Title: A Phase 2 Open-Label Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV VLP], Aluminum Hydroxide Adjuvanted)

NCT number: NCT05065983

Document: EBSI-CV-317-010 Protocol

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CLINICAL STUDY PROTOCOL

A Phase 2 Open-Label Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus-Like Particle Vaccine [CHIKV VLP], alum-adjuvanted)

Protocol Number: EBSI-CV-317-010

Version 4.0

[redacted]2021

Sponsor: Emergent Travel Health, Inc.

[redacted]

CONFIDENTIAL

SPONSOR SIGNATORY

Signatory: *See electronic signature page*

[redacted]

Date (DDMMMYYYY)

[redacted]

Emergent BioSolutions Inc.

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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-317-010, Version 4.0:	A Phase 2 Open-Label Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus-Like Particle Vaccine [CHIKV VLP], alum-adjuvanted)
Clinical Site(s):	**Institution Name:** **Institution Address:**

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

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

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] Investigator Signature [redacted] Date (DDMMMYYYY)

[redacted]

PROTOCOL SYNOPSIS

Name of Sponsor/Company: Emergent Travel Health, Inc.	
Name of Investigational Product: PXVX0317	
Name of Active Ingredient(s): PXVX0317 vaccine is comprised of field-formulated CHIKV VLP 40 micrograms (µg) with 300 µg Alhydrogel® 2% administered via intramuscular (IM) injection in the deltoid muscle.	
Protocol Number: EBSI-CV-317-010	
Title of Study: A Phase 2 Open-Label Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus-Like Particle Vaccine [CHIKV VLP], alum-adjuvanted)	
Study Center(s): Single center in the United States of America (US)	
Study Duration for Each Subject: 7 months **Estimated Study Duration:** 8 months **Estimated Enrollment Period:** 1 month **Anticipated Start Date:** [redacted] **Estimated End Date:** [redacted]	**Phase of Development:** 2

Objectives

Primary Objectives:

- To assess the induction of anti-CHIKV neutralizing antibody responses following a single adjuvant dose of PXVX0317 (40 µg CHIKV VLP adjuvanted with 300 µg Alhydrogel) as measured 21 days (Day 22) after vaccination
- To assess the induction of anti-CHIKV neutralizing antibody responses following a single adjuvant dose of PXVX0317 as measured 7 days (Day 8), 14 days (Day 15), and 56 days (Day 57) after vaccination

Secondary Objective:

- To assess the safety of a single dose of PXVX0317 in healthy adults

Exploratory Objectives:

- To obtain plasma and sera at Days 22 and 57 from subjects immunized with a single dose of PXVX0317 to support nonclinical studies
- To characterize the kinetics of anti-CHIKV IgM, IgG, and neutralizing responses following a single dose of PXVX0317 as measured 7, 14, 21, and 56 days (Days 8, 15, 22, and 57 respectively) after vaccination

- To characterize T and B cell responses following a single dose of PXVX0317 with collections of peripheral blood mononuclear cells (PBMCs) 7, 14, 21, and 56 days (Days 8, 15, 22, and 57) after vaccination

Methodology

Study Design: This is an open-label, single arm Phase 2 study in healthy adults 18 to 45 years of age. Approximately 25 subjects are targeted for enrollment.

This study has a screening period of 30 days, a treatment and observation period from Day 1 to Day 22, and a follow-up period through Day 183. Subjects will receive CHIKV VLP at a dose of 40 µg in combination with 300 µg Alhydrogel on Day 1, and have follow-up visits on Day 8, Day 15, and Day 22. A plasmapheresis procedure and collection of serum will occur on Day 22. A phone call follow-up visit will occur on Day 29, followed by a second plasmapheresis procedure and collection of serum at Day 57. A phone call follow-up visit to assess safety and concomitant medications will occur on Day 64. An end of study (EOS) phone call follow-up visit to assess safety and concomitant medications will occur on Day 183.

Details of visits, visit windows, and procedures are provided in the Schedule of Events below. After signing the informed consent form, subjects will undergo screening procedures up to 30 days before a single-dose injection on Day 1. Subjects will be observed in clinic for 30 minutes after injection and vital signs obtained at least 30 minutes but no more than 60 minutes after injection. Local and systemic solicited adverse events (AEs) occurring within 7 days after injection (to Day 8) will be recorded by the subject using a memory aid. Subjects will be specifically asked to record local injection site events (pain, redness, swelling) and systemic events (oral temperature ≥100.4º F, chills, fatigue, headache, myalgia, joint pain, and nausea). Memory aids will be reviewed at the Day 8 visit. Unsolicited AEs will be monitored from Day 1 through Day 29; serious adverse events (SAEs) and adverse events of special interest (AESI) will be monitored for the duration of the study (to Day 183). Information on all concomitant medications used through Day 29 will be recorded; after Day 29, only information on concomitant medications used for the treatment of an SAE or AESI will be recorded. After screening, blood will be collected for immunogenicity assessments at Days 1 (pre-vaccination), 8, 15, 22, and 57.

Subjects who discontinue study participation before Day 22 (or who do not undergo Day 22 plasmapheresis for other reasons) may be replaced.

A final analysis of data collected throughout the study from all subjects will be performed after the last subject has completed the study and the immunogenicity and safety data have been cleaned and locked.

Serology: Serum neutralizing antibody (SNA) responses to CHIKV VLP will be determined by a luciferase based anti-CHIKV neutralization assay (CHIKV-luc). Titers are expressed as the reciprocal of the serum dilution achieving 80% neutralization (NT_{80}). Anti-CHIKV specific IgG and IgM antibody levels will be determined by enzyme-linked

immunosorbent assay (ELISA). Peripheral blood mononuclear cells will be collected for exploratory analyses of T and B cell immune responses.

Number of Subjects (Planned): 25

Study Population: Healthy adults 18 to 45 years of age

Inclusion Criteria:

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

1. Able and willing to provide informed consent voluntarily signed by subject.
2. Any gender, 18 to 45 years of age (inclusive).
3. Generally healthy, in the opinion of the Investigator, based on medical history, physical examination, and screening laboratory assessments.
4. Women who are **either**:
   i. Not of childbearing potential (CBP): pre-menarche, anatomically sterile, or post-menopausal (defined as ≥12 months without menses).

   or:

   ii. Meeting **all** the below criteria:
   - Negative urine pregnancy test at screening visit **and**
   - Negative urine pregnancy test immediately prior to dosing at Day 1 **and**
   - Using an acceptable method of contraception (if female of childbearing potential) for the duration of participation, such as:
     - Hormonal contraceptives (e.g., implants, pills, patches) initiated ≥30 days prior to dosing or
     - Intrauterine device (IUD) inserted ≥30 days prior to dosing or
     - Double barrier type of birth control (male condom with female diaphragm, male condom with cervical cap).

Exclusion Criteria:

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

1. Currently pregnant, breastfeeding, or planning to become pregnant during the study.
2. Body Mass Index (BMI) ≥35 kg/m^2.
3. Positive laboratory evidence of current infection with human immunodeficiency virus (HIV-1, HIV-2), hepatitis C virus (HCV) or hepatitis B virus (HBV).
4. History of severe allergic reaction or anaphylaxis to any component of the investigational product (IP).

5. History of known congenital or acquired immunodeficiency that could impact response to vaccination (e.g., leukemia, lymphoma, generalized malignancy, functional or anatomic asplenia, alcoholic cirrhosis).
6. Prior or anticipated receipt of immunomodulatory or immunosuppressive therapy from six months prior to screening through Day 64.
7. Receipt or anticipated receipt of blood or blood-derived products from 90 days prior to screening through Day 64.
8. Acute disease within the last 14 days (subjects with an acute mild febrile illness can be considered for a deferral of vaccination two weeks after the illness has resolved and treatment has been completed).
9. Clinically significant cardiac, pulmonary, respiratory, rheumatologic, or other chronic disease, in the opinion of the Investigator. This may include chronic illness requiring hospitalization in the last one month prior to screening.
10. Enrollment in an interventional study and/or receipt of another investigational product from 30 days prior to screening through the duration of study participation.
11. Receipt or anticipated receipt of any vaccine from 30 days prior to screening through Day 64.
12. Prior receipt of an investigational CHIKV vaccine/product.
13. Detectable baseline anti-CHIKV IgG antibody as determined by ELISA.
14. Any other condition that, in the opinion of the Investigator, could adversely impact the subject's participation or the conduct of the study, creates an unacceptable risk to the subject, or may interfere with the conduct of the study or validity of the data.
15. Restricted venous access that would prevent the collection of plasma and serum necessary for participation.
16. Weight <110 pounds.

Investigational Product (IP), Dosage and Mode of Administration: PXVX0317 vaccine is comprised of field-formulated CHIKV VLP 40 µg with 300 µg Alhydrogel 2% administered via intramuscular (IM) injection in the deltoid muscle.

Reference Therapy, Dosage and Mode of Administration: There will be no control group for this study.

Study Endpoints/Outcome Measure(s)

Primary: The geometric mean titer (GMT) of anti-CHIKV neutralizing antibody determined by luciferase-based CHIKV-luc assay at Day 22.

Immunogenicity Endpoint: Anti-CHIKV neutralization response data will be evaluated from Day 1 through Day 57. The primary analysis will be based on anti-CHIKV neutralization titers measured at 21 days after injection (Day 22) for the modified intent to

treat (mITT) population, defined as all subjects who are vaccinated and have at least one post-injection anti-CHIKV SNA NT_{80} result.

The derived immunogenicity endpoints are listed briefly below.

Geometric Mean Titer (GMT)*:* Anti-CHIKV neutralization titers will be logarithmically transformed (base10). The GMTs and associated 95% confidence intervals (CI) will be computed by exponentiating the corresponding log-transformed means and 95% confidence limits.

