randomized if they have tested positive for COVID-19 (using any type of test, even if asymptomatic) for 14 days or until negative molecular test result eg, PCR.

If any one of these occur at the time of the scheduled study vaccination, randomization is permitted later, if within the screening window (30 days), at the discretion of the investigator and after consultation with the MM. If randomization and vaccination cannot occur within the allowed screening window, rescreening will be required.

Investigational Product, Dosage, and Mode of Administration:

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

Primary Endpoints

Coprimary Immunogenicity Endpoints:

- Difference in anti-CHIKV SNA seroresponse rate (PXVX0317 minus placebo) and associated 95% CI (confidence interval) at Day 22.
- Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for PXVX0317 and 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 9.6.2 for immunogenicity analysis details and Section 9.4 for success criteria and multiplicity controls.

Safety Endpoints:

- Incidence of solicited adverse events (AEs) through Day 8 for PXVX0317 and placebo.
- Incidence of unsolicited AEs through Day 29 for PXVX0317 and placebo.
- Incidence of serious adverse events (SAEs), medically attended adverse events (MAAEs; medically attended visits include hospital, emergency room [ER], urgent care clinic, or other visits to or from medical personnel), and adverse events of special interest (AESI; new onset or worsening arthralgia that is medically attended) through Day 183 for PXVX0317 and placebo.

Secondary Immunogenicity Endpoints:

- **Key Secondary Immunogenicity Endpoints:** Difference in anti-CHIKV SNA seroresponse rate (PXVX0317 minus placebo) with associated 95% CIs at Day 15 and Day 183, in that order (see Section 9.6.2).
- Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at 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 Immunogenicity Endpoint:

- Geometric mean titers and associated two-sided 95% CIs for neutralizing antibodies against various CHIKV genotypes measured at Day 22 (and at baseline if necessary) for a subset of participants in the PXVX0317 group.

Procedures and Assessments:

Study procedures/assessments will occur at: Screening (Visit 1), to occur no more than 30 days prior to Day 1 of IP administration; Day 1 (Visit 2, Baseline, Randomization, and IP Administration); Day 15 (Visit 3); Day 22 (Visit 4); Day 29 (Visit 5, telephone contact); Day 92 (Visit 6, telephone contact); Day 183 (Visit 7, End of Study) or Early Discontinuation Visit (EDV). The per-participant estimated total study duration is 212 days (includes the 30-day screening window).

Solicited AEs (collected from Day 1 through Day 8) will consist of local adverse reactions (injection site pain, redness, and swelling) and systemic adverse reactions (fever, chills, fatigue, headache, myalgia, arthralgia, and nausea). Unsolicited AEs will be collected from

Day 1 through Day 29. Serious adverse events, MAAEs, and AESI will be collected from Day 1 through Day 183 End of Study Visit.

The immune response to PXVX0317 will be measured by CHIKV SNA responses up to 182 days after dosing (Day 183). CHIKV SNA levels will be measured by a validated CHIKV luciferase-based assay (hereafter referred to as human SNA assay) developed by the sponsor (see Section 7 for human SNA assay description).

Statistical Methods:

Seroresponse rates: Proportions (percentages) of participants in PXVX0317 and placebo groups with a seroresponse at Day 15, Day 22, and Day 183 will be summarized with two-sided 95% CIs based on the Wilson score method. This analysis will be primarily based in the immunogenicity evaluable population (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 chi-square tests at each visit based on both age groups combined with a two-sided alpha=0.05.

The difference in CHIKV SNA seroresponse rates (PXVX0317 minus placebo) among baseline seronegative participants will be calculated along with the two-sided 95% CI for the difference based on the Newcombe hybrid score method at Day 15, Day 22, and Day 183. At Day 22, the lower bound of the two-sided 95% CI for the difference in seroresponse rates (PXVX0317 minus placebo) for both age strata combined must meet or exceed [redacted] for clinical relevance to be demonstrated.

Geometric mean titers: Geometric mean titers will be derived from a linear analysis of variance (ANOVA) model with $\log_{10}$-transformed CHIKV SNA titers as the dependent variable and treatment group and study site as fixed effects. The adjusted least square means and their two-sided 95% CIs calculated based on the ANOVA model will be back transformed and reported as the PXVX0317 and placebo group GMT values at Day 15, Day 22, and Day 183. Significance testing will be carried out at a two-sided significance level of 0.05 using both age strata combined.

Geometric mean fold increase: Geometric mean fold increase in CHIKV SNA titers from Day 1 to Day 15, Day 22, and Day 183 will be reported for PXVX0317 and placebo groups.

Four-fold rise over baseline in CHIKV SNA titer: Proportions (percentages) of participants in PXVX0317 and placebo groups with a CHIKV SNA titer of at least 4-fold rise over baseline at Day 15, Day 22, and Day 183 will be summarized with two-sided 95% CIs based on the Wilson score method.

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

Sample Size Considerations: 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 nonevaluable participants, the power to show superiority over placebo with 180 PXVX0317 vaccine and 180 placebo evaluable participants is >99.9% for the combined age groups.

The difference in seroresponse rate between PXVX0317 and placebo groups that is considered clinically relevant is [redacted]. With 180 PXVX0317-treated participants and a target seroresponse rate of [redacted] vs a rate of 5% for placebo, the width of a two-sided 95% CI would be ±5.4%. If the target PXVX0317 seroresponse is [redacted], the width would be ±6.9%. Therefore, the difference in seroresponse rates must be above [redacted] for the lower bound of the 95% CI for the difference to be [redacted].

Analysis Populations

Enrolled population: All screened participants who sign the informed consent form (ICF), are entered in the electronic data capture (EDC) database and meet all eligibility criteria.

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. Safety endpoints will utilize the safety population, analyzed as treated.

Modified intent to treat population: All randomized participants who are vaccinated and have at least one postinjection CHIKV SNA titer result, analyzed as randomized.

Immunogenicity evaluable population: All participants in the mITT population who: i) provide evaluable serum sample results for the Day 22 time point within the required time frame (Days 19 to 27); ii) have no measurable CHIKV SNA at Day 1; iii) have no important protocol deviation or other reason to be excluded as defined prior to unblinding. The IEP is the primary population for all immunogenicity analyses.

Preliminary Analysis: There will be a safety and immunogenicity preliminary analysis on data for all participants through the Day 29 visit to facilitate health authority presubmission preparation. The analyses will be performed by a third-party vendor and unblinded results will be reported only at the treatment group summary level preserving the double-blind status on the participant level. No p-value penalty will be assessed because the Day 22 primary immunogenicity endpoint data will be final at the time of preliminary analysis and no action regarding the study will be made based on these findings.

SCHEDULE OF EVENTS

Table 1 Schedule of Events

	Screening Visit 1	Day 1 Visit 2	Day 15 Visit 3	Day 22 Visit 4	Day 29 Visit 5 (phone)	Day 92 Visit 6 (phone)	Day 183/Early Discontinuation[k] Visit 7
Window (days)	**Within 30 days of Day 1**	**0**	**-1/+3**	**-1/+3**	**±1**	**±3**	**-14/+7**
Written informed consent	X						
Eligibility criteria	X	X[a]					
Demographics	X						
Medical history[b]	X	X[a,c]					
Vital signs[d]	X	X[e]					
Physical examination	X[f]	X[a,f]	X[f]	X[f]			X[f]
Viral marker testing (HIV-1/2, anti-HCV[g], HBsAg)	X						
Randomization		X[a]					
Administration of IP		X					
Diary or memory aid (electronic or paper) training and device distribution[h]		X[a]					
Acute observation[i]		X					
Diary or memory aid (electronic or paper) collection and review			X				
Investigator assessment of reactogenicity			X				
Unsolicited AEs		X	X	X	X		
SAEs		X	X	X	X	X	X
AESI and MAAEs		X	X	X	X	X	X
Concomitant medications	X	X[a]	X	X	X	X[j]	X[j]
Blood for CHIKV SNA		X[a]	X	X			X
Blood for testing against CHIKV genotypes		X[a]		X			X

	Screening Visit 1	Day 1 Visit 2	Day 15 Visit 3	Day 22 Visit 4	Day 29 Visit 5 (phone)	Day 92 Visit 6 (phone)	Day 183/Early Discontinuation[k] Visit 7

[a] To be done prior to IP administration.
[b] To include any history or current presence of joint pain.
[c] To be updated if necessary.
[d] Vital signs include temperature, heart rate, blood pressure, respiratory rate. Height and weight will be obtained at the screening visit only for BMI calculation. Vitals signs should be measured after at least five minutes of rest. Oral temperature should be measured.
[e] To be taken before and after IP administration. Vital signs will be performed prevaccination and 30 minutes to one-hour postvaccination on Day 1.
[f] Complete physical exam at screening and then targeted exams or per PI discretion at other visits.
[g] If HCV antibody is positive HCV ribonucleic acid (RNA) testing can be performed.
[h] Smart phone (e-Diary), paper diary (memory aid), digital thermometer, and ruler will be provided.
[i] Participants will be monitored by study staff for signs of an acute adverse reaction for at least 30 minutes after injection.
[j] Concomitant medications associated with SAEs/AESI/MAAEs only.
[k] For EDV occurring within seven days postvaccination, from 7 to 21 days postvaccination, or ≥22 days postvaccination, the Visit 3, Visit 4, or Visit 7 schedule will be followed, respectively.