Proportion (percentage) of subjects with anti-CHIKV titer exceeding defined cut-off values: The proportion (percentage) of subjects with antibody responses exceeding stipulated cut-off values (e.g., titers of 15 or 100), together with a two-sided 95% CI based on the Wilson method, will be tabulated at all serum collection time points (Days 8, 15, 22, and 57).

Proportion (percentage) of subjects with at least a four-fold rise over baseline in anti-CHIKV titer: The proportion (percentage) of subjects with post-vaccination titers at least four times higher than pre-vaccination, together with a two-sided 95% CI based on the Wilson method, will be tabulated at all serum collection time points (Days 8, 15, 22, and 57).

Safety Endpoints: Subjects who receive a study injection will have data collected for safety analysis. The safety objectives will be assessed by analysis of the incidence rate for all local and systemic post-injection solicited events and other AEs collected during the 7 days following injection. Safety analysis will also include the occurrence of any unsolicited AEs through Day 29, and SAEs and AESIs collected through Day 183 end of study. In addition, any AEs occurring within 30 minutes of the plasmapheresis procedures at Days 22 and 57 will be collected. Data listings will include all subjects, including screen failures. The Principal Investigator will assess causality and severity.

Statistical Methods

Sample Size Considerations: The sample size (N=25) is based on the number of subjects needed to provide adequate plasma and sera for non-clinical studies.

SCHEDULE OF EVENTS

Visit	Screen (Visit 1)	Day 1 (Visit 2)	Day 8 (Visit 3)	Day 15 (Visit 4)	Day 22 (Visit 5)	Day 29 (Visit 6) (phone call)	Day 57 (Visit 7)	Day 64 (Visit 8) (phone call)	Day 183 (Visit 9) (phone call) EOS Visit	Early D/C
Window	**-30 days**	**0**	**+3d**	**±2d**	**+5d**	**-1/+5d**	**+5d**	**-1/+5d**	**-14/+7d**	**n/a**
Informed Consent	X									
Medical History	X	X[5]								
Demographics	X									
Physical Exam[1]	X									
HBsAg, anti-HCV[12], HIV 1/2 Ag/Ab[13]	X									
CHIKV IgG Antibody ELISA (kit at site)[13]	X									
Inclusion/Exclusion Criteria	X	X[5]			X[8]		X[8]			
Vital Signs	X	X[3]								
Pregnancy Test[4]	X	X[5]			X		X			X[2]
Study Vaccine Administration		X								
Acute Observation (30 min)[9]		X			X		X			
Issue Memory Aid, Thermometer & Ruler		X								
Review Memory Aid			X							X[6]
Adverse Event Evaluation[10]		X	X	X	X[7]	X	X[7]	X	X	X
Prior/Con Med Evaluation[11]	X	X[3]	X	X	X	X	X	X	X	X
Anti-CHIKV Neutralization (Serum)[13]		X[5]	X	X	X		X			X

PBMC Collection (B+T cells)[13]		X[5]	X	X	X		X			X
Blood for ELISA (IgM/IgG)[13]		X[5]	X	X	X		X			X
Plasmapheresis and Serum Collection (for nonclinical studies)[13]					X		X			

D/C, discontinuation; n/a, not applicable; HBsAg, Hepatitis B surface antigen; anti-HCV, Hepatitis C virus antibody; HIV1/2 Ag/Ab, human immunodeficiency virus antigen/antibody; CHIKV, chikungunya virus; Con Med, concomitant medication; ELISA, enzyme-linked immunosorbent assay; min, minute; PBMC, peripheral blood mononuclear cells; EOS, End of Study.

[1]Complete physical examination at Screening Visit only; Directed physical examination, if indicated by updated medical history at Visit 2 and as needed in subsequent visits.

[2]Only if before Day 57.

[3]To be taken prior and after Study Vaccine administration.

[4]Urine pregnancy test at Screening, prior to Study Vaccine administration and prior to plasmapheresis.

[5]Done pre-vaccination.

[6]If the visit occurs within seven days after Study Vaccine administration.

[7]Adverse events occurring 30 mins post plasmapheresis at Day 22 and 57 will be collected.

[8]Confirmation of continued eligibility (inclusion/exclusion criteria met) prior to plasmapheresis procedure.

[9]Subjects will be monitored by study staff for signs of an acute adverse reaction for 30 mins after injection but no more than 60 mins after injection.

[10]Solicited AEs from Days 1 to 8, Unsolicited AEs to Day 29; After Day 29, collect SAE and AESI only for the duration of the study (Day 183 EOS).

[11]After Day 29, concomitant medications are collected only for SAEs and AESI.

[12]If anti-HCV positive HCV RNA testing will be performed.

[13]Table summarizing bloodwork by procedure, assay, collection tubes, and the total volume of plasma and/or serum by visit is provided in Appendix I.

TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
Ag/Ab	Antigen/Antibody
BMI	Body Mass Index
CBP	Childbearing Potential
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHIKV	Chikungunya Virus
CHIKV VLP	Chikungunya Virus-Like Particle
CI	Confidence Interval
CS	clinically significant
ºC	Degrees Celsius
ºF	Degrees Fahrenheit
DMP	Data Management Plan
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
EOS	End of Study
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
HBSag	Hepatitis B Surface antigen
HCV	Hepatitis C Virus
HEK	Human Embryonic Kidney
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IM	Intramuscular
IND	Investigational New Drug
IP	Investigational Product
IPD	Important Protocol Deviation
IRB	Institutional Review Board

IUD	Intrauterine Device
LLOQ	Lower Limit of Quantitation
MedDRA	Medical Dictionary of Regulatory Activities
µg	Microgram
mL	Milliliter
mITT	Modified Intent-to-Treat Population
MM	Medical Monitor
NIH	National Institutes of Health
NT	Neutralization Titer
PBMC	peripheral blood mononuclear cells
PFU	Plaque Forming Unit
PT	Preferred term
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SNA	Serum Neutralizing Antibody
SOC	System Organ Class
SUSAR	Serious and Unexpected Suspected Adverse Reactions
VLP	Virus-Like Particle
VRC	Vaccine Research Center
US	United States of America
WHO	World Health Organization
w/w	Weight per Weight

1 BACKGROUND INFORMATION

1.1 Name and Description of Investigational Product

PXVX0317 is a chikungunya virus-like particle (CHIKV VLP) vaccine. The vaccine is comprised of CHIKV VLP adsorbed on Alhydrogel 2% adjuvant (2% weight per weight [w/w]) aqueous suspension of aluminum hydroxide) and stabilized with formulation buffer, which is field-formulated as a single dose of 0.8 milliliters (mL) in a syringe to be administered intramuscularly (IM) in the deltoid muscle. For additional product information, see Section 5.1 and the PXVX0317 Investigator's Brochure (IB) (1).

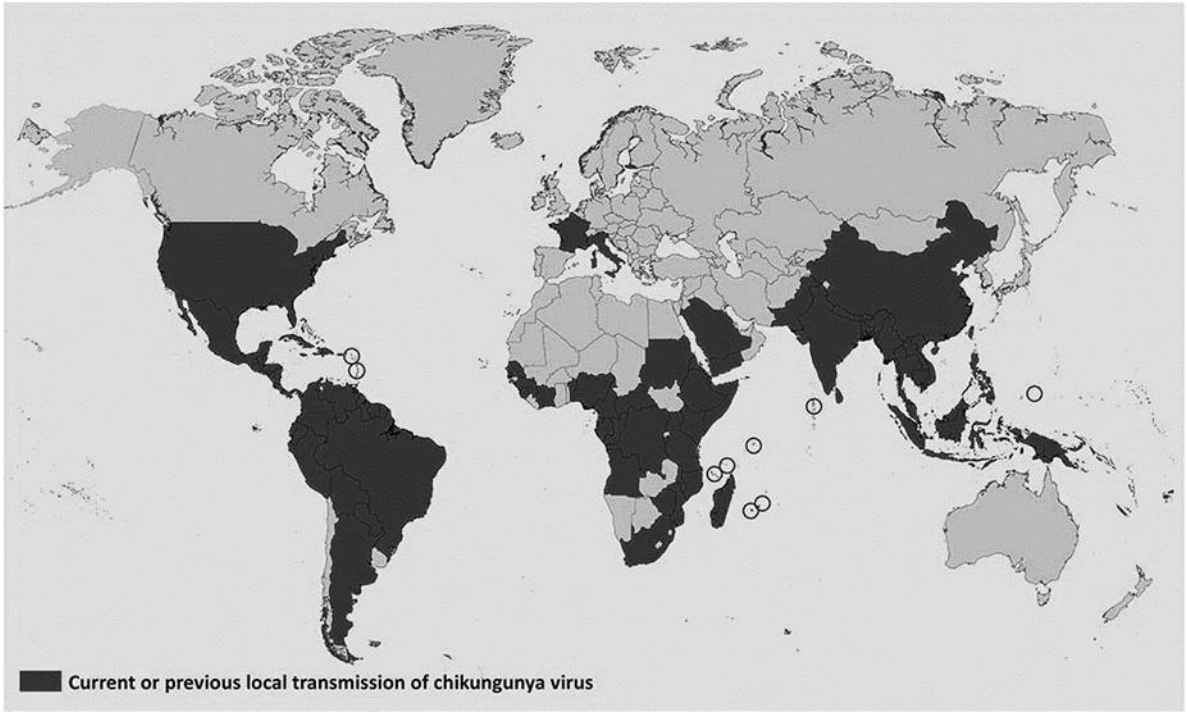
The target indication for PXVX0317 vaccine is active immunization to prevent disease caused by chikungunya virus.

1.2 Chikungunya Virus and Disease Background

Chikungunya virus is an arthropod-borne alphavirus of the family *Togaviridae*. The CHIKV virion contains a positive-sense single-strand ribonucleic acid (RNA) genome with a long open reading frame coding for capsid (C) and envelope (E1, E2, E3, and 6K) structural proteins, together with four non-structural proteins (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 (2), this disease has been endemic in Africa and parts of Asia with transmission to humans occurring through *Aedes aegypti* and more recently via *Aedes albopictus* mosquitoes (3).