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APPENDIX

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event
AESI	Adverse Event of Special Interest
ANOVA	Analysis of Variance
ATC	Anatomic Therapeutic Chemical
BMI	Body Mass Index
C	Capsid
CBP	Childbearing Potential
CFR	Code of Federal Regulations
CHIKV	Chikungunya Virus
CHIKV-luc	Modified version of CHIKV containing a reporter gene that expresses a luciferase protein
CI	Confidence Interval
DMP	Data Management Plan
E	Envelope
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDV	Early Discontinuation Visit
ER	Emergency Room
FDA	Food and Drug Administration
FRNT	Focus Reduction Neutralization Test
GCP	Good Clinical Practices
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ID	Identification
IEP	Immunogenicity Evaluable Population
IgG	Immunoglobulin G
IM	Intramuscular
IP	Investigational Product

IRB	Institutional Review Board
LLOQ	Lower Limit of Quantitation
LTF	Lost to Follow-up
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary of Regulatory Activities
mITT	Modified Intent to Treat
MM	Medical Monitor
NHP	Nonhuman Primates
NIH	National Institutes of Health
nsP	Nonstructural Proteins
NT_{80}	80% Neutralization Titer
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Units
PI	Principal Investigator
PT	Preferred Term
RBC	Red Blood Cell
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
SMC	Safety Monitoring Committee
SNA	Serum Neutralizing Antibody
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
US	United States of America
VLP	Virus-like Particle
VRC	Vaccine Research Center
WBC	White Blood Cell Count

1 BACKGROUND INFORMATION

1.1 Name and Description of Investigational Product(s)

PXVX0317 is a CHIKV VLP vaccine. The vaccine is comprised of 40 µg CHIKV VLP adsorbed on aluminum hydroxide 2% adjuvant (2% [w/w] aqueous suspension of aluminum hydroxide, Alhydrogel®) 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 muscle.

Placebo (diluent) is a sterile aqueous solution with the same excipient composition as the PXVX0317 drug product without CHIKV VLP or aluminum hydroxide components.

For additional study vaccine information, see Section 5.1 and the PXVX0317 Investigator's Brochure (IB).

1.1.1 Indication

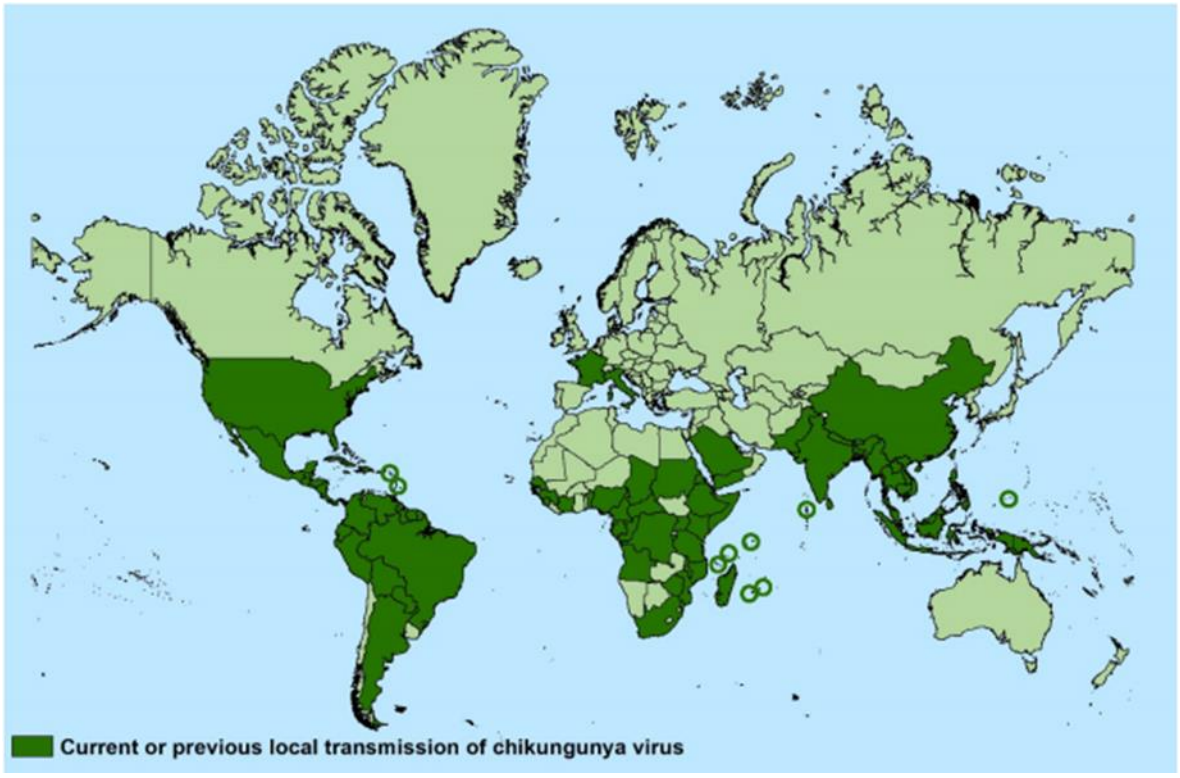
The target indication for PXVX0317 vaccine is for active immunization to prevent disease caused by CHIKV.

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 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 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 (adapted from (3))



Following an incubation period of 2 to 12 days, acute clinical manifestations include high fever, rash, gastrointestinal complications, headache, muscle pain, nausea, fatigue, myalgia, and joint pain (5, 6, 7). The most classic symptom of chikungunya 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). The frequency and severity of arthritic sequelae are higher in older adults (10).

Currently there are no approved vaccines to prevent CHIKV infection or disease. However, protection against subsequent infection has been shown to correlate with the presence of CHIKV antibodies that neutralize the virus *in vitro* (11, 12).

1.3 Summary of Findings from Nonclinical Studies

The sponsor conducted a study in mice that demonstrated comparability between the immune responses induced by the VRC-CHKVLP059-00-VP (discussed in Section 1.4) and PXVX0317 vaccines. Following this, the sponsor conducted a pilot challenge study in cynomolgus macaques nonhuman primates (NHP) to determine the CHIKV challenge dose required to induce signs of arthritis, as quantified by degree of inflammation of the joints and surrounding tissues in infected animals. A scoring system was developed to measure the degree of joint infiltration and a challenge dose of 10^7 plaque forming units (PFUs) of CHIKV La Reunion outbreak strain LR2006-OPY1 was selected as the optimal dose to

induce signs of arthritis in NHPs. An NHP active vaccination and challenge study with the PVXV0317 vaccine followed. Nonhuman primates received two IM immunizations (on study Days 0 and 28) with VLP (1.25, 6, or 20 µg) adjuvanted with alum (300 µg), VLP alone (20 µg), or alum alone, followed by challenge with 10^7 (PFU) of CHIKV strain LR2006-OPY1.

Data from the study demonstrate that all three dose levels of the PXVX0317 vaccine, including 20 µg without alum, induced a robust immune response as measured by CHIKV luciferase serum neutralizing antibody assay for NHP serum (NHP SNA Assay), even after a single immunization. Importantly, the addition of alum increased the prechallenge SNA titers compared to the VLP vaccine without alum. Furthermore, vaccination completely protected NHPs from viremia, as measured by plaque assay on serum from challenged animals. Joint pathology scores in the active vaccination study demonstrate that vaccination with PXVX0317 protects NHPs from joint infiltration in a dose-dependent manner. Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) analysis (by NHP RT-qPCR assay) of joint tissues showed significantly lower levels of viral RNA in tissues of vaccinated animals, which rarely had detectable RNA, compared with animals that had received only alum. Taken together, these data suggest that vaccination induced CHIKV SNA protects cynomolgus macaques from developing CHIKV disease following viral challenge.

In addition, Emergent conducted a passive transfer and challenge study in cynomolgus macaque NHPs. Human Immunoglobulin G (IgG) purified from plasma of volunteers vaccinated with PXVX0317 was passively transferred to NHPs at three dose levels (5, 15, and 100 mg/kg, resulting in SNA titers at time of challenge of 1:38, 1:101, and 1:644, respectively) followed by CHIKV (strain LR2006-OPY1) challenge. All three dose levels of IgG purified from plasma of PXVX0317 vaccinated participants protected NHPs from viremia as measured by plaque assay. Human IgG administration also appears to protect NHPs from joint pathology in a dose-dependant manner. Results indicate that purified human IgG at an SNA titer of 1:38 is sufficient to protect NHPs against viremia as measured by plaque assay following CHIKV challenge.

1.4 Literature and Data Relevant to the Trial

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) (13, 14) and phase 2 (VRC 704) (15, 16) clinical studies. 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) (17). 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 and EBSI-CV-317-002), a phase 3 study (EBSI-CV-317-004) and this phase 3 study.

1.4.1 Clinical Study VRC 311

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 (13, 14). 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 (13, 14).

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 (13, 14).

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 (13, 14). 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 7 for assay details).

1.4.2 Clinical Study VRC 704

The NIH VRC 704 was a phase 2 study conducted at multiple CHIKV-endemic sites in the Caribbean (15, 16). The study was a double-blind, placebo-controlled study with 200 participants (planned) receiving 20 µg of CHIKV VLP and 200 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 (FRNT) reported as EC_{50} values. EC_{50} is the dilution of sera that inhibits 50% infection in viral neutralization assay. Chikungunya virus VLP appeared safe and well tolerated in participants who were followed through Week 72, with no related SAEs or other safety concerns (15, 16). Chikungunya virus 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 (15, 16).

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 safe 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%) (15, 16). 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 (15, 16).

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.

1.4.3 Clinical Study PXVX-CV-317-001

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 18 to <46 years of age (17). The dosages of CHIKV VLP ranged from 6 µg to 40 µg, adjuvanted or unadjuvanted. These doses were below or approximately equivalent to those used in the NIH's VRC 311 and VRC 704 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 neutralization titer (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. Participants receiving the single 40 µg dose demonstrated only slightly lower GMT levels (1712 at Day 57, ie, 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 24-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 post vaccination as well as the highest GMTs at Day 182. Seroresponse was well maintained at 760 days in Group 8 participants. Based on these data the 40 µg CHIKV VLP + 300 µg alum adjuvant single dose regimen was selected going forward in the PXVX0317 clinical development.

1.4.4 Clinical Study EBSI-CV-317-002

The phase 2 clinical study (EBSI-CV-317-002) conducted at two sites in the US compared the safety and immunogenicity of a 40 µg CHIKV VLP + 300 µg alum adjuvant single dose in prior recipients of other alphavirus vaccines vs alphavirus vaccine-naïve controls (19). The 30 prior alphavirus vaccine recipients and 30 gender and age-matched vaccine-naïve controls were vaccinated and followed for six months. There were no differences between the groups in CHIKV neutralizing antibody GMTs at Day 22 and no new safety signals were identified.

For more information refer to the IB.