Beginning in 2014, CHIKV disease cases were reported among United States of America (US) travelers returning from affected areas in the Americas and local transmission was identified in Florida, Puerto Rico, and the US Virgin Islands. According to the US Centers for Disease Control and Prevention (CDC) as of September 17, 2019, 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 (4)). 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 (5).

Figure 1 Chikungunya virus Global Burden



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 (6, 7, 8). The most classic symptom of CHIKV is a debilitating polyarthralgia that is present in greater than 90% of cases (9). This acute phase resolve within several weeks, but joint pain and arthritis may persist for months or years in over 25% to 40% of infected individuals (10).

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

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

1.3 Justification for Use of Investigational PXVX0317 Vaccine

The National Institutes of Health (NIH) Vaccine Research Center (VRC) initiated the development of the CHIKV VLP vaccine, designated VRC-CHKVLP059-00-VP. The VRC completed Phase 1 (VRC 311) (13, 14) and Phase 2 (VRC 704) (15, 16) clinical studies (15, 16). 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). [redacted] was acquired by

Emergent BioSolutions, Inc. in [redacted] has been renamed Emergent Travel Health Inc. (Emergent), and was the Sponsor of two Phase 2 studies (PXVX-CV-317-001 (17), EBSI-CV-317-002 (18), and this Phase 2 study (EBSI-CV-317-010).

1.3.1 Summary of Animal Studies

The Sponsor conducted a study in mice that demonstrated comparability between the immune responses induced by the VRC-CHKVLP059-00-VP and PXVX0317 vaccines. A model development challenge study was conducted in cynomolgus macaque nonhuman primates (NHPs) to determine the appropriate CHIKV challenge dose to induce symptoms of arthralgia. This study revealed that high CHIKV challenge doses induced inflammation of the joints and surrounding tissues in infected animals, and a scoring system was developed to measure the degree of joint infiltration. Subsequently, an active vaccination and challenge study was conducted with the PXVX0317 vaccine. NHPs 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 high-dose challenge with 10^7 plaque forming units (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 the CHIKV-luc assay, even after a single immunization. Importantly, the addition of alum increased the pre-challenge SNA titers compared to the VLP vaccine without alum. Furthermore, vaccination completely protected NHPs from infectious viremia, as measured by plaque assay on serum from challenged animals, which measures replicating virus. Joint pathology scores in the active vaccination study demonstrate that vaccination with PXVX0317 protects NHPs from joint infiltration in a dose-dependent manner. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis, which measures presence of viral RNA, showed significantly lower levels of viral RNA in plasma and joint tissues of vaccinated animals compared with animals that had received only alum. Taken together, these data suggest that vaccination induced anti-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 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 LR2006-OPY1 challenge. The in-life portion of the study has been completed. All three dose levels of IgG from plasma of PXVX0317 vaccinated subjects completely protected NHPs from infectious viremia as measured by plaque assay. IgG administration also appeared to protect NHPs from joint pathology and viral RNA in plasma, as measured by RT-PCR, in a dose-dependant manner. IgG also protected NHPs from the presence of viral RNA in joint tissues in a non-dose-dependant manner.

1.3.2 Summary of Findings from Clinical Studies

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 ELISA (13, 14). Post-hoc analysis of SNA by a luciferase-based assay was also performed by the Sponsor (19).

All injections were well tolerated, with no Serious Adverse Events (SAE) 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 (CHIKV-luc) (19), confirming both the immunogenicity of the VLP and the suitability of CHIKV-luc for future studies (see Section 6.1 for assay details).

VRC 704

The NIH's 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 subjects receiving 20 µg of CHIKV VLP and 200 receiving placebo in a 2-dose series at Weeks 0 and 4. The study was initiated in 2016 and completed in 2018. Approximately 20% of subjects demonstrated detectable 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. CHIKV VLP appeared safe and well tolerated in subjects who were followed through Week 72, with no related SAEs or other safety concerns (15, 16). CHIKV VLP appeared highly immunogenic, with a GMT of 2004.5 and 99.5% of recipients having neutralizing antibodies at Week 8. A boosting effect of SNA after administration of CHIKV VLP was also observed in subjects with baseline CHIKV neutralizing antibodies (15, 16).

Specimens from VRC 704 were also analyzed by the Sponsor, using the CHIKV-luc assay. A subgroup analysis was performed on subjects 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 adverse event (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%) subjects were reported, all were assessed as unrelated to the investigational product (IP) (i.e., 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.

PXVX-CV-317-001

The Phase 2 clinical study (PXVX-CV-317-001) conducted in the US compared multiple dose and dosing regimens of CHIKV VLP in healthy adults aged 18 to <46 years (17). The dosages of CHIKV VLP ranged from 6 µg to 40 µg, adjuvanted. These doses were below or approximately equivalent to those used in VRC 311 (13, 14) and VRC 704 (15, 16) clinical studies. PXVX0317 vaccine was immunogenic across all dose groups as measured by CHIKV-luc assay. Immunogenicity data reported as 80% antibody neutralization titer (NT_{80}) values supporting the benefit of adjuvant was evident after one dose but not two doses. There was a clear dose-response relationship in GMT. Subjects receiving 20 µg on the standard schedule (Days 1 and 29), either unadjuvanted or adjuvanted, had the highest Day 57 GMT at 2057 and 2024 respectively, similar to those of CHIKV VLP recipients in the VRC 704 study (15, 16). Subjects receiving the single 40 µg dose (Group 8) demonstrated only slightly lower GMT levels (1713 at Day 57, i.e., 28 days after vaccination); all other dose groups demonstrated a GMT range of 920 to 1563 at Day 57. Seroconversion rates showed that with a single dose administered, up to 98% of study participants produced a neutralizing antibody response by Day 8. Further, the immune response was shown to be persistent through the 12-month visit, including in the one dose 40 µg CHIKV VLP regimen. There was a clear dose-response relationship in GMT, with the Group 8 40 µg CHIKV VLP + 300 µg alum adjuvant single dose resulting in 86% sero-response (titers ≥40) 7 days post-vaccination as well as the highest GMTs at Day 182 and Day 365. Sero-response was well maintained at 365 days in all groups. Group 8 was followed through Day 760 and demonstrated persistent sero-response for 2 years post vaccination (17). Two additional open-label groups were sequentially added to the study in order to provide plasma for NHPs in planned passive transfer studies; one group received two doses of the adjuvanted 20 µg on the standard 4-week schedule (N=20) and one group received a single alum-adjuvanted 40 µg dose at Day 1 (N=10).

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 versus alphavirus vaccine-naïve controls (18). The thirty prior alphavirus vaccine recipients and thirty gender and age-matched vaccine-naïve controls were vaccinated and followed for 6 months. There were no differences between the groups in anti-CHIKV neutralizing antibody GMTs at Day 22 and no new safety signals were identified. Final data analysis is pending.

1.3.3 Rationale for Dosage and Route of Administration

The regimen of PXVX0317 selected for this study is 40 µg of VLP with alum adjuvant administered in a single IM dose on Day 1. The alum dose of 300 µg is within the range of doses of alum adjuvants used in many licensed vaccines, including VLP-based vaccines. This dose also creates a concentration ratio to PXVX0317 that achieves high (~90%) levels of adsorption, thought to enhance both immunogenicity and short-term stability. The IM route of administration is consistent with that in previous clinical studies (VRC 311 (13, 14), VRC 704 (15, 16), PXVX-CV-317-001, and EBSI-CV-317-002 (17)). This regimen was selected by an interim analysis of the PXVX-CV-317-001 study data. All regimens tested in PXVX-CV-317-001 were given to groups of 50 to 53 healthy adults and all resulted in 100% seroconversion by Day 57. The group receiving a single dose of 40 µg plus alum showed the highest anti-CHIKV SNA GMT at Day 182 and Day 365.

The PXVX0317 vaccine (40 µg CHIKV VLP + 300 µg alum adjuvant) single dose has been selected for further development by the Sponsor in a planned Phase 3 clinical study (EBSI-CV-317-004).

2 STUDY OBJECTIVES AND PURPOSE

2.1 Primary Objectives

- To assess the induction of anti-CHIKV neutralizing antibody responses following a single adjuvant dose of PXVX0317 as measured 21 days (Day 22) after vaccination.
- To assess the induction of anti-CHIKV neutralizing antibody responses following a single adjuvant dose of PXVX0317 as measured 7 days (Day 8), 14 days (Day 15), and 56 days (Day 57) after vaccination.

2.2 Secondary Objective

- To assess the safety of a single dose of PXVX0317 in healthy adults.

2.3 Exploratory Objectives

- To obtain plasma and sera at Days 22 and 57 from subjects immunized with a single dose of PXVX0317 (40 µg CHIK VLP adjuvanted with 300 µg Alhydrogel) to support nonclinical studies.
- To characterize the kinetics of anti-CHIKV IgM, IgG, and neutralizing responses following a single dose of PXVX0317 as measured 7, 14, 21, and 56 days (Days 8, 15, 22, and 57) after vaccination.
- To characterize T and B-cell responses following a single dose of PXVX0317 with collections of PBMCs 7, 14, 21, and 56 days (Days 8, 15, 22, and 57 respectively) after vaccination.

3 STUDY DESIGN

3.1 Study Description

This is an open-label, single arm Phase 2 study in healthy adults 18 to 45 years of age. This study has a screening period of 30 days, a treatment and observation period from Day 1 to Day 22, and a follow-up period through Day 183. Subjects will receive CHIKV VLP at a dose of 40 µg in combination with 300 µg Alhydrogel on Day 1, have follow-up visits on Day 8, Day 15, and Day 22, with an additional plasmapheresis procedure and collection of serum on Day 22. A phone call follow-up visit will occur on Day 29, followed by a second plasmapheresis procedure and collection of serum at Day 57. A phone call follow-up visit to assess safety and concomitant medications will occur on Day 64. An EOS phone call follow-up visit to assess safety and concomitant medications will occur on Day 183.

3.1.1 Study Centers

This is a single center study in the US.

3.1.2 Number of Subject (Planned)

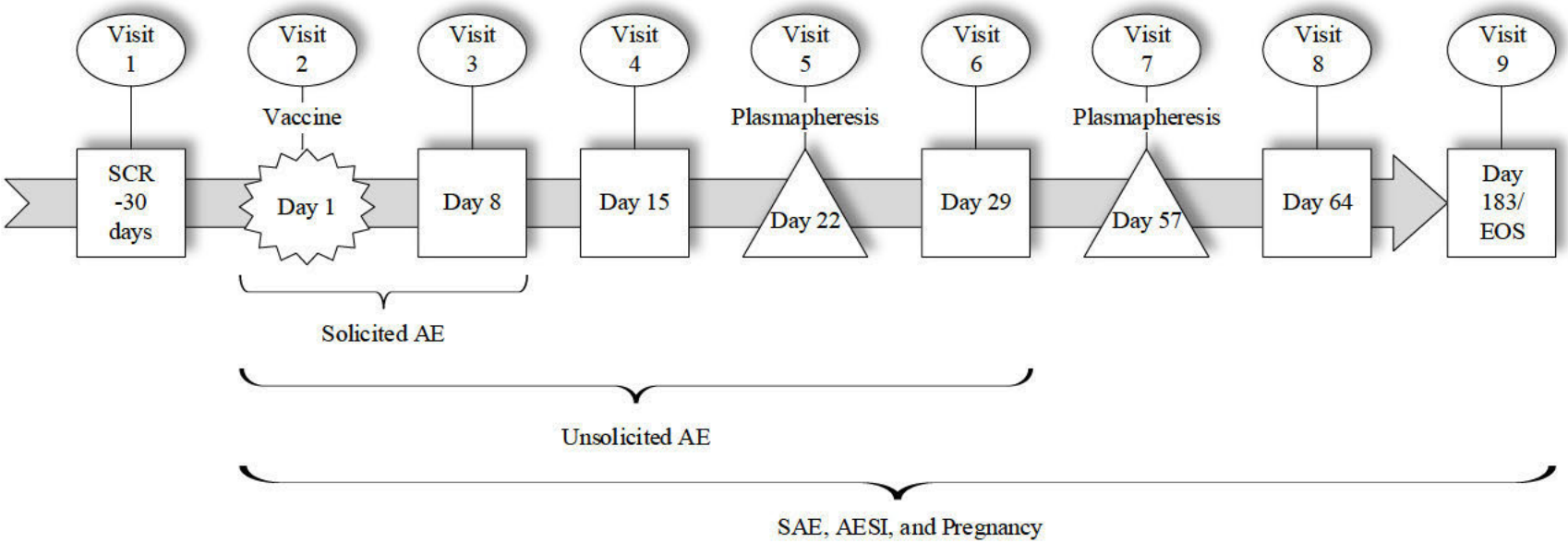
Twenty-five subjects are targeted for enrollment.

3.1.3 Estimated Study Duration

The per subject estimated total study duration is 213 days. The screening window will be no greater than 30 days prior to Day 1 (administration of IP). Plasmapheresis will be performed on Day 22 and Day 57 with an EOS phone call on Day 183.

3.2 Schematic Diagram of Study Design

Figure 2 EBSI-CV-317-010 Schematic Diagram of Study Design



Note: Days 29, 64, and 183 are phone calls

3.3 Description of Study Assessments

The underlying subsections (Section 3.3.1 through Section 3.3.13) describe the planned study procedures and assessments. For the per visit timing of these procedures and assessments refer to Section 5.2 and the study Schedule of Events. Information on subject consent is described in Section 11.1.

3.3.1 Review of Eligibility Criteria

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

3.3.2 Medical History and Demography

Medical history information will be collected from subjects at the Screening Visit and confirmed at the Day 1 Visit (at baseline i.e., prior to IP administration) and will include (but not be limited to) demographic information (date of birth, race, ethnicity, and sex of subject), current and past medical conditions (including presence of joint pain), prior and concomitant medications (see Section 5.3) taken within 30 days of Screening Visit (or within 90 days for blood products, or within 6 months for immunosuppressive/immunomodulatory medications).

3.3.3 Physical Examination

A complete physical examination will be performed on subjects 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 exam may be performed on subjects at additional time points if indicated by AE, SAE, or Adverse Event of Special Interest (AESI) reports.

3.3.4 Vital Signs

Vital signs collected from subjects will include blood pressure, heart rate, respiratory rate, and temperature. The first set of screening vitals are to be collected and transcribed into the screening electronic Case Report Form (eCRF) for inclusion of the subject into the study. Repeat measurements on abnormal vital parameters are allowed twice for confirmation of eligibility to receive the vaccination. Vital signs will be taken prior to and after study vaccine administration and should be within normal limits prior to planned vaccination.

Measurement of body weight, height for body mass index (BMI) calculations will be obtained at the Screening Visit.

3.3.5 Laboratory Tests

At the Screening Visit, blood samples will be collected for serum testing for HBsAg, HCV antibody, HIV-1/HIV-2 antigen/antibody (Ag/Ab), and CHIKV IgG antibodies, and urine will be collected for pregnancy testing for women of childbearing potential (CBP).

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

3.3.6 Immunogenicity Sample

Anti-CHIKV SNA levels will be assessed using a validated CHIKV-luc assay that measures neutralization titer of 80% (NT_{80}). CHIKV IgM and IgG levels will be assessed by serum ELISA and PBMCs by assays performed on whole blood. Blood samples will be taken on Day 1 (prior to IP administration), Day 8, Day 15, Day 22, and Day 57 (or at Early Discontinuation/Withdrawal). For information on the immunogenicity assessment see Section 6.1.

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

3.3.7 Pregnancy Testing and Contraception

Female subjects of CBP will undergo a urine pregnancy test at the Screening Visit and prior to IP administration on Day 1, prior to plasmapheresis on Day 22 and Day 57, and at any Early Discontinuation Visit. The subject must have a negative urine pregnancy test on Day 1, prior to administration of IP, and prior to both plasmapheresis visits.

Women of CBP must also use an acceptable method of contraception from prior to Day 1 through the Day 183 EOS Visit. Acceptable methods include highly effective forms of contraception such as: hormonal contraceptives (e.g., implants, pills, patches) containing combined estrogen and progestogen, or progestogen-only initiated ≥30 days prior to dosing **or** IUD inserted ≥30 days prior to dosing **or** use of double barrier type of birth control (male condom with female diaphragm, male condom with cervical cap). The Investigator must confirm that contraception methods (e.g., hormonal contraceptive or intrauterine device) were initiated ≥30 days prior to Day 1 to be considered fully effective.

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

3.3.8 Investigational Product Administration

Investigational Product is 0.8 mL in volume and administered by IM injection into the deltoid muscle with a field-formulated syringe attached to 25 gauge 1" (or 1.5") needle, using universal precautions and sterile technique in accordance with General Best Practice Guidelines for Immunization: Best Practices Guidance of the Advisory Committee on Immunization Practices (ACIP) (27) per Table 1.

Table 1 Needle Length for IM Injections by Sex and Weight

Sex, Weight	Needle length	Injection Site
Men and women, <60 kg (<130 lbs)	1 inch (25 mm)	Deltoid muscle of arm
Men and women, 60-70 kg (130-152 lbs)	1 inch (25 mm)	
Men, 70-118 kg (152-260 lbs)	1-1.5 inches (25-38 mm)	
Women, 70-90 kg (152-200 lbs)		
Men, >118 kg (260 lbs)	1.5 inches (38 mm)	
Women, >90 kg (200 lbs)		

Source: Adapted from Table 6-2, General Best Practice Guidelines for Immunization: Best Practices Guidance of the Advisory Committee on Immunization Practices (ACIP) (27).

Investigational Product is to be administered by a staff member only under the direct supervision of the Investigator or a qualified sub-Investigator identified on the FDA Form 1572. Under no circumstances will the Investigator allow PXVX0317 vaccine to be used other than as specified in the protocol.

For further details on the IP refer to Section 5.1.

3.3.9 Acute Observation After IP Administration

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

3.3.10 Solicited Adverse Events

Solicited AEs will be collected from IP administration until Day 8. Solicited AEs for this study are local events of pain, redness, and swelling at the injection site and systemic events of oral temperature ≥38°C (≥100.4 degrees Fahrenheit [°F]), chills, fatigue, headache, myalgia, arthralgia, and nausea (see Section 7.1.2 and Section 7.1.2.1).

Subjects will be trained to complete a memory aid to observe, measure, and record these solicited AEs. To measure oral temperature, a digital thermometer will be provided to the subject to measure their temperature each day and record them in their memory aid. To record injection site local reactions, a ruler will be provided to the subject to measure 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 memory aid. The Investigator will then assess all solicited AEs for severity (Section 7.1.7) and the action taken, and causality (Section 7.1.8). The results of the Investigator's assessment will be recorded as a separate source document and will be entered on the solicited AE CRF. Symptoms continuing beyond the solicited AE collection period will be collected and recorded on the AE CRF.

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

3.3.11 Unsolicited Adverse Events

Unsolicited AEs (AEs not listed in the memory aid) will be collected from Day 1 through 28 days post-vaccination (Day 29) (see Section 7.1.3). Adverse events related to plasmapheresis procedures will be collected at the Day 22 and Day 57 visit. Serious AEs and AEs that result in changes to the plasmapheresis procedure(s), such as early termination of collection, must be reported within 24 hours of awareness to the Medical Monitor (MM).

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

3.3.12 Serious Adverse Events and Adverse Events of Special Interest

Serious AEs and AESI will be collected for all subjects from Day 1 through Day 183 EOS Visit (see Section 7.1.4 and Section 7.1.5).