1.5 Seroresponse Rate

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

1.6 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 immunogenicity. The IM route of administration is consistent with that in previous clinical studies (VRC 311, VRC 704, PXVX-CV-317-001, 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 CHIKV SNA GMT at Day 182 and Day 365. Similar findings were observed in EBSI-CV-317-002.

The PXVX0317 vaccine (40 µg CHIKV VLP + 300 µg aluminum hydroxide adjuvant) single dose was selected for further development by the sponsor in this phase 3 clinical study (EBSI-CV-317-005).

1.7 Population to be Studied

The target population is adults ≥65 years of age. This study will generate safety and immunogenicity data in elderly adults, in whom PXVX0317 has not been previously evaluated. This study is important due to the high relative disease burden in older adults, as well as potential concerns about immunosenescence and attendant hyporesponsiveness to vaccination in this age group.

1.8 Summary of Known and Potential Risks and Benefits

1.8.1 Risks Related to Study Intervention

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

Most solicited AEs reported by participants enrolled in study PXVX-CV-317-001 were mild to moderate in severity. The percentage of participants who reported ≥Grade 3 AEs for the following categories were as follows: any AEs (7.9%), any solicited AE (6.1%), any systemic solicited AE (5.9%), any local solicited AE (0.2%), any unsolicited AE through Day 57 (2.9%), any treatment-related solicited AE (3.6%), any treatment-related systemic solicited AE (3.4%), any treatment-related local solicited AE (0.2%). There were no reports of ≥Grade 3 treatment-related unsolicited AEs through Day 57. No participants had a treatment-related SAE, and no participants permanently discontinued study due to AE or died during the study.

Among participants from PXVX-CV-317-001 Group 8 (n=50) participants who received the 40 µg adjuvanted dose selected for further development, 48.0% reported solicited AEs occurring within seven days postvaccination with active vaccine at Day 29. Approximately 22% of participants reported solicited systemic AEs and 40.0% reported solicited local AEs. Solicited local AEs reported included only injection site pain; no injection site redness or swelling was reported after receipt of the active dose. Solicited systemic AEs reported included malaise (4.0%), fatigue (4.0%), headache (8.0%), myalgia (12.0%), nausea (4.0%), and joint pain (2.0%).

There were 13 unrelated SAEs reported for 10 participants in study PXVX-CV-317-001 and study EBSI-CV317-002. One case of anaphylaxis occurred on study Day 309 in a participant that was suspected to be due to a nut allergy. No participants had a treatment-related SAE, and no participants permanently discontinued study due to AE or died during the study.

1.8.2 Risk of Allergic Reaction

With any vaccine, there is a risk of severe allergic reaction. Although such reactions have not been reported as related to this vaccine, they have occurred with other vaccines containing some of the same ingredients. Symptoms of allergic reaction may include, but are not limited to, rash, wheezing and difficulty breathing, difficulty swallowing, dizziness and fainting, swelling around the mouth, throat or eyes, a fast pulse, and/or sweating. Participants will be directed to inform study staff immediately if experiencing any of these symptoms.

1.8.3 Risks with Blood Draws

Blood drawing may cause pain, bruising, feeling faint, fainting, needle site infections, swelling, and rarely other infections. Bruising at the site of blood drawing can be prevented

by applying pressure for several minutes. To reduce the risk of infection, the area will be wiped clean with alcohol and sterile equipment will be used.

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

At the Screening Visit, participants will be tested for HBV, HCV, and HIV. Receiving information that any health screening tests are abnormal or that tests for HIV and HBV or HCV are positive may upset participants. The study doctors will discuss their health results face-to-face (and notify their primary doctor at their request). Counseling will be available to the study participant if they wish. A positive HIV status could limit a participant's ability to obtain future life and health insurance. In rare circumstances, a positive HIV test has led to discrimination in employment and housing, etc. Participants will be advised of this in the ICF.

1.8.4 Risks to Pregnancy

PXVX0317 has not been thoroughly evaluated for potential risks to unborn or nursing children.

1.8.5 Risks to Confidentiality

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

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

1.8.6 Benefits

There is no expected benefit to participants from this study. A potential benefit of participation in this clinical trial might be protection against CHIKV disease; however, the clinical efficacy of this vaccine for that indication has not yet been proven. This study will accumulate additional safety data on this vaccine and uniquely provide a first glimpse at the safety and immunogenicity of this vaccine in the context of elderly adults. This data will be important to the design of field studies. If, for example, the vaccine is less immunogenic in the elderly, this should be considered for determining any potential modified dosing/dose regimen recommendations.

1.9 Hypothesis

The study hypothesis being tested is that the immune response to PXVX0317 is superior to that of placebo in adults ≥65 years of age at Day 22, that the immune response to PXVX0317 in adults ≥65 years of age at Day 22 is clinically relevant, and that the safety profile in adults ≥65 years of age is comparable to that in individuals 18 to <65 years of age. A clinically relevant immune response in adults ≥65 years of age is defined as the lower bound of the two-sided 95% CI for the Day 22 seroresponse rate [redacted].

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIK SNA titer [redacted].

2 STUDY OBJECTIVES AND PURPOSE

2.1 Study Purpose

The purpose of this phase 3, randomized, double-blind, placebo-controlled study is to evaluate the safety and immunogenicity to PXVX0317 in adults ≥65 years of age.

2.2 Coprimary Objectives

- 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) in adults ≥65 years of age.

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

- To evaluate the safety of PXVX0317 in adults ≥65 years of age.

2.3 Secondary Objectives

- To compare the anti-CHIKV SNA response to PXVX0317 and placebo at Day 15 and Day 183, as measured by GMT and seroresponse rate.
- To compare the anti-CHIKV SNA response to PXVX0317 and placebo in participants ≥65 to <75 and ≥75 years of age as measured by GMT and seroresponse rate.

2.4 Exploratory Objective

To evaluate the ability of PXVX0317 vaccine-induced CHIKV antibodies to neutralize various CHIKV genotypes.

3 STUDY DESIGN

3.1 Study Description

This is a phase 3, randomized, double-blind, placebo-controlled, parallel-group study with two treatment groups.

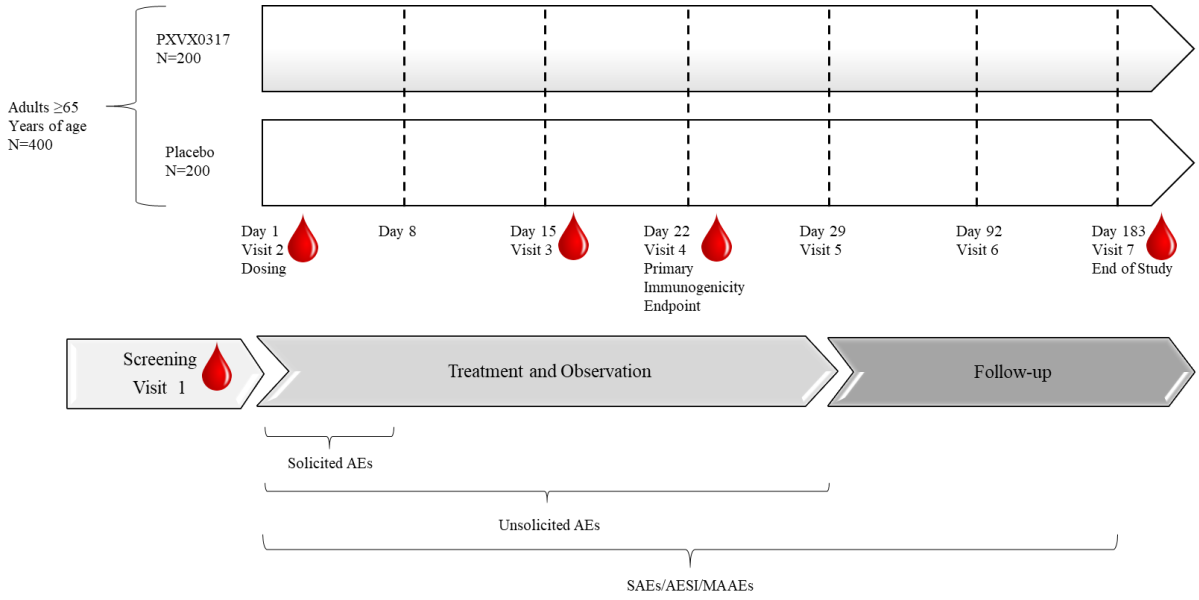
At least 400 healthy US elderly participants ≥65 years of age and older will be enrolled, stratified in two age subgroups (≥65 to <75 and ≥75 years of age). Participants will be randomized in a 1:1 ratio to receive either a single IM dose of PXVX0317 or placebo at Day 1, respectively.

The per participant estimated total study duration is 212 days (including screening). 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 the Day 183 End of Study Visit. For details on schedule of events, refer to Table 1

An independent SMC will provide safety oversight. The SMC will review aggregated, blinded safety data after the first 50 participants have completed seven days of safety follow-up. The remaining participants will be enrolled following the safety review by the SMC and based on the sponsor's consideration of the SMC's recommendation. The need for any further reviews will be determined by the SMC.

3.1.1 Schematic Diagram of Study Design, Procedures, and Stages

Figure 2 EBSI-CV-317-005 Schematic Diagram of Study Design



Note: Days 29 and 92 are phone call follow-ups.

3.1.2 Study Center(s)

This will be a multicenter study conducted in the US with up to 10 sites.

3.2 Description of Study Assessments

The underlying subsections (Section 3.2.1 through Section 3.2.11) describe the planned study procedures and assessments. For the per visit timing of these procedures and assessments refer to Section 6 and the study schedule of events (Table 1). Information on participant consent is described in Section 12.1.

3.2.1 Review of Eligibility Criteria

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

3.2.2 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 5.2) taken within 30 days of Screening Visit (or within 90 days for blood products, or within six months for immunosuppressive/immunomodulatory medications).

3.2.3 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, SAE, or AESI reports or at the investigator's discretion.

3.2.4 Vital Signs

Vital signs collected from participants will include blood pressure, heart rate, respiratory rate, and temperature. Vital signs should be measured after the participant has been at rest for at least five minutes. 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 for IP administration. Vital signs will be taken prior to vaccination and 30 minutes to one hour after IP administration.

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

3.2.5 Laboratory Tests

At the Screening Visit, blood samples will be collected for serum testing for hepatitis B surface antigen (HBsAg), HCV antibody (HCV RNA polymerase chain reaction (PCR), if needed), HIV-1/HIV-2.

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

3.2.6 Immunogenicity Samples

3.2.6.1 Immunogenicity Sample for Human SNA Assay

Chikungunya virus SNA levels will be assessed using a validated human SNA assay that measures NT_{80} titer. Blood (serum) samples will be taken on Day 1 (prior to IP administration), Day 15, Day 22, and Day 183 End of Study Visit (or at Early Discontinuation/Withdrawal).

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

3.2.6.2 Immunogenicity Sample for Various CHIKV Genotypes

Neutralizing antibodies against various CHIKV genotypes will be measured for a subset of participants in the PXVX0317 group using FRNT50. Blood (serum) samples will be collected on Day 1, Day 22, and Day 183 End of Study Visit.

3.2.7 Investigational Product Administration

Investigational product is 0.8 mL in volume and administered by IM injection into the deltoid muscle 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) (20) per Table 2. Investigational product is to be administered only under the direct supervision of the investigator or a qualified subinvestigator identified on the FDA Form 1572.

Table 2 Needle Length and Injection Site of Intramuscular Injections for Adults ≥19 Years of Age (by Sex and Weight)

Sex, Weight	Needle length	Injection Site
Men and women, <60 kg (<130 lbs)	1 inch (25 mm)[1]	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)		

Source: Adapted from Table 6-2, General Best Practice Guidelines for Immunization: Best Practices Guidance of the Advisory Committee on Immunization Practices (ACIP) (20).

[1]Some experts recommend a 5/8-inch needle for men and women who weigh <60 kg, if used, skin must be stretched tightly (do not bunch subcutaneous tissue).

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.

3.2.8 Acute Observation After Investigational Product Administration

The participant will be monitored by study staff for signs of an acute adverse reaction for at least 30 minutes after injection and vital signs will be obtained 30 minutes to one hour after vaccination.

3.2.9 Assess Solicited Adverse Events

Electronic or paper diaries (memory aids) will be used to collect solicited AEs following vaccination. Participants will record events in their diary daily, for at least seven days after vaccination. Solicited AEs to be assessed in 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.

Participants will be trained to complete a diary (electronic or paper) to observe, measure, and record these solicited AEs daily. A digital thermometer will be provided to the participants to measure their temperature each day. The highest temperature measured each day should be recorded in the participant's diary or memory aid (electronic or paper). 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.

Study staff will review the signs and symptoms recorded in the diary or memory aid (electronic or paper) daily and will follow-up with participants if any data is missing. The investigator will then assess all solicited AEs for severity (Section 8.1.8) and the action taken, and causality (Section 8.1.9). 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 safety definition, evaluation, reporting periods and documentation are outlined in Section 8.

3.2.10 Assess Unsolicited Adverse Events

Unsolicited AEs (AEs not listed in the diary or memory aid [electronic or paper]) will be collected from Day 1 through 28 days post vaccination.

Details on safety definition, evaluation, reporting periods and documentation are outlined in Section 8.

3.2.11 Assess Serious Adverse Events, Medically Attended Adverse Events, and Adverse Events of Special Interest

Serious Adverse Events, AESI, and MAAE will be collected for all participants from Day 1 through Day 183 End of Study Visit.

Details on safety definition, evaluation, reporting periods, and documentation are outlined in Section 8.

3.3 Study Endpoints

3.3.1 Primary Endpoints

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).

- 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.

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIK SNA [redacted]. See Section 9.6.2 for immunogenicity analysis details and Section 9.4 for success criteria and multiplicity controls.

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 SAEs, MAAEs, and AESI through Day 183 for PXVX0317 and placebo.

3.3.2 Secondary Immunogenicity Endpoints

- **Key Secondary Immunogenicity Endpoints:** Difference in anti-CHIKV SNA seroresponse rate (PXVX0317 minus placebo) with associated 95% CIs at Day 15 and Day 183, in that order (see Section 9.6.2).
- Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at 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.

3.3.3 Exploratory Immunogenicity Endpoint

Geometric mean titers and associated two-sided 95% CIs for neutralizing antibodies against various CHIKV genotypes measured at Day 22 (and at baseline if necessary) for a subset of participants in the PXVX0317 group.

3.4 Measures Taken to Minimize/Avoid Bias

3.4.1 Masking/Blinding Procedures

Study participants, the investigator, and study site personnel will remain blinded to all randomization assignments throughout the study. 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 semi-transparent 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 3.4.1.1).

- Assays will be run in a blinded manner. The assay titer results will not be delivered to the sponsor data management and analysis teams 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.

Members of the SMC may have access to unblinded data as defined in the SMC Charter for safety/other data review.

Under certain circumstances (eg, safety reasons, required reporting to regulatory agencies), unblinding of IP for a particular participant will be allowed. This safety unblinding will be documented as a protocol deviation. Otherwise, unblinding of the study will only occur after the clinical database has been locked.

Refer to the Pharmacy Manual for procedures in case of accidental unblinding of study-affiliated personnel.

3.4.1.1 Breaking the Blind for Individual Participants

If the PI determines that knowledge of a participant's treatment assignment is urgently needed to guide treatment or ensure the participant's safety, the PI may perform emergency unblinding via the RTSM system. The PI must attempt to notify the sponsor MM prior to unblinding and must notify the sponsor 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.

Documentation of breaking the blind must be entered in the study participants' source documents with the following information recorded: (i) date and time the blind was broken; (ii) the rationale behind the unblinding decision/occurrence; (iii) the names of the personnel involved; and (iv) date of contact with the sponsor MM(s). The unblinding will be documented as a protocol deviation.

3.4.2 Treatment Assignment/Randomization

Participant eligibility will be confirmed and documented by the investigator immediately prior to randomization of each participant on Day 1. Study staff will indicate on a randomization eCRF within the 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 planned administration to the participant.

Participants will be considered enrolled once a randomization number has been assigned within the EDC system. Participants will be stratified by age subgroups (65 to <75 years and ≥75 years of age), with a target of 25% enrollment of participants ≥75 years of age. Participants will be randomized to PXVX0317 or placebo in a 1:1 ratio.

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 PI, nor the sponsor will know participants' individual treatment assignments until all participants have either prematurely discontinued or completed their participation in the study through the Day 183 End of Study Visit and the database has been cleaned and locked.

3.5 Description of Stopping Rules

There are no specific predefined stopping rules for this study; however, an independent SMC will provide safety oversight. The SMC will review aggregated, blinded safety data after the first 50 participants have completed seven days of safety follow-up. The remaining participants will be enrolled following the safety review by the SMC and based on the sponsor's consideration of the SMC's recommendation. The need for any further reviews will be determined by the SMC.

3.6 Accountability Procedures

The PI (or designee) is responsible for maintaining accurate inventory records of the IP and placebo.

3.7 Maintenance of Study Intervention Randomization

Please see Section 3.4.

3.8 Data Directly Recorded on the Electronic Case Report Form

Data recorded directly from the e-Diary will be considered source data.

3.9 End of Study

A participant is considered to have completed this study if he/she has been dosed with IP and completed the final planned study visit at Day 183. Participants who do not complete the Day 183 visit are considered to have been withdrawn from the study. For participants that terminate early, efforts should be made to conduct an EDV.

The End of Study Date is defined as the date of the participant's last study visit on Day 183 or EDV.

3.9.1 Terminating the Study

The sponsor reserves the right to stop or 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 applicable regulatory authority of any suspension or termination, along with justification for terminating the study.

The sponsor will notify the investigators, FDA, and central institutional review board (IRB) of study's completion or early termination. The investigators will notify local IRBs 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.

3.9.2 Terminating the Study at an Individual Investigational Site

An investigational site may be terminated from the study at the discretion of the sponsor or IRB for reasons such as noncompliance, lack of recruitment, fraud or blacklisting by the regulatory authorities, relocation of the investigator, etc.

The sponsor may decide to replace a terminated investigational site.

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 voluntarily signed by participant. Must verbalize understanding of the general procedures of, and reason for the study.
2. Males or females, ≥65 years of age.
3. Able to complete all scheduled visits and comply with all study procedures.
4. Women who are not of CBP: 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 post menopausal sex-hormonal treatment).
5. Participants must be in stable health in the opinion of the investigator for at least 30 days prior to screening (eg, no hospital admission for acute illness in the last 30 days prior to screening).

4.2 Exclusion Criteria

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

1. Participation or planned participation in an investigational clinical trial (eg, vaccine, drug, medical device, or medical procedure) within 30 days of Day 1 and for the duration of the study. **Note:** Participation in an observational trial or follow-up phase of a trial may be allowed; however, these instances should be discussed with the sponsor's MM prior to enrollment.
2. Prior receipt of any CHIKV vaccine.
3. Positive laboratory evidence of current infection with HIV, HCV, or HBV.
4. Body Mass Index ≥35 kg/m^2.
5. History of any known or suspected allergy or history of anaphylaxis to any component of the IP.
6. History of any known congenital or acquired immunodeficiency or immunosuppressive condition that could impact response to vaccination (eg, leukemia, lymphoma, malignancy, functional or anatomic asplenia, alcoholic cirrhosis). **Note:** History of basal cell and squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix considered cured would not be exclusionary. History of a malignancy considered cured from over five years from the date of screening with minimal risk of recurrence is not exclusionary.
7. Prior 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 90 days of screening through Day 22 is exclusionary. The use of inhaled, intranasal, topical, or ocular steroids is allowed.
8. Bleeding disorder or receipt of anticoagulants in the 21 days prior to screening, contraindicating IM vaccination, as judged by the investigator.
9. Moderate or severe acute illness with or without fever (oral temperature ≥100.4°F [≥38.0°C]).
10. Receipt or anticipated receipt of immunoglobulin from 180 days prior to screening through Day 22.
11. Medical condition (such as dementia) that, in the opinion of the investigator, could adversely impact the participant's participation in or conduct of the study.
12. Evidence of substance abuse that, in the opinion of the investigator, could adversely impact the participant's participation in or conduct of the study.
13. Identified as an investigator or employee of an investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (ie, parent, spouse) of the investigator or employee with direct involvement in the proposed study.