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

3.3.13 Plasmapheresis Procedure

[redacted] will be the designated apheresis site. Per the blood bank guidelines, plasmapheresis can be done once every 28 days. Plasmapheresis will be performed using either the [redacted] Plasmapheresis system while connected to the [redacted] data management system (preferred option) or the [redacted] Blood Component Collection System while connected to [redacted] Information Systems per their standard operating procedures (SOPs).

Subjects will be advised to consume a well-balanced meal and a 12-ounce glass of a decaffeinated beverage prior to the procedure. The amount of plasma collected may range from ~600 to 900 mL (depending on donor weight) and the amount of red blood cell loss will be ~10 to 15 mL. Subject identity will be confirmed before the procedure begins. Prior to venipuncture, the designated phlebotomist will prepare each subject's arm for phlebotomy by sterilizing the designated insertion site on the subject's arm and by ensuring adequate venous access. Each subject should expect plasma drawing to take approximately 1 to 2 hours. Subjects will be monitored for any reactions or discomfort during and after the procedure. Subject tolerance to the anticoagulant must be monitored as well.

3.4 End of Study

An individual subject is considered to have completed study participation after completion of the Day 183 visit and any required safety follow-up.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Subject Inclusion Criteria

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

1. Able and willing to provide informed consent voluntarily signed by subject
2. Any gender, 18 to 45 years of age (inclusive).
3. Generally healthy, in the opinion of the Investigator, based on medical history, physical examination and screening laboratory assessments.
4. Women who are either:
   i. Not of CBP: pre-menarche, anatomically sterile, or post-menopausal (defined as ≥12 months without menses).

      or:

   ii. Meeting **all** the below criteria:
   - Negative urine pregnancy test at screening visit **and**
   - Negative urine pregnancy test immediately prior to dosing at Day 1 **and**
   - Using an acceptable method of contraception (if female of CBP) for the duration of participation, such as:
     - Hormonal contraceptives (e.g., implants, pills, patches) initiated ≥30 days prior to dosing or
     - Intrauterine device (IUD) inserted ≥30 days prior to dosing or
     - Double barrier type of birth control (male condom with female diaphragm, male condom with cervical cap).

4.2 Subject Exclusion Criteria

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

1. Currently pregnant, breastfeeding, or planning to become pregnant during the study.
2. Body Mass Index ≥35 kg/m^2.
3. Positive laboratory evidence of current infection with human immunodeficiency virus (HIV-1, HIV-2), hepatitis C virus (HCV) or hepatitis B virus (HBV).
4. History of severe allergic reaction or anaphylaxis to any component of the IP.
5. History of known congenital or acquired immunodeficiency that could impact response to vaccination (e.g., leukemia, lymphoma, generalized malignancy, functional or anatomic asplenia, alcoholic cirrhosis).

6. Prior or anticipated receipt of immunomodulatory or immunosuppressive therapy from six months prior to screening through Day 64.
7. Receipt or anticipated receipt of blood or blood-derived products from 90 days prior to screening through Day 64.
8. Acute disease within the last 14 days (subjects with an acute mild febrile illness can be considered for a deferral of vaccination two weeks after the illness has resolved and treatment has been completed).
9. Clinically significant cardiac, pulmonary, respiratory, rheumatologic, or other chronic disease, in the opinion of the Investigator. This may include chronic illness requiring hospitalization in the last one month prior to screening.
10. Enrollment in an interventional study and/or receipt of another investigational product from 30 days prior to screening through the duration of study participation.
11. Receipt or anticipated receipt of any vaccine from 30 days prior to screening through Day 64.
12. Prior receipt of an investigational CHIKV vaccine/product.
13. Detectable baseline anti-CHIKV IgG antibody as determined by ELISA.
14. Any other condition that, in the opinion of the Investigator, could adversely impact the subject's participation or the conduct of the study, creates an unacceptable risk to the subject, or may interfere with the conduct of the study or validity of the data.
15. Restricted venous access that would prevent the collection of plasma and serum necessary for participation.
16. Weight <110 pounds.

4.3 Withdrawal of Subjects

4.3.1 Subject Consent Withdrawal

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

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

4.3.2 Investigator-Based Subject Withdrawal

The Investigator may withdraw a subject from further participation in the study, at their discretion, if medically necessary or for reasons of noncompliance. The reason for withdrawal of any subject 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 subject, except in the event of a medical emergency.

The Investigator (and/or Sponsor) may withdraw a subject from the study for any of, but not limited to, the following reasons:

- Noncompliance with the protocol. If the subject is non-compliant with protocol requirement, the issue should be discussed with the subject and, if not resolved, consideration given to withdrawing the subject.
- Lost to follow-up; requires documentation of at least three unsuccessful attempts to contact subjects. Lost to follow-up will be determined after the date of the subject's projected last visit.
- Other reason(s) which, in the opinion of the Investigator, indicates that continued participation in the study is not in the best interest of the subject.

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

4.3.3 Follow-up for Withdrawn Subjects

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

4.3.4 Documentation of Withdrawal/Discontinuation

Reasons for withdrawal of individual subjects from the study prior to final protocol required visit and/or final safety follow-up are to be recorded on the CRF. 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 subjects, lost to follow-up will be determined after the date of the subject's projected last visit)
- Adverse event
- Pregnancy
- Protocol deviation (that results in discontinuation)

- Non-compliance
- Physician (i.e., Investigator) decision
- Other

Safety follow-up for AEs should occur for all subjects, for information on safety follow-up for withdrawn subjects (see Section 4.3.3 Follow-up for Withdrawn Subjects).

4.3.5 Subject Replacement

Subjects who undergo Early Discontinuation or withdrawal of consent before receiving treatment or Day 22 plasmapheresis may be replaced at the Sponsor's discretion. Subjects who undergo Early Discontinuation after Day 22 plasmapheresis will not be replaced.

5 TREATMENT OF SUBJECTS

5.1 Investigational Product(s)

5.1.1 CHIKV VLP

CHIKV VLP refers to the virus-like particle (VLP) component of PXVX0317 produced by transient transfection of human embryonic kidney (HEK) 293 cells with a DNA expression plasmid encoding Capsid (C) and E3, E2, 6K, and E1 proteins. After expression of the plasmid-encoded proteins, VLPs self-assemble and are released into the cell culture medium as ~70 nm particles. The 6K and E3 proteins have not been specifically detected in the VLPs. No replication-capable viral genetic material is incorporated into the VLPs. Virus-like particles are then concentrated from the cell supernatant and purified. After purification, the VLPs are diluted into a sucrose-containing aqueous buffer, sterile-filtered and filled into sterile single-dose glass vials to create the final CHIKV VLP drug product. The production process does not use animal-derived raw materials or antibiotics. Process residuals (including host cell and recombinant DNA, host cell protein, and Benzonase®) were assessed to be at acceptable levels. All excipients are generally regarded as safe (GRAS).

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

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

Table 2 CHIKV VLP Drug Product

Composition Ingredient	Function	Concentration
Active Ingredient		
CHIKV VLP	Active Ingredient	0.051 mg/mL
Excipients		
Sucrose, Ph. Eur./NF	Cryoprotectant / stabilizer	74.4 mg/mL
Sodium citrate dihydrate, Ph. Eur./USP	Stabilizer	7.4 mg/mL
Potassium phosphate monobasic, NF	Buffering agent	0.5 mg/mL
Potassium phosphate dibasic, Ph. Eur./ USP	Buffering agent	1.1 mg/mL
WFI quality water, USP	Solvent	q.s. to 0.8 mL

WFI = Water for injection; Ph. Eur. = European Pharmacopeia; NF = National Formulary; USP = United States Pharmacopeia; q.s. = quantum satis (the amount which is enough)

5.1.2 Alum Adjuvant

The adjuvant is a commercially available sterile, non-pyrogenic formulation of 2% w/w aluminum hydroxide gel (10.0 mg/mL aluminum), aqueous, branded as Alhydrogel 2% adjuvant [redacted]. It meets the requirements of the European Pharmacopoeia monograph for aluminum hydroxide, hydrated for adsorption and has a pH of 6-7. The adjuvant is packaged in a 250 mL HDPE bottle with a rubber stopper and each bottle is for single-use only. The bottle should be shaken well before use.

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

5.1.3 Labeling

PXVX0317 will be shipped in individual vials and adjuvant will be shipped in 250 mL HDPE bottles. PXVX0317 vials and adjuvant bottles will be labelled with the names of the Sponsor and product, the concentration, lot number, storage conditions and "Caution: New Drug – Limited by Federal Law to Investigational Use Only".

5.1.4 Investigational Product Shipment

CHIKV VLP vials will be shipped directly to the site from [redacted] for Emergent Travel Health in a shipping container with dry ice. During shipment, the temperature of the IP will be monitored to ensure the required temperature conditions are maintained. On receipt of the shipment the site will perform a visual inspection of the vials and transfer to a temperature-controlled freezer at ≤–70°C within 4 minutes after opening the shipment box. See the Pharmacy Manual for additional information.

Bottles of Alhydrogel (250 mL in HDPE bottles) will be shipped directly to the site from [redacted] for Emergent Travel Health under ambient conditions.

The Principal Investigator (or designee) will be responsible for checking the number of vials/bottles and the condition of the vials/bottles received, and entering this information into the drug accountability records, and reporting the condition of shipment to the Sponsor. Investigational Product received in good condition will be automatically released for use upon confirmation of receipt as such; shipments received with any excursions/issues will be placed under quarantine by the site and released for use by the Sponsor following investigation. For additional information on IP shipments please refer to the study Pharmacy Manual.

5.1.5 Storage Conditions

PXVX0317 vials must be stored in a qualified, temperature-controlled freezer at ≤–70°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 each working day. 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.

The recommended storage condition for the adjuvant is room temperature not to exceed 30°C. Avoid freezing.

Please refer to the Pharmacy Manual for additional details.