14. Receipt or anticipated receipt of any vaccine from 30 days prior to Day 1 through Day 22.
15. Receipt or anticipated receipt of blood or blood-derived products from 90 days prior to screening through Day 22.
16. Any planned elective surgery that may interfere with study participation or conduct.
17. Any other medical condition that, in the opinion of the investigator, could adversely impact the participant's participation in or conduct of the study.

Reasons for Delay of Study Vaccination:

i. Any fever (oral temperature ≥100.4°F [≥38.0°C]) within 24 hours of planned study vaccination.
ii. Any condition that may interfere with assessment of reactogenicity and/or other safety assessments.
iii. Signs and/or symptoms of an acute infectious illness.
iv. Participants should not be randomized if they have tested positive for COVID-19 (using any type of test, even if asymptomatic) for 14 days or until negative molecular test result eg, PCR.

If any one of these occur at the time of the scheduled study vaccination, randomization is permitted later, if within the screening window (30 days), at the discretion of the investigator and after consultation with the MM. If randomization and vaccination cannot occur within the allowed screening window, rescreening will be required.

4.3 Participant Withdrawal Criteria

The participants must be available, without coercion, for all parts of the study. If continued participation jeopardizes the participant's health, the participant should be withdrawn from the study. The investigator is encouraged to consult the sponsor prior to the withdrawal of any participant, except in the event of a medical emergency. The reason for withdrawal of any participant must be clearly documented on the study source documents and the appropriate eCRF.

All participants have the right to withdraw at any time during the study without prejudice.

4.3.1 Withdrawal of Consent

All participants are free to withdraw from participation in this study at any time, for any reason, specified or unspecified, and without penalty or loss of benefits to which the participant is otherwise entitled. The investigator will ask (but cannot require) such participants to provide the reason(s) for withdrawal of consent.

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 requirement, the issue should be discussed with the participant and, if not resolved, consideration given to withdrawing the participant.
- Lost to follow-up (LTF); requires documentation of at least three unsuccessful attempts to contact participants, one of which must be a registered letter. Lost to follow-up will be determined after the date of the participant's projected last visit. Study disposition date will be the last date of contact with the participant prior to LTF.
- 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.

Reasons for withdrawal of individual participants from the study prior to the final protocol required visit and/or final safety follow-up are to be recorded on the eCRF and participant source documents.

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 EDV 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. The participant must first agree to the collection of early withdrawal and safety assessments prior to the assessments, and this agreement must be documented in the source documents. Additional information regarding ongoing AEs may be provided as a follow-up report.

4.3.4 Participant Replacement

Participants withdrawn from the study or who withdraw consent prior to or after dosing will not be replaced. Data from participants withdrawn from the study or who withdraw consent after dosing will be kept in the exposed population (all enrolled participants who received IP).

4.3.5 Documentation of Withdrawal

Reasons for withdrawal of individual participants from the study prior to final protocol required visit and/or 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, LTF will be determined after the date of the participant's projected last visit)
- Adverse event
- Death
- 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.

5 STUDY INTERVENTION

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. Chikungunya virus VLP refers to the virus-like particle component of PXVX0317 produced by transient transfection of human embryonic kidney (HEK293) cells with a DNA expression plasmid encoding C and 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 0.85 mL fill volume to allow for 0.8 mL of deliverable volume. The glass syringe is sealed with a rubber closure and plastic cap. The rubber closure does not contain natural latex.

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 0.85 mL fill volume to allow for 0.8 mL of 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 Packaging and Labeling

Investigational product (PXVX0317 and placebo) will be shipped in 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 or placebo (to maintain the blind both names will be included)
- Dose volume and container – single dose of 0.8 mL in pre-filled single-dose syringe
- Route of administration – IM
- Randomization kit number (in lieu of lot numbers and manufacture date and/or expiry date to maintain the blind)
- Protocol number – EBSI-CV-317-005
- Recommended storage temperature/conditions – stored refrigerated at 2.0 to 8.0°C
- Sponsor – Emergent Travel Health Inc.

5.1.4 Storage Conditions

Investigational product syringes must be stored at 2.0 to 8.0°C in a secured area until ready for use. The temperature in the storage area must be monitored by checking and recording current, maximum/minimum temperature readings inside the vaccine storage unit once daily. Any excursions must be promptly reported to the sponsor; product should be quarantined by the site and may be released for use by the sponsor only after investigation and confirmation of continued stability. Accountability will be maintained in accordance with GCP to include dates on which IP kit was received, administered to participants, returned to the sponsor, or destroyed at the site. The study monitors will periodically check supplies held at the clinical sites to verify accountability. Templates for accountability records are included in the Pharmacy Manual.

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

5.1.5 Preparation

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

5.1.6 Investigational Product Preparation

On Day 1 before IP administration, the interim medical history (ie, from Screening Visit to Day 1) will be reviewed and updated in the participant file, a targeted physical exam may be performed, and blood samples will be collected. Immediately prior to IP administration, staff will verify that the participant is still eligible and randomize the participant in the EDC (Section 3.4.2). Once these procedures are performed and if the participant is still eligible, IP will be administered.

All doses of IP are 0.8 mL in volume and are administered by IM injection in the deltoid muscle with a 1 mL syringe and 25 gauge 1" (or 1.5", at the investigator's discretion) needle using universal precautions and sterile technique; see Table 2 for more details. Investigational product is to be administered only under the direct supervision of the PI or a qualified subinvestigator identified on the FDA Form 1572. The injection will be administered into the deltoid muscle by a staff member delegated by the investigator. Right or left deltoid muscle may be used at the discretion of the administering staff member with consideration for participant preference and features that may obscure interpretation of any local AEs such as tattoos, scars, or other lesions.

For further details see the Pharmacy Manual.

The investigator is responsible for maintaining accurate inventory records of IP (PXVX0317 or placebo). The investigator or designee will inventory all IP kit shipments upon receipt, acknowledge possession by signing all required documentation, and returning these to the sponsor. The investigator must ensure that all drug supplies are kept in a secure location in the site pharmacy in accordance with recommended storage conditions. Inventory and ongoing record of test material supplies using the Drug Accountability Form provided within the Clinical Trial Material Tracking Module of the sponsor's RTSM system. This inventory record for the IP will include:

- Protocol name, number, and sponsor
- Kit number
- Study site and investigator name
- Use-by/Expiry/Retest date
- Name and title of qualified individual administering IP.

These records will be reviewed by representatives of the sponsor and may be reviewed by regulatory agencies.

5.1.6.1 Investigational Product Resupply

Investigational product will be automatically resupplied via the sponsor's RTSM system.

5.1.6.2 Used or Partially Used Investigational Product

Remaining unused IP kits will either be returned to sponsor or destroyed according to written investigational site procedures with a record of disposition maintained, per sponsor instructions.

5.2 Prior and Concomitant Medications

At the Screening Visit, the details of prior and concomitant medications taken within 30 days of the Screening Visit (or within 90 days for blood products, or within six months for immunosuppressive/immunomodulatory medications) will be collected. Concomitant medications will be collected at each onsite study visit or by phone interview (or EDV) for all groups. The details of all concomitant medications including those associated with solicited AEs and unsolicited AEs through Day 29 will be collected. Concomitant medications associated with SAEs, MAAEs, and/or AESI will be collected through the end of the study.

5.2.1 Prohibited and/or Restricted Medications

The history of all prohibited medications used at any time during study participation (regardless of association with an AE) will be collected. The sponsor MM should be consulted for any questions about prohibited or restricted medication usage.

Participants must not have received or be planning to receive:

- Systemic immunomodulatory or immunosuppressive medications from six months prior to screening through Day 22.
- Systemic corticosteroid use at a dose or equivalent dose of 20 mg of prednisone or more daily for 14 days or more within 90 days of screening through Day 22. The use of inhaled, intranasal, topical, or ocular, is allowed.
- Any vaccine from 30 days prior to Day 1 through Day 22.
- Blood or blood-derived products from 90 days prior to screening through Day 22.
- Receipt or anticipated receipt of immunoglobulin from 180 days prior to screening through Day 22.
- Investigational agents from 30 days prior to Day 1 through the duration of study participation.

Medically required concomitant medications should be used for the treatment of AEs regardless of whether their use will need to be documented as a protocol deviation (Section 10.7).

5.3 Procedures for Monitoring Participant Compliance

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

6 STUDY PROCEDURES AND ASSESSMENTS PER VISIT

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

Within 30 days prior to Day 1 of IP administration, eligible participants will first undergo informed consent counseling. Once written informed consent has been obtained, participants will undergo a screening visit to ascertain their eligibility in this study. The screening visit assessments will include:

- Informed consent (Section 12.1).
- Review of eligibility criteria (Section 3.2.1).
- Medical history including demographics (Section 3.2.2).
- Prior and current medications (Section 5.2).
- Vital signs: blood pressure, heart rate, respiratory rate, and temperature (Section 3.2.4).
- Complete physical examination including height and weight (general appearance, eyes-ears-nose-throat, head-neck, lungs-chest, heart, abdomen, musculoskeletal, lymph nodes, skin, extremities, and neurological assessment) (Section 3.2.3).
- Blood collection for laboratory testing for HIV, HBV, and HCV (Section 3.2.5).

Each participant who signs the ICF will receive a sequential identification (ID) number unique to the site number and the participant. When screening information is entered into the EDC system a participant will be assigned a participant ID number with the following format: 4-digit site number and 4-digit ID number, eg, 1111-1111. Participant numbers will be sequential since screen failed participants will be entered into the EDC system. Each site will maintain a screening enrollment log to record the enrollment or the reason(s) for screen failure for all participants who receive a participant ID number. Reason for screen failure will also be captured in the EDC disposition eCRF. Screening procedures are listed in the schedule of events (Table 1).

6.2 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 rescreened 14 days after resolution of their acute illness.
- If the participant tests positive for COVID-19 (using any type of test, even if asymptomatic) the participant can be rescreened after 14 days or after a negative

molecular test, eg, PCR, if outside of the initial screening window (within 30 days of Day 1).

- Participants who have received vaccines within 30 days of Day 1 may be rescreened provided no additional doses are anticipated up to Day 22.
- Participants who have received blood products or IP from participation in another clinical study that has ended may be rescreened after the appropriate duration has passed (90 days for blood products; 30 days for IP; six months prior to screening for systemic immunomodulatory or immunosuppressive medications).
- Eligible participants who are not able to be vaccinated within 30 days of their screening period may be rescreened.

Any other request for rescreening should be discussed with the sponsor MM.

Rescreening will involve undergoing 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.

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

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
- Targeted physical examination (as needed)
- Blood collection for anti-CHIKV SNA and CHIKV antibodies to neutralize strains representing all CHIKV genotypes (Section 3.2.6)
- Randomization (Section 3.4.2)
- Confirm and review any AEs that may have occurred after ICF signing
- Diary or memory aid (electronic or paper) training, ruler and thermometer distribution and training (Section 3.2.9)
- IP administration (Section 3.2.7)

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

- In-clinic acute observation (Section 3.2.8)
- Vital signs (Section 3.2.4)
- Solicited AEs, unsolicited AEs, MAAEs, SAEs and AESI evaluation (Sections 3.2.9, 3.2.10, 3.2.11)

6.4 Scheduled Study Visits

Visit 3, Day 15 (-1/+3 days):

- Review diary or memory aid (electronic or paper)
- PI assessment of reactogenicity (Sections 8.1.8, 8.1.9)
- Solicited AEs, unsolicited AEs, MAAEs, SAEs, and AESI evaluation (Sections 3.2.9, 3.2.10, 3.2.11)
- Review concomitant medications
- Targeted physical examination (as needed)
- Blood collection for anti-CHIKV SNA

Visit 4, Day 22 (-1/+3 days)

- Unsolicited AEs, MAAEs, SAEs, and AESI evaluation
- Review concomitant medications
- Targeted physical examination (as needed)
- Blood collection for anti-CHIKV SNA and CHIKV antibodies to neutralize strains representing all CHIKV genotypes

Visit 5, Day 29 (±1 day) (Telephone Contact)

- Unsolicited AEs, MAAEs, SAEs, and AESI evaluation
- Review concomitant medications

Visit 6, Day 92 (±3 days) (Telephone Contact)

- MAAEs, SAEs, and AESI evaluation
- Review concomitant medications (only if associated with MAAEs/SAEs/AESI)

6.5 Unscheduled Visits

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

6.6 End of Study Visit (Visit 7, Day 183 [-14/+7 days])

- MAAEs, SAEs, and AESI evaluation
- Review concomitant medications (only if associated with MAAEs/SAEs/AESI)
- Targeted physical examination (as needed)
- Blood collection for anti-CHIKV SNA and CHIKV antibodies to neutralize strains representing all CHIKV genotypes

6.7 Early Discontinuation Visit

For EDV occurring within seven days postvaccination, from seven to 21 days postvaccination, or ≥22 days postvaccination, the Visit 3, Visit 4, or Visit 7 schedule will be followed, respectively.

7 ASSESSMENT OF IMMUNOGENICITY

7.1 Anti-CHIKV SNA Analysis

The immune response to PXVX0317 will be measured by anti-CHIKV SNA analysis, for which serum samples will be collected on Day 1 predose, Day 15, Day 22, and Day 183 End of Study Visit (or at EDV).

The immunogenicity analysis will be performed using the validated human SNA assay, a high-throughput *in vitro* infectivity assay developed by the sponsor for assessing titers of neutralizing antibodies against CHIKV in serum samples. The induction of the humoral immune response (immunogenicity) by PXVX0317 vaccine, specifically anti-CHIKV SNA levels, will be measured by a bioluminescence assay 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 micro-plate 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.

7.2 Cross-reactive Neutralizing Antibodies to Various CHIKV Genotypes

Cross-reactive neutralizing antibodies to various CHIKV genotypes will be measured using FRNT50, as an exploratory objective. Foci from infected cells are enumerated following exposure to mixtures of virus and immune serum. The foci are identified by examining stained cells with antiviral antibodies and detected with a colorimetric substrate to horse radish peroxidase. Reduction of stained foci are quantitated using a plate reader and neutralizing data analyzed using GraphPad prism analysis software. Anti-CHIKV neutralization antibody titer is the reciprocal of the antibody dilution that protects a percentage of cells from infection compared to virus only control.

8 SAFETY ASSESSMENTS AND REPORTING

8.1 Definitions

8.1.1 Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation participant administered a medicinal 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 considered 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 PI (or designee) as not clinically significant 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, is considered an AE.*
- *Surgical procedures are not AEs. They are the action taken to treat a medical condition. The medical condition that was treated with surgery may be the AE depending on whether it occurred prior to IP treatment. 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, ER, urgent care clinic, or other visits to or from medical personnel for any reason. Routine scheduled study visits will not be considered medically attended visits.*

8.1.2 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/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"/Death not otherwise specified (Death NOS).*
- *Overnight stays at hospital/clinic that occurs during a trial for social reasons (eg, transportation difficulties, respite care) is not considered to be a hospitalization event.*

8.1.3 Adverse Drug Reaction

A response to a medicinal product which is noxious and unintended. Response in this context means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility.

8.1.4 Suspected Unexpected Serious Adverse Reaction

Suspected Unexpected Serious Adverse Reaction (SUSAR) is a serious, unexpected AE that is suspected of being related to the IP and occurring during the clinical study.

8.1.5 Adverse Events of Special Interest

An AESI is a noteworthy event for the particular product or class of products that a sponsor may wish to monitor carefully. It could be serious or nonserious and could include events that might be potential precursors or prodromes for more serious medical conditions in susceptible individuals.

In this study, new onset or worsening arthralgia that is medically attended 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, ER, urgent care clinic, or other visits to or from medical personnel. Routine scheduled study visits will not be considered medically attended visits.

8.1.6 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. Solicited AEs to be assessed for this study are local injection site events of pain, redness, and swelling at the injection site and systemic events of fever with oral temperature ≥38.0°C (≥100.4°F; if more than one temperature reading is taken, the highest reading obtained each day should be recorded), fatigue, chills, headache, myalgia, arthralgia, and nausea.

Solicited AEs will be recorded by the participants using a diary (electronic or paper) for seven days postvaccination.

8.1.7 Unsolicited Adverse Event

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

8.1.8 Severity of Adverse Events

The investigator will grade all AEs for severity. For the study Grading Scale see Appendix I.

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 at ER visit or hospitalization

- For solicited AEs, 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.
- Events reported as not present (0 mm is entered) will be reported as Grade 0.

8.1.9 Causality of Adverse Events

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

The following guidelines are provided for this assessment:

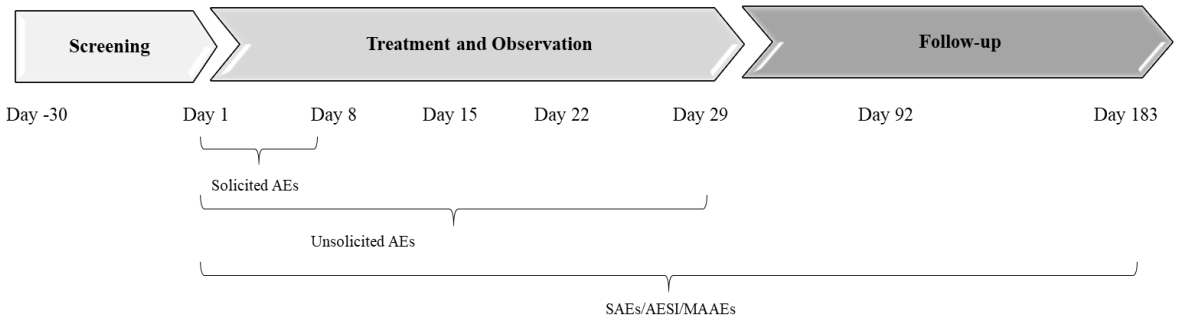
- **Unrelated:** No relationship between the IP and the reported event. The event will be considered "not related" to the IP.
- **Possibly related:** The event follows a reasonable temporal sequence from the time of administration of IP and/or follows a known response pattern to the IP but could also 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 PI's (or designee) clinical judgment the association of the event with the IP seems likely.
- **Definitely related**: A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to IP administration, and which cannot be explained by concurrent disease or other drugs or chemicals.

If the relationship between the AE and the IP is determined to be “possible” “probable,” or “definitely”, the event will be considered to be “related” to the IP.

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

For reporting of solicited AEs, unsolicited AEs, SAEs/AESI/MAAEs please see Sections 8.1.1, 8.1.5 – 8.1.7.

Figure 3 Adverse Event Period of Reporting



Occurrence of SAEs will be monitored throughout the study and will cover all participating individuals. Study participants will be provided with a 24-hour telephone number to contact study personnel in case of an untoward reaction.

8.2.1 Safety Laboratory Tests

Not applicable.

8.2.1.1 Drug Screen

Not applicable.

8.2.1.2 Alcohol Screen

Not applicable.

8.2.1.3 Viral Testing

At the Screening Visit, blood samples will be collected for serum testing for HBsAg, HCV antibody, and HIV-1/HIV-2 antigen/antibodies. If HCV antibody is positive HCV RNA testing can be performed.

8.3 Reporting Requirements for Immediately Reportable Events

8.3.1 Principal Investigator's Reporting Requirements

The following events must be reported within 24 hours of awareness by the PI (or designee) to the sponsor:

- Any SAE regardless of causal association with the IP.
- Any AESI regardless of causal association with the IP.

Applicable events must be entered into the validated EDC system. In the event of a system failure, the appropriate forms (listed below) will be completed and sent by email to the following address:

Global Pharmacovigilance Department
Emergent BioSolutions Inc.
[redacted]

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

For SAEs/AESIs, the Serious Adverse Event/Adverse Event of Special Interest Report Form will be completed (abbreviated hereafter SAE/AESI Report Form). The SAE/AESI Report Form is NOT the same as the AE eCRF. 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 IRB by the PI (or designee) as required by ICH GCP E6(R2).