5.1.6 Other Supplies

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

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

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

5.1.7 Preparation

1. 0.03 mL Alhydrogel is added to a first CHIKV VLP vial (0.8 mL) and mixed by swirling (first dose vial).
2. 0.03 mL Alhydrogel is added to a second CHIKV VLP vial (0.8 mL) and mixed by swirling (second dose vial).
3. Both vials are held at room temperature for 15 minutes.

4. 0.4 mL of CHIKV VLP is withdrawn from the first dose vial into a syringe, syringe plunger is pulled to 0.8 mL mark and then CHIKV VLP injected into the second dose vial. A total of 0.8 mL is then withdrawn from the second dose vial into syringe.

All doses are prepared into Sponsor-provided syringes using sterile technique and all doses must be administered no later than 1 hour post thaw of CHIKV VLP. For further information on Study Vaccine, including secondary packaging, receipt, accountability, and detailed preparation instructions, please refer to the Pharmacy Manual.

5.1.8 Drug Accountability

The Investigator is responsible for maintaining accurate inventory records of IP. The Investigator (or designee) will inventory all IP shipments upon receipt. The Investigator or designee must ensure that all drug supplies are kept in a secure location in the site pharmacy in accordance with recommended storage conditions (Section 5.1.5). Inventory and ongoing record of test material supplies will be documented using the Drug Accountability Form provided. These records will be reviewed by representatives of the Sponsor and may be reviewed by regulatory agencies.

5.2 Study Procedures and Assessments by Visit

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

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

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

- Informed consent (Section 11.1)
- Review of eligibility criteria (Section 3.3.1, Section 4.1, Section 4.2)
- Medical history (including presence of joint pain) (Section 3.3.2)
- Demographics (date of birth, race, ethnicity, and sex of subject) (Section 3.3.2)
- Prior and current medications (Section 3.3.2, Section 5.3)
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature) as well as height and body weight (for BMI calculation) (Section 3.3.4)
- Complete physical exam (general appearance, eyes-ears-nose-throat, head-neck, lungs-chest, heart, abdomen, musculoskeletal, lymph nodes, skin, extremities, and neurological assessment) (Section 3.3.3)

- Blood collection for laboratory viral marker testing (HBsAg, anti-HCV antibody, HIV-1/HIV-2 Ag/Ab) (Section 3.3.5)
- Blood collection for anti-CHIK IgG antibody (ELISA kit) testing
- For women of CBP: urine sample for pregnancy test (Section 3.3.5, Section 3.3.7)

5.2.1.1 Re-Screening

Re-screening may occur given the following circumstances:

- If the participant has an acute febrile illness at the time of their scheduled enrollment, they may be re-screened 14 days after resolution of their acute illness
- If the participant is asymptomatic but tests positive by reverse transcription polymerase chain reaction (RT-PCR) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) they may be re-screened provided 14 days have elapsed since the participant tested RT-PCR positive for SARS-CoV-2, the participant continues to be asymptomatic, and the participant is otherwise eligible for enrollment
- Participants who have received vaccines within 30 days of screening may be re-screened provided no additional doses are anticipated up to Day 64
- Participants who have received blood products or investigational products from participation in another clinical study may be re-screened after the appropriate duration has passed (90 days for blood products; 30 days for investigational drugs)
- Eligible subjects who are not able to be vaccinated within 30 days of their screening period may be re-screened

Re-screened subjects must undergo all screening procedures again, including re-consenting and use of the same subject ID number. Subjects may be re-screened one time only. Re-screening is not otherwise permitted.

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

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

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

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

- In-clinic acute observation for 30 mins after injection but no more than 60 mins after injection (Section 3.3.9)
- Vital signs
- Memory aid, digital thermometer, and ruler distribution, set-up, and training (Section 3.3.10)
- Solicited AE, unsolicited AE, SAE, and AESI (Sections 3.3.10 - 3.3.12, Section 7) evaluation
- Review concomitant medications

5.2.3 Day 8 (+3) Visit 3

- Review memory aid
- Solicited AE, unsolicited AE, SAE, and AESI evaluation
- Review concomitant medications
- Blood collection for anti-CHIKV SNA, ELISA, PBMC
- Directed physical exam (as needed)

5.2.4 Day 15 (±2) Visit 4

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

5.2.5 Day 22 (+5) Plasmapheresis Visit 5

- Review of inclusion/exclusion criteria
- For women of CBP: urine pregnancy test
- Unsolicited AE, SAE, and AESI evaluation
- Review concomitant medications
- Directed physical exam (as needed)
- Blood collection for anti-CHIKV SNA, ELISA, PBMC
- Serum collection for nonclinical studies
- Plasmapheresis procedure performed at blood bank
    - AE follow-up 30 minutes post plasmapheresis

5.2.6 Day 29 (-1/+5) Telephone Contact for Visit 6

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

5.2.7 Day 57 (+5) Plasmapheresis Visit 7

- Review of inclusion/exclusion criteria
- For women of CBP: urine pregnancy test
- SAE and AESI evaluation
- Review concomitant medications (only if associated with SAE/AESI)
- Directed physical exam (as needed)
- Blood collection for anti-CHIKV SNA, ELISA, PBMC
- Serum collection for nonclinical studies
- Plasmapheresis procedure performed at blood bank
    - AE follow-up 30 minutes post plasmapheresis

5.2.8 Day 64 (-1/+5d) Telephone Contact for Visit 8

- SAE and AESI evaluation
- Review concomitant medications (only if associated with SAE or AESI)

5.2.9 Day 183 (-14/+7d) Telephone Contact for Visit 9 End of Study

- SAE and AESI evaluation
- Review concomitant medications (only if associated with SAE or AESI)

5.2.10 Early Discontinuation/Withdrawal Visit

All subjects who discontinue study participation before the Day 57 visit will be requested to undergo an Early Discontinuation/Withdrawal Visit. The following will be conducted:

- Review AEs, AESI and SAEs
- Review concomitant medications associated with new or ongoing AEs and any AESIs or SAEs
- Blood collection for anti-CHIKV SNA, ELISA, PBMC
- For women of CBP: urine pregnancy test

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

a. Review of the memory aid

b. AE (solicited) evaluation

5.2.11 Unscheduled Visits

Any study procedure, excluding vaccination or plasmapheresis, 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/SAE/AESI.

5.2.12 COVID-19 Related Considerations

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

i. The conduct of the study will be in accordance with state and local travel limitations/restrictions.

ii. Study staff at the plasma center will take appropriate precautions to protect study participants.

iii. Safety assessments will be performed by phone call when appropriate.

iv. If travel restrictions or COVID-19 related illness impact the conduct of the study, specific measures will be taken to mitigate risk to study staff and participants and monitor protocol deviations due to COVID-19 illness and/or COVID-19 control measures.

5.3 Prior and Concomitant Medications

At the Screening Visit, the details of prior and concomitant medication usage will be collected (through 30 days prior to screening, or 90 days prior for blood products, or within 6 months for immunosuppressive/immunomodulatory medications). Concomitant medications will be collected at each on site visit or by phone interview (or early discontinuation/ withdrawal). The details of all concomitant medications including those associated with solicited AEs and unsolicited AEs will be collected through Day 29. Concomitant medications associated with a SAEs and AESI will be collected through the end of the study.

5.3.1 Required Concomitant Medications

Not applicable.

5.4 Procedures for Monitoring Subject Compliance

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

6 IMMUNOGENICITY ASSESSMENT

6.1 Immunogenicity Analysis

To the extent possible and practicable, serum and plasma samples will be utilized to understand antibody-mediated mechanisms of protection elicited by vaccination with CHIKV VLP.

The immunogenicity analysis will be performed using a high-throughput *in vitro* infectivity assay developed by the Sponsor for assessing titers of neutralizing antibodies against CHIKV in serum samples. The assay is based on using a modified version CHIKV that expresses luciferase (CHIKV-luc) and measures the reduction of luciferase activity in infected cultures of Vero cells following treatment of virus with test serum. Using the CHIKV-luc assay, antibody neutralization titers can be determined via characterization of reductions of luciferase activity in the presence of immune serum. The quantitation of reporter gene expression, a correlate of the level of virus infection of cells, is determined by detection of luciferase in assay wells using a micro-plate luminometer. The CHIKV neutralizing antibody titre 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. Additionally, neutralization analyses may include fractionated antibody subtypes and/or F(ab)/$F(ab)_2$ products. Antibody responses, including IgM and IgG isotypes will also be assessed and quantified with a commercial anti-CHIKV ELISA test.

The IgG and IgM antibody levels specific to CHIKV on days 1, 8, 15, 22, and 57 will be assessed by ELISA. Human serum samples from each timepoint will be tested using InBios Anti-CHIKV IgM/IgG ELISA Enzyme Immunoassays. These kits are ELISAs, where a capture antibody specific for total human IgG or IgM is coated onto polystyrene plates. Human serum samples are added, and any IgG or IgM present in the sample becomes immobilized on the plate. An antigen consisting of the chikungunya virus E1/E2 glycoproteins is then added to the plate. Any anti-CHIKV E1/E2 antibodies present in the human serum samples will bind to the antigen and immobilize it to the plate. Next, a horseradish peroxidase (HRP)-conjugated anti-CHIKV E1/E2 antibody is added, which binds to any immobilized antigen in the wells. TMB (3,3',5,5'-tetramethylbenzidine) is used to develop the plate, and absorbance is read on a spectrophotometer at 450 nm. A calibrator control is included with the kit to establish a positive/negative cut-off absorbance. The reportable value, the Immune Status Ratio (ISR) value, is calculated by dividing the mean optical density (OD) of the test sample (for IgG or IgM) by the mean OD of the calibrator.

Peripheral blood mononuclear cells will be collected for analysis of cell-mediated immunity. CHIKV antigen-specific B and T cell subsets will be identified and characterized by evaluating cytokine and antibody secretion using ELISpot and flow cytometry assays. The cell mediated immune response will be linked to previously determined antibody levels to define a mechanistic model for humoral response.