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

8.3.2 Sponsor's Reporting Requirements

As specified in 21 CFR 312.32, SUSARs will be reported by the sponsor of the IP to the FDA and to all participating investigators in an individual case 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 the FDA no later than seven days after the sponsor's initial receipt of the information in accordance with 21 CFR 312.32(c)(2). All SAEs that are unexpected (e.g., adverse drug reactions) must be reported to the IRB as required by ICH GCP E6(R2).

8.4 Follow-up of Adverse Events

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

8.4.1 Follow-up of Nonserious Adverse Events

Nonserious AEs that are identified from the time IP administration through the 28-day post-IP administration period and are 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.

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

This study requires that participants be monitored for SAE/AESI up to Day 183 End of Study Visit.

The PI will provide or arrange appropriate care for participants for whom SAEs or a potential AESI are experienced. Withdrawal of participants from the study to treat SAEs/AESI is at the discretion of the treating PI. See Section 4.3 Participant Withdrawal Criteria.

All SAEs/AESI will be followed by the PI until at least one of the following conditions is met:

- The SAE/AESI is resolved or stable if expected to remain chronic.
- The participant is referred to a specialist or other physician for treatment and follow-up. The PI (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/AESI:

- Participant withdraws consent
- Participant is referred to appropriate long-term medical care
- Participant is considered LTF

It is expected that the investigational site will obtain supporting medical records from appropriate physicians and record this information on the SAE/AESI Report Form and AE eCRF. Additional information received related to any SAE/AESI must be forwarded within 24 hours of awareness to the Emergent Global Pharmacovigilance Department.

8.4.3 Follow-up of Pregnancies

Not applicable, as study population is ≥65 years of age.

8.4.4 Unanticipated Problems

For investigational sites in the US, 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.

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

8.5 Safety Data Monitoring

An independent SMC will provide safety oversight. The SMC will review aggregated, blinded safety data after the first 50 participants have completed at least seven days of safety follow-up. The remaining participants will be enrolled following the safety review by the SMC and based on the sponsor's consideration of the SMC's recommendation. Any further safety reviews will be at the discretion of the SMC and per the SMC Charter.

9 STATISTICAL CONSIDERATIONS

This section is a summary of the planned statistical analyses. The details are described in the study Statistical Analysis Plan (SAP).

9.1 Sample Size Rationale

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 nonevaluable participants, the power to show

superiority over placebo with 180 PXVX0317 vaccine and 180 placebo evaluable participants is >99.9% for the combined age groups.

The difference in seroresponse rate between PXVX0317 and placebo groups that is considered clinically relevant is [redacted]. With 180 baseline seronegative PXVX0317-treated participants and a target seroresponse rate of [redacted] vs a rate of 5% for placebo, the width of a two-sided 95% CI would be ±5.4%. If the target PXVX0317 seroresponse is [redacted], the width would be ±6.9%. Therefore, the difference in seroresponse rates must be above [redacted] for the lower bound of the 95% CI for the difference to be [redacted].

9.2 Analysis Populations

Analysis will be based on the following study populations:

Enrolled Population: All screened participants who sign an ICF, are entered in the EDC database, and meet all eligibility criteria.

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

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

Safety Population: All participants in the exposed population who provide safety assessment data. Safety endpoints will utilize the safety population, analyzed as treated.

Modified Intent-to-Treat population: All randomized participants who are vaccinated and have at least one postinjection anti-CHIKV SNA titer result, analyzed as randomized.

Immunogenicity Evaluable Population: All participants in the mITT population who: i) provide evaluable serum sample results for the Day 22 time point within the required time frame of Day 19 through Day 27, inclusive, ii) have no measurable anti-CHIKV SNA at Day 1, iii) have no important protocol deviation or other reason to be excluded as defined prior to unblinding. The IEP is the primary population for all immunogenicity analyses.

9.3 Statistical Methods

Tables will be displayed by treatment group (PXVX0317 and placebo) columns for the analysis populations described in Section 9.6.

Continuous endpoints will be summarized by descriptive statistics including number of nonmissing observations (n), mean, standard deviation, median, minimum, and maximum. In the analysis of the human SNA assay data as a continuous variable, anti-CHIKV SNA NT_{80} values will be logarithmically transformed ($base_{10}$), and the GMTs and associated 95% CIs for each treatment group will be computed by exponentiating the corresponding log-transformed means and two-sided 95% CIs. Categorical variables will be summarized by frequency counts (n) and percentages of participants (%) in each category, including missing or unknown when appropriate.

9.4 Multiplicity Controls

9.4.1 Coprimary Immunogenicity Endpoints

The familywise error rate will be fixed at a two-sided alpha of 0.05 by the requirement that both 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 both 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.

9.4.2 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 both 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 and 183 will be tested sequentially in order, each for the IEP across both age groups combined, as follows:

1. Day 15 seroresponse superior to placebo.
2. Day 183 seroresponse superior to placebo.

After testing the coprimary immunogenicity endpoints and, if both 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.

9.5 Handling of Missing Data

Participants with missing immunogenicity data at Days 15, 22, or 183 will be excluded from the corresponding analysis; missing immunogenicity data will not be imputed. Human SNA assay anti-CHIKV SNA NT_{80} 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 and for missing AE relatedness and/or severity will be described in the study SAP.

9.6 Planned Analyses

9.6.1 Study Population Characteristics

9.6.1.1 Participant Disposition

Participant disposition will be summarized for all screened participants who sign and date the ICF. Randomized participants will be displayed by treatment group, including number and percentages of participants still in the study at each scheduled visit, those competing the study and reason for not completing the study. Screen failure reasons will be listed by participant. The number and percentage of participants enrolled by site will be provided by treatment group for all randomized participants.

Randomization details including site, stratum, date and time of randomization, kit number, randomized treatment group assignment and actual treatment received will be listed by participant. Participants randomized but not treated and those receiving the wrong treatment will be listed. Participants who were randomized despite not meeting entry criteria and those who received an excluded concomitant medication will be listed.

9.6.1.2 Protocol Deviations

Protocol deviations will be categorized as important or not important and evaluated for exclusion of participants from analysis populations prior to database lock and unblinding. Important protocol deviations will be tabulated by category and by treatment group for the randomized population as well as listed by participant.

9.6.1.3 Populations Analyzed

The number and percentage of participants in each analysis population (see Section 9.2; randomized, exposed, safety, mITT, IEP) will be summarized by treatment group for all randomized participants. Reasons for exclusion from analysis populations will be summarized by treatment group for all randomized participants and listed by participant.

9.6.1.4 Demographics and Baseline Characteristics

Demographic and baseline characteristics including age, age stratum, sex, race, ethnicity, baseline height, weight, and BMI will be tabulated by treatment group for the randomized, safety, mITT, and IEP populations.

9.6.1.5 Medical History

Medical history will be coded to the MedDRA dictionary system organ class (SOC) and preferred term (PT). A summary table and a listing of medical history will be supplied by treatment group for the randomized population.

9.6.1.6 Prior and Concomitant Medications

Prior and concomitant medications and vaccines will be coded to the World Health Organization Drug Global Dictionary anatomic therapeutic chemical (ATC) classification and preferred drug name. All prior and concomitant medications and vaccines will be tabulated together by ATC classification, preferred drug name and treatment group for the safety population. A participant data listing of all prior and concomitant medications will be generated.

9.6.2 Immunogenicity Analyses

Summary statistics for immunogenicity results by scheduled visit for each treatment group will be provided for the IEP and mITT populations. Chikungunya virus SNA GMT will be tabulated by visit for each treatment group. Chikungunya virus SNA GMT will also be plotted over time for each treatment group for the IEP.

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 both 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.

9.6.2.1 Coprimary Immunogenicity Analysis

9.6.2.1.1 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 9.4) 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.

9.6.2.1.2 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.

The primary comparison will be in the IEP across both 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.

9.6.2.2 Secondary Immunogenicity Analysis

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

Seroresponse rates and seroresponse rate differences (PXVX0317 minus placebo) with associated 95% CIs based on antibody titers measured at Days 15 and 183 will be analyzed as described above for Day 22. 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.

9.6.2.2.2 Geometric Mean Titer at Days 15 and 183

For the comparison of PXVX0317 to placebo, GMTs based on antibody titers measured at Days 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.

9.6.2.2.3 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 using the IEP. The significance of the treatment group difference will be assessed using chi-square tests at each visit for the IEP based on both age groups combined with an alpha=0.05.

9.6.2.3 Geometric Mean Titer for Different CHIKV Genotypes at Day 22 for PXVX0317 Group

This exploratory endpoint will be evaluated in the CHIKV genotype subset population for both age strata combined.

9.6.3 Investigational Product Administration and Treatment Compliance

Investigational product administration data will be summarized for the randomized population and listed by participant.

Treatment compliance is not applicable since this study involves a single, in-clinic IP administration.

9.6.4 Safety Analyses

The safety of PXVX0317 in adults ≥65 years of age will be evaluated using solicited AEs occurring from IP administration on Day 1 until Day 8 and unsolicited AEs from Day 1 through Day 29, AESI, MAAEs, and SAEs though Day 183 End of Study Visit, and vital signs.

9.6.4.1 Adverse Events

Adverse events will be coded to the MedDRA dictionary SOC and PT. They will be graded for severity according to Section 8.1.8. Only treatment-emergent AEs will be collected (ie, those after the start of IP administration). Adverse events will be reported using both age strata pooled, but selected AE analyses will be repeated for the separate age strata. All AE tabulations and participant listings will be based on the safety population.

Solicited AEs, unsolicited AEs, MAAEs, AESI, and SAEs will be summarized separately by treatment group and SOC, PT, and maximum severity for the safety population for both age strata combined. Only participants with at least one solicited AE observation (ie, any nonmissing values but excluding "not done/unknown") will be summarized. Solicited AEs continuing beyond seven days after IP administration will be combined with the unsolicited AEs; a combined summary table of solicited and unsolicited AEs by treatment group and SOC, PT, and maximum severity will also be provided for the safety population for both age strata pooled.