7 SAFETY ASSESSMENTS AND REPORTING

7.1 Definitions

7.1.1 Adverse Event

An AE is any untoward medical occurrence in a clinical study subject administered a pharmaceutical product or intervention regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product/intervention, whether or not considered related to the medicinal product/intervention.

Note: A diagnosis should be preferentially captured as an AE term and signs and symptoms should be captured only in the absence of a unifying diagnosis. If there are multiple diagnoses, then all diagnoses should be captured. The worsening of an existing sign, symptom or disease is also considered to be an AE. An abnormal laboratory finding deemed by the Principal Investigator as not clinically significant (NCS) will not be captured as an AE, but an abnormal laboratory finding that worsens after dosing with the study drug, from not clinically significant to clinically significant (CS), is considered an AE. Surgical procedures are not AEs. They are the action taken to treat a medical condition. Interventions that were planned prior to study entry for medical conditions that started prior to study entry but did not worsen during the clinical study are not reported as AEs.

7.1.2 Solicited Adverse Event

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

7.1.2.1 Reactogenicity

Reactogenicity (solicited systemic and injection site reactions) will be assessed by the subjects using paper memory aids. Information will be solicited on the following injection site reactions: pain, redness, and swelling. In addition, information will be solicited for AEs, on the following systemic reactions: temperature increases (oral) ≥38°C (≥100.4°F), chills, fatigue, headache, myalgia, arthralgia, and nausea.

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

7.1.3 Unsolicited Adverse Event

An unsolicited AE is an AE that is spontaneously reported by the subject or discovered by the Investigator.

7.1.4 Adverse Event of Special Interest

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 subjects. Medically attended visits include hospital, emergency room, urgent care clinic, or other visits to or from medical personnel.

7.1.5 Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose: results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly or birth defect.

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

Note: 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 AE". Overnight stays at hospital/clinic that occurs during a study for social reasons (e.g., transportation difficulties, respite care) is not considered to be a hospitalization event.

7.1.6 Suspected Unexpected Serious Adverse Reaction

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

7.1.7 Severity of Adverse Events

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

- **Mild (Grade 1):** No interference with activity
- **Moderate (Grade 2):** Some interference with activity
- **Severe (Grade 3):** Significant; prevents daily activity

- **Potentially Life-Threatening (Grade 4):** As determined by emergency room visit or hospitalization

For the study Grading Scale see Appendix II.

7.1.8 Causality of Adverse Events

The Investigator is responsible for the assessment of the causality of an AE and Sponsor's Medical Monitor will also assess SAE causality, independent of the Investigator.

The following guidelines are provided for this assessment:

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

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

7.2 Eliciting Adverse Events

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

7.3 Reporting Requirements for Immediately Reportable Events

7.3.1 Principal Investigator's Reporting Requirements

The following events must be reported within 24 hours of awareness by the Principal Investigator (or designee) to Sponsor's Medical Monitor and Global Pharmacovigilance Department (Global PV):

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

The appropriate form(s) (listed below) will be completed and sent by email to the following address:

[redacted]

Emergent BioSolutions Inc.

Email: [redacted]

Telephone (toll free): [redacted]

For SAE/AESI, the "Serious Adverse Event/Adverse Event of Special Interest Report Form" will be completed (abbreviated hereafter as "SAE/AESI Report Form"). The SAE/AESI Report Form is **not** the same as the AE eCRF. Supporting documentation that may be emailed to accompany the form(s) can include source documentation or medical records (e.g., discharge summary for hospitalizations, lab reports) which support a diagnosis. Participant identifiers (e.g., 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 (e.g., adverse drug reactions) must be reported to the Institutional Review Board (IRB) by the Investigator (or designee) as required by ICH GCP E6.

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

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

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

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

7.3.2 Sponsor's Reporting Requirements

A SUSAR is a suspected adverse reaction that is both serious and unexpected (Section 7.1.6). As specified in 21 Code of Federal Regulations (CFR) 312.32, SUSARs will be reported by

the Sponsor of the Investigational New Drug Application (IND) to the FDA and to all participating Principal Investigators in an IND safety report as soon as possible, no later than 15 calendar days after the Sponsor becomes aware of the suspected adverse reaction (21 CFR 312.32(c)(1)).

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

7.4 Reporting of Other Information - Unanticipated Problems

As outlined by the Office for Human Research Protection (OHRP), 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 subject 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 subjects 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 to protect the safety, welfare, or rights of subjects or others. Only a small subset of AEs occurring in human subjects participating in a clinical study will meet these three criteria for an unanticipated problem. There are other types of incidents, experiences, and outcomes that occur during the conduct of clinical study that represent unanticipated problems but are not considered AEs. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with AEs. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs.

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

7.5 Follow-up of Adverse Events

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

7.5.1 Follow-up of Nonserious Adverse Events

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

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

This study requires that participants be monitored for SAE/AESI up through the Day 183 EOS Visit. From Day 1 (Visit 2), confirmed SAEs and confirmed AESI will be recorded on the AE eCRF. All SAEs that are ongoing at the time of Day 183/ Early Withdrawal visits will have final outcome recorded as "Not Recovered/Not Resolved".

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

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

- The SAE is resolved or stable if condition is expected to remain chronic
- The participant is referred to a specialist or other physician for treatment and follow-up. The Principal Investigator (or designee) will follow the subject's condition even if the subject 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:

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

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

8 STATISTICAL CONSIDERATIONS

8.1 Sample Size Determinations

The sample size is based on practical, rather than statistical, considerations. Twenty-five subjects will provide adequate plasma and serum for future non-clinical purposes.

8.2 Analysis Populations

Analysis will be based on the following study populations:

Exposed Population: All subjects who receive IP.

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

Modified Intent-to-Treat (mITT) Population: All subjects who are vaccinated and have at least one post-injection anti-CHIKV SNA NT_{80} result.

8.3 Study Endpoints

8.3.1 Primary Endpoints

- Anti-CHIKV SNA seroresponse rate and associated 95% confidence interval (CI) at Day 22.
- Anti-CHIKV SNA GMT and associated 95% CIs at Day 22.
- Anti-CHIKV SNA seroresponse rate and anti-CHIKV SNA GMTs with associated 95% CIs at Days 8, 15, and 57.
- Anti-CHIKV ELISA IgG and IgM GMTs with associated 95% CIs at Days 8, 15, 22, and 57.
- Geometric mean fold increase (GMFI) in anti-CHIK SNA titer from Day 1 to subsequent collection time points.
- Geometric mean fold increase (GMFI) in anti-CHIK ELISA IgG and IgM from Day 1 to subsequent collection time points.
- Number and percentage of subjects with an anti-CHIKV SNA titer ≥15, 40, 60, 80, 100, 160, 640, and 4-fold rise over baseline thresholds.

8.3.2 Secondary Endpoints

- Incidence of solicited AEs through Day 8.
- Incidence of unsolicited AEs through Day 29.

- Incidence of AESIs and SAEs through end of the study.

8.3.3 Exploratory Endpoints

The kinetics of the anti-CHIKV immune responses (via ELISA and neutralizing assay) will be characterized using the responses over time described under the primary and secondary endpoints. The exploratory endpoints (both categorical and continuous) for the T- and B-cell responses from PBMC samples will be described using descriptive statistics.

8.4 Handling Missing Data

Subjects with missing immunogenicity data at Days 8, 15, 22 or 57 will be excluded from the corresponding analysis; missing immunogenicity data will not be imputed. CHIKV-luc assay anti-CHIKV SNA NT_{80} values or ELISA values below the lower limit of quantitation (LLOQ) will be replaced by LLOQ/2 in the immunogenicity analyses. Imputation rules for partial or missing dates will be described in the study statistical analysis plan (SAP).

8.5 Analysis of Disposition, Demographic and Baseline Characteristics

Disposition will be summarized for all subjects. Demographic and baseline characteristics including age, sex, race, ethnicity, BMI, will be tabulated for the safety and mITT populations. Continuous endpoints will be summarized by descriptive statistics; categorical endpoints will be summarized by the number of subjects, frequency counts and percentages.

Medical history will be coded to System Organ Class (SOC) and preferred term (PT) using the MedDRA dictionary and summarized by treatment group for the randomized population. Protocol deviations will be categorized as important or not important and evaluated for exclusion of data from analyses. Protocol deviations will be presented for the exposed population.

8.6 Immunogenicity Analysis

Summary statistics for immunogenicity results by scheduled visit will be provided for the mITT, unless otherwise specified. In the analyses of the CHIKV-luc assay data and ELISA data as continuous variables, 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. Geometric mean fold increase will also be displayed by post-vaccination scheduled visit.

Proportions of subjects with CHIKV-luc assay anti-CHIKV SNA $NT_{80} \geq 40$ seroresponse rate and secondary response rates at other thresholds (e.g., 15, 60, 80, 100, 160, 640, 4-fold rise over baseline) will be reported with associated two-sided 95% Wilson method CIs by scheduled visit.

Reverse cumulative distribution plots of CHIKV-luc assay anti-CHIKV SNA NT_{80} versus proportion of subjects will be generated by scheduled visit. Geometric mean titer will also be plotted over time.

8.7 Safety Analysis

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

8.7.1 Exposure

The frequencies and percentages of subjects treated will be summarized for the safety population.

All safety analyses will be based on the safety population.

8.7.2 Adverse Events

Adverse events will be coded to SOC and PT using the MedDRA dictionary. Solicited AEs, unsolicited AEs, AESIs, and SAEs will be summarized separately by maximum severity for the safety population.

8.7.2.1 Solicited Adverse Events

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

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

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

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

- Grade 0/absent = 0–24 mm
- Grade 1/mild= >24–50 mm
- Grade 2/moderate= >50–100 mm

- Grade 3/severe= >100 mm
- Grade 4/potentially life threatening = necrosis or exfoliative dermatitis

Refer to Appendix II.

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

The following summaries of solicited events will be performed:

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

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

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

8.7.2.2 Unsolicited AEs

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

The original verbatim terms used by Investigators to identify AEs in the eCRFs will be mapped to PT using the MedDRA dictionary. The unsolicited AEs will then be grouped by MedDRA PT into frequency tables according to SOC. All reported AEs, as well as AEs judged by the Investigator as at least possibly related to IP, will be summarized according to SOC and PT. When an unsolicited AE occurs more than once for a subject, the maximum severity and strongest relationship to treatment will be counted.

Only treatment-emergent AEs will be summarized (i.e., excluding those after a subject has given informed consent, but before vaccination).

Unsolicited AEs will be summarized by alphabetic SOC and descending PT as follows:

- Any unsolicited AE
- Related ("possibly" or "probably" related) unsolicited AEs
- SAEs
- Related ("possibly" or "probably" related) SAEs
- AESI
- Unsolicited AEs leading to withdrawal
- Any AE leading to death

Listings of all AEs will be provided by subject.

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

8.7.3 Clinical Laboratory Data

No clinical laboratory data will be collected in this study.

8.7.4 Physical Examinations

Physical examinations by body system include a complete examination at screening and directed examination at Day 1. Physical examination data will be listed.

8.7.5 Vital Signs

Vital signs including temperature, blood pressure, respiratory rate, and heart rate at screening and pre- and post-vaccination on Day 1 will be listed.

8.7.6 Prior and Concomitant Medications

Prior and concomitant medications will be coded to preferred drug name using the WHO DRUG dictionary and summarized for the safety population.

8.7.7 Other Safety Variables

None.

8.7.8 Subgroup Analysis

Analyses of the primary immunogenicity and safety endpoints will be summarized by sex and race.

8.7.9 Interim Analysis

No formal interim analysis is planned.

9 DATA HANDLING AND RECORD KEEPING

9.1 Source Documents and Access

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

records in any form, including those maintained in the Institution's Electronic Health Record system, if applicable.

The Investigator/Institution will maintain adequate and accurate source documents and study records that include all pertinent information related to subjects' 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, subject 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 (e.g., via an audit trail).

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

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

9.2 Data Management

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

9.3 Data Collection and Discrepancy Management

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

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

9.4 Laboratory Data

No external laboratory data transfers will be done. Sites will manually enter results to HBsAg/HCV antibody/HIV-1/HIV-2/screening CHIKV IgG ELISA and pregnancy testing into applicable eCRFs. Anti-CHIKV SNA titer results and ELISA tests done after screening will be handled by Sponsor.

9.5 File Management at the Investigational Site

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

9.6 Records Retention at the Investigational Site

Per ICH 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 Sponsor has notified the regulatory authority(ies) that clinical investigation with this product is discontinued.

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

9.7 Deviations from the Protocol

The Principal Investigator agrees to conduct the clinical study in compliance with the protocol agreed to by Sponsor and approved by the site's IRB.

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

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

The occurrence of protocol deviations will be routinely monitored by for evaluation of Investigator compliance with the protocol, GCP, and regulatory requirements. The Sponsor will review all protocol deviations on an ongoing basis and will be responsible for

determining if the deviation should be categorized as an Important Protocol Deviations (IPDs). 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 study drug and termination of further enrollment at the investigational site, or termination of the investigational site from the study.

10 QUALITY CONTROL AND ASSURANCE

10.1 Monitoring

The assigned clinical study monitor will verify eCRFs entries against source documentation at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to ICH-GCP Guidelines and local and federal regulations applicable to the conduct of the clinical study. The Principal Investigator must make source documentation accessible to the study monitor as needed to verify the information in the eCRFs. The Investigator agrees to meet with the study monitor at regular intervals to discuss study progress and ensure that any problems detected in the course of data monitoring are resolved appropriately.

10.2 Auditing

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

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

In the event the Investigator is contacted by a regulatory agency in relation to this study, the Investigator and investigational site staff must be available to respond to reasonable requests and inspection queries made during the inspection process. The Investigator must provide Sponsor with copies of all correspondence that may affect the review of the current study (e.g., Form FDA 483, inspectional observations, warning letters). Sponsor will provide any needed assistance in responding to regulatory inspections.

11 ETHICS AND RESPONSIBILITY

This study must be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and in compliance with the protocol, current ICH GCP

Guideline, national regulatory authorities, and all other applicable local laws and regulatory requirements. Each investigational site will seek approval by an IRB according to regional requirements. The IRB will evaluate the ethical, scientific and medical appropriateness of the study. Further, in collecting and handling participant data and completing eCRFs, the Investigator and investigational site staff will take measures to ensure adequate care in protecting participant privacy. To this end, a subject identification number will be used to identify each participant.

11.1 Informed Consent

The Investigator must obtain signed written informed consent from study participants prior to starting any study-related activities. All prospective subjects must sign and date an IRB-approved Informed Consent Form (ICF).

11.2 Institutional Review Board (IRB)

Before the start of the study, the IB, the protocol, proposed ICF(s), subject compensation (if any), Sponsor-approved study materials and advertisements, and any other written information to be provided to the subject, will be submitted to a properly constituted IRB for review. Sponsor must receive a copy of the written approval from the IRB for all of the above applicable documents prior to recruitment of subjects into the study and shipment of IP.

The IRB must provide written approval for all amendments to any of the above documents prior to implementation of these amendments at the investigational site. The Investigator is obliged to report SAEs and AESI, as well as any unanticipated problems to the IRB in addition to other information as required by the IRB.

The names (or title, if IRB procedures prohibit publishing of names) and associated backgrounds of the members of IRB (to assist in assuring that the board membership is properly constituted and operates according to 21 CFR part 56) will be given to the Sponsor prior to the start of the study along with a signed and dated statement stating that the protocol and ICF and, where applicable, any other document listed above, have been approved by them.

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

11.3 Compensation for Injury

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

11.4 Subject Confidentiality

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

Based on ICH GCP guidelines and regulatory requirements, the Principal Investigator is required to allow authorized personnel of Sponsor (or designee), the IRB, and members of the appropriate regulatory authority(ies) to review subject's files that are related to EBSI-CV-317-010. Subjects must be informed that their records may be reviewed by Sponsor (or designee) the IRB and the appropriate regulatory authority(ies) through direct access to the subject's original medical records.

11.5 Future Use of Stored Samples

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

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

12 ADMINISTRATIVE AND LEGAL REQUIREMENTS

12.1 Sponsorship

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

12.2 Protocol Amendments

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

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

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

12.3 Clinical Study Registration

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

12.4 Publication Policy

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

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

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

All publication rights are delineated in the Clinical Trial Agreement.

12.5 Terminating the Study

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

12.6 Terminating the Study at an Individual Site

A study site may be terminated from the study at the discretion of the Principal Investigator, Sponsor, or IRB. Sponsor may decide to replace a terminated site.

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APPENDIX I SUMMARY OF BLOODWORK

Study Visit	Screening		Day 1		Day 8		Day 15		Day 22		Day 57	
Procedure	Blood Draw		Blood Draw including PBMCs		Blood Draw including PBMCs		Blood Draw including PBMCs		Blood Draw including PBMCs and Plasmapheresis		Blood Draw including PBMCs and Plasmapheresis	
Assay	ELISA[1] (IgG)		ELISA[2] (IgM and IgG)		ELISA[2] (IgM and IgG)		ELISA[2] (IgM and IgG)		ELISA[2] (IgM and IgG)		ELISA[2] (IgM and IgG)	
	HBsAg, anti-HCV, HIV 1/2 Ag/Ab (local lab)		CHIKV-luc		CHIKV-luc		CHIKV-luc		CHIKV-luc		CHIKV-luc	
			ELISpot and flow cytometry		ELISpot and flow cytometry		ELISpot and flow cytometry		ELISpot and flow cytometry		ELISpot and flow cytometry	
Tubes	SST	CPT	SST	CPT	SST	CPT	SST	CPT	SST	CPT	SST	CPT
	3	0	2	8	2	8	2	8	10	8	10	8
Volume (mL, max)	15	0	10	64	10	64	10	64	50	64	50	64
Total Volume Serum (mL, max)	15		74		74		74		114		114	
Total Volume Plasma (mL)	0		0		0		0		~690-880 mL per subject (weight based)		~690-880 mL per subject (weight based)	

SST, serum-separating tube; CPT, cell preparation tubes; ELISA, enzyme-linked immunosorbent assay; PBMC, peripheral blood mononuclear cell; ELISpot, enzyme-linked immune absorbent spot; HBsAg, Hepatitis B surface antigen; anti-HCV, Hepatitis C virus antibody; HIV1/2 Ag/Ab, human immunodeficiency virus antigen/antibody.

[1] Screening will be performed by PPD using the EUROIMMUN Anti-CHIKV IgG ELISA kit.

[2] Study sample testing will be performed by Emergent using InBios Anti-CHIKV IgG and IgM ELISA kits.

APPENDIX II GRADING SCALE

EVENT	MILD (Grade 1)	MODERATE (Grade 2)	SEVERE (Grade 3)	POTENTIALLY LIFE THREATENING (Grade 4)
Fever	≥100.4-101.1°F (≥38.0-38.4°C)	≥101.2-102°F (≥38.5-<39°C)	≥102.1°F-104°F (≥39°C-40°C)	>104°F (>40°C)
Headache	No interference with activity	Some interference with activity, may require repeated use of non-narcotic pain reliever for more than 24 hours	Significant, prevents daily activity, any use of narcotic pain reliever	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Nausea	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Chills	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization
Arthralgia	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization
Injection site pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Use of any narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Injection site erythema/redness	25-50 mm*	>50 mm-100 mm*	>100 mm*	Necrosis or exfoliative dermatitis
Injection site induration/swelling	25-50 mm* and does not interfere with activity	>50 mm-100 mm* or interferes with activity	>100 mm* or prevents daily activity	Necrosis

*Revised by Sponsor