Additional AE displays will include the following:

- Solicited AEs ≥Grade 3
- Treatment-related ("possibly", "probably" or "definitely") solicited AEs
- Solicited AEs: day of first onset, duration, day postinjection, and occurrence of at least one solicited AE by category (local, systemic)
- Unsolicited AEs ≥Grade 3
- Treatment-related ("possibly", "probably" or "definitely") unsolicited AEs

- Medically attended AEs
- Treatment-related MAAEs
- AEs leading to study discontinuation
- Fatal AEs, if any
- Treatment-related ("possibly", "probably" or "definitely") SAEs

Separate participant data listings of solicited and unsolicited AEs sorted by participant identifier and AE start date will be generated. Diary compliance will not be summarized.

9.6.4.2 Vital Signs

Pre- and post-IP administration vital signs data will be listed by participant, visit, and time point for each treatment group for the safety population.

9.6.5 Subgroup Analyses

Subgroup analyses of key safety and immunogenicity endpoints may be performed by age subgroups (65 to <75 and ≥75 years of age), sex, race, ethnicity, and baseline serostatus (if more than 10% of participants are seropositive at baseline).

9.6.6 Planned Interim Analyses and Criteria for Study Termination

The SMC (Sections 3.5 and 8.5) will review aggregated, blinded safety data after the first 50 participants have completed seven days of safety follow-up and periodically thereafter as per SMC recommendation according to the SMC Charter.

There will be a safety and immunogenicity preliminary analysis on data for all participants through the Day 29 Visit to facilitate health authority presubmission preparation. The analyses will be performed by a third-party vendor and results will be reported only at the treatment group summary level preserving the double-blind status on the participant level. No p-value penalty will be assessed because the Day 22 primary immunogenicity endpoint data will be final at the time of preliminary analysis and no action regarding the study will be made based on these findings.

10 DATA HANDLING AND RECORD KEEPING

10.1 Source Documents and Access

Source data are all information, original records of clinical findings, and observations in a clinical trial necessary for the reconstruction and evaluation of the trial. 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 PI/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 PI/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 the sponsor (or designee), the IRB, the FDA, EMA, other government agencies as appropriate and will not otherwise be released except as required by law. Refer to Section 12.5 Participant Confidentiality. All information provided to the PI by the sponsor is to be considered confidential unless otherwise stated.

10.2 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).

10.3 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.

10.4 Laboratory Data

No external laboratory data transfers will be done. Sites will manually enter results of HBsAg/HCV antibody/HIV-1/HIV-2 into applicable eCRFs. Chikungunya virus SNA titer results will be handled by the sponsor.

10.5 File Management at the Investigational Site

The PI will ensure that the essential study documents are maintained in accordance with the ICH GCP Guidelines and as required by applicable local and federal regulations. The PI/institution will take measures to prevent accidental or premature destruction of these documents.

10.6 Records Retention at the Investigational Site

Per ICH 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 PI 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 PI 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 its designee[s]) and relevant regulatory agencies. If the PI withdraws from the study (eg, due to relocation or retirement), all study-related records will be transferred to a mutually agreed upon designee within the sponsor's specified timeframe. Notice of such transfer will be given to the sponsor in writing.

10.7 Deviations from the Protocol

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

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the protocol.

The PI or investigational 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 PI 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.

11 QUALITY CONTROL AND ASSURANCE

11.1 Monitoring

The assigned clinical study monitor will verify eCRF 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 PI must make source documentation accessible to the study monitor as needed to verify the information in the eCRFs. The PI 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.

11.2 Auditing

The sponsor's Quality Assurance Department (or designee) may conduct investigational site audits before study initiation, during the study, or after study completion. Audits will include, but are not limited to, review of source documents, verification of eCRFs against source documents and review of essential documents to ensure compliance with protocol and applicable local and federal regulations. The PI agrees to participate in site audits and assist in the prompt resolution of any issues found during audits.

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

12 ETHICS AND RESPONSIBILITY

This study must be conducted in accordance with the ethical principles described in the current Declaration of Helsinki, and in compliance with the protocol, current ICH GCP Guidelines, and applicable local and federal regulations, and all other applicable local laws. 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 PI and

investigational site staff will take measures to ensure adequate care in protecting participant privacy. To this end, a participant ID number will be used to identify each participant.

12.1 Informed Consent

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout their study participation.

The sponsor (or designee) will generate and provide a master ICF template to each investigational site for development of a site-specific ICF.

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

The participant will be asked to read and review the document. The PI will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

12.2 Institutional Review Board

Before the start of the study, the protocol, the IB, proposed ICF(s), participant compensation (if any), sponsor-approved study materials and advertisements, and any other written information to be provided to the participant, and applicable amendments, 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 and shipment of PXVX0317.

The PI (or designee) is responsible for informing and obtaining appropriate approval from the IRB of any amendment to the protocol or ICF in accordance with local requirements.

The PI (or designee) is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the IP, if required by the IRB. The sponsor will provide this information to the PI.

Initial IRB approval, and all materials approved by the IRB for the study including the ICF and recruitment materials, must be maintained by the PI and made available for inspection.

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

12.3 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. Compensation for injury will be described in the ICF.

12.4 Documentation Required Prior to Study Initiation

The PI (or designee) is responsible for forwarding the following documents to the sponsor for review prior to study initiation:

- Signed protocol signature page, form FDA 1572 (or equivalent, depending on applicable local and federal regulations), financial disclosure form (principal and subinvestigators), Clinical Trial Agreement, and any other required regulatory documents.
- Copy of IRB-approved ICF and assent form (as applicable).
- Copy of the written IRB approval for the following documents: protocol, IB, ICF, participant compensation (if any), any study materials and advertising, and any other written information or tools (including diaries) to be provided to the participant.
- Current signed and dated Curriculum Vitae and a copy of medical license (if applicable) of the PI and subinvestigator, and other investigational site personnel as required by the sponsor.
- Written statement that the IRB is properly constituted and operates according to ICH GCP Guidelines and applicable local and federal regulations. Investigators participating in this study, if IRB members, should state in writing that they have abstained from voting in regard to this protocol.
- Laboratory normal ranges and documentation of laboratory certification (as applicable).

12.5 Participant Confidentiality

The PI 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 eCRF or on any other study documents provided to the sponsor (or designee(s)). 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 investigational site.

Based on ICH GCP Guidelines and regulatory requirements, the PI is required to allow authorized personnel of the sponsor (or its designee), the IRB, and members of the appropriate regulatory authority(ies) to review participant's files that are related to study EBSI-CV-317-005. Participants must be informed that their records may be reviewed by the sponsor, its designee(s), the IRB and the appropriate regulatory authority(ies) through direct access to the participant's original medical records.

Redacted copies of participant medical records that are related to study EBSI-CV-317-005 may be collected by the sponsor for the purposes of AE follow-up and reporting, and/or monitoring. Redacted copies of medical records may be transferred via encrypted email (AES or triple DES); via secure fax line; or uploaded to a secure, encrypted password-protected environment hosted by a third-party service provider to which only assigned site personnel and monitors have access, with a list of users maintained for the duration of the study and audit trails available on all activities.

Copies of redacted medical records related to monitoring will be permanently destroyed at study closure.

12.6 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.

13 ADMINISTRATIVE AND LEGAL REQUIREMENTS

13.1 Sponsorship

This clinical study is sponsored by Emergent Travel Health Inc., [redacted] who is the manufacturer of PXVX0317.

13.2 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 PI, the sponsor, and the IRB prior to implementation. The PI must receive written IRB approval for all protocol amendments prior to implementing protocol amendments at the investigational site; copies of IRB correspondence including approval/disapproval letters from the IRB must be provided to the sponsor.

However, 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).

13.3 Clinical Study Registration

The sponsor is responsible for clinical trial registration and reporting to Clinicaltrials.gov and other registries in accordance with applicable regulations.

13.4 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 a PI (or a number of PIs) 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 investigational site(s) PI(s) 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 ClinicalTrials.gov (or other applicable registries) 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.

14 REFERENCES

1. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond). 1956;54(2):177-191.
2. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol. 2007;88(Pt 9):2363-2377.
3. CDC. Geographic Distribution: countries and territories where chikungunya cases have been reported [Internet]. Centers for Disease Control and Prevention (US); 2020Oct30 [cited 2021Jul13]. Available from: https://www.cdc.gov/chikungunya/geo/.
4. Staples JE, Hills SL, Powers AM. CDC yellow book [Internet]. Centers for Disease Control and Prevention (US); revised 2020Jan10. Chapter 4, Travel-related infectious diseases: chikungunya; [cited 2019 Sep 26]; p.78. Available from: https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/chikungunya.
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):1401-1407.
6. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis. 2007;7(5):319-327.
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):2011-2014.
10. Gérardin P, Fianu A, Malvy D, Mussard C, Boussaïd K, Rollot O, et al. Perceived morbidity and community burden after a chikungunya outbreak: the TELECHIK survey, a population-based cohort study [Internet]. BMC Med. 2011;9(5). Available from: https://doi.org/10.1186/1741-7015-9-5.
11. EBSI, Emergent BioSolutions Inc. PXVX-EPI-CV-001 Pediatric Dengue Cohort Study in Nicaragua 2019.

12. 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.

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 Identifier: NCT01489358. 2016 [cited 2019 September 26]. Available from: https://clinicaltrials.gov/ct2/show/record/NCT01489358.

14. 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.

15. 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. 2019 [cited 2019 September 26]. Available from: https://clinicaltrials.gov/ct2/show/record/NCT02562482?term=VRC+704&rank=1.

16. 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.

17. 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.gov Identifier: NCT03483961. 2018 [cited 2019 September 26]. Available from: https://clinicaltrials.gov/ct2/show/NCT03483961.

18. Mendy J. Poster: Development of a high-throughput luciferase-based chikungunya neutralization assay (CHIKV-luc). ASTMH 2018. Emergent Travel Health Inc.

19. 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 Identifier: NCT03992872. 2020. Available from: https://www.clinicaltrials.gov/ct2/show/NCT03992872.

20. 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.

15 APPENDICES

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 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	--
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 intravascular coagulation (DIC)

WBC - White blood cell count
*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: 31 Mar 2023

Document Approval Task Verdict: Approve	[redacted] Scientist, Clinical Research
Document Approval Task Verdict: Approve	[redacted] Sr Director, Biostatistics & Data Sciences
Document Approval Task Verdict: Approve	[redacted] Sr. Director, Clinical Development
Document Approval Task Verdict: Approve	[redacted] VP, Clinical Development