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 Statistical Analysis Plan (SAP)

SAP Version: v3.0

SAP Version Date: 07-Dec-2021

Statistical Analysis Plan

Protocol EBSI-CV-317-010, A Phase 2 Open-Label Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus-Like Particle Vaccine [CHIKV VLP], alum-adjuvanted)

Author:

[redacted]	[redacted]	*See electronic signature*	
Name	Position	Signature	YYYY/MMM/DD

Reviewed and Approved:

[redacted]	[redacted]	*See electronic signature*	
Name	Position	Signature	YYYY/MMM/DD
[redacted]	[redacted]	*See electronic signature*	
Name	Position	Signature	YYYY/MMM/DD

Protocol Version	**Date**
Original Protocol 1.0	[redacted]
Amendment 2.0	
Amendment 3.0	
Amendment 4.0	

SAP Version	**Date**
1.0 (Final)	[redacted]
2.0 (Final)	
3.0 (Final)	

Summary of Changes

Version	Summary of Major Change(s) and Impact	Revision Date
Version 1.0	First approved version of SAP.	[redacted]
Version 2.0	SAP updated for protocol amendment 3.0, including addition of telephone follow-up visit Day 183. Protocol amendment 2.0 was administrative and did not impact the SAP.	[redacted]
Version 3.0	SAP updated for protocol amendment 4.0. This amendment removed the pregnant partner follow-up and did not impact the SAP.	[redacted]

Table of Contents

1 INTRODUCTION....9

2 PROTOCOL SUMMARY....9

2.1 Study Objectives....9
2.1.1 Primary Objectives....9
2.1.2 Secondary Objective....9
2.1.3 Exploratory Objectives....9
2.2 Study Design and Conduct....9
2.3 Randomization and Blinding....14
2.3.1 Method of Randomization....14
2.3.2 Randomization Errors....14
2.3.3 Blinding and Unblinding....14

3 STUDY ENDPOINTS....14

3.1 Primary Immunogenicity Endpoints....14
3.2 Safety Endpoints....14
3.3 Exploratory Immunogenicity Endpoints....14

4 POWER AND SAMPLE SIZE CONSIDERATIONS....15

5 DATA CONSIDERATIONS....15

5.1 Protocol Deviations....15
5.1.1 Important Deviations....15
5.1.2 Reporting of Protocol Deviations....15
5.2 Analysis Populations....15
5.3 Analysis Groups....16
5.4 Analysis Time Points....16
5.5 Definition of Baseline....16
5.6 Multiple Records in an Analysis Window....16
5.7 Coding Dictionaries....16
5.8 Adverse Event Toxicity/Severity Grading....16

6 STATISTICAL CONSIDERATIONS....17

6.1 Statistical Considerations....17
6.1.1 Seroresponse Rates....18
6.1.2 Geometric Mean Titers and Geometric Mean Fold Increase....18
6.2 Units and Precision....18
6.3 Derived Variables....18
6.4 Statistical Hypotheses....21
6.5 Handling of Missing Data and Other Data Issues....21
6.5.1 Missing or Partial Dates....21
6.5.2 Missing Outcome and Covariates....22
6.5.3 Non-Quantifiable Laboratory Data....22
6.5.4 Implausible Subject Reported Outcomes....22
6.6 Adjustment for Covariates....22

6.7 Multicenter Study 22
6.8 Subgroup Analysis 23
6.9 Multiplicity Adjustment 23

7 STUDY POPULATION CHARACTERISTICS 23

7.1 Subject Disposition 23
7.2 Protocol Deviations 23
7.3 Populations Analyzed 23
7.4 Demographics and Baseline Characteristics 23
7.4.1 Demographics 23
7.4.2 Baseline Disease Characteristics 23
7.5 Medical History 24
7.6 Prior and Concomitant Medications and Vaccines 24
7.7 Treatment Compliance 24

8 IMMUNOGENICITY ANALYSIS 24

8.1 Primary Immunogenicity Endpoints 25
8.1.1 CHIKV SNA Seroresponse Rate and Associated 95% CI at Day 22 25
8.1.2 CHIKV SNA GMT and Associated 95% CI at Day 22 25
8.1.3 CHIKV SNA Seroresponse Rates and GMTs with Associated 95% CIs at Days 8, 15, and 57 25
8.1.4 CHIKV ELISA IgG and IgM GMTs and Associated 95% CIs at Days 8, 15, 22, and 57 25
8.1.5 Geometric Mean Fold Increase in CHIKV SNA Titers from Day 1 to Days 8, 15, 22, and 57 25
8.1.6 Geometric Mean Fold Increase in CHIKV ELISA IgG and IgM Titers from Day 1 to Days 8, 15, 22, and 57 26
8.1.7 Subjects with CHIKV SNA Titer ≥15, 40, 60, 80, 100, 160, 640, and 4-fold Rise over Baseline Thresholds at Days 8, 15, 22, and 57 26
8.2 Secondary Immunogenicity Endpoints 26
8.3 Exploratory Immunogenicity Endpoints 26
8.3.1 Kinetics of CHIKV Immune Responses over Time 26
8.3.2 PBMC T and B Cell Responses at Days 1, 8, 15, 22, and 57 26
8.4 Pharmacokinetic and Pharmacodynamic Analysis 26

9 SAFETY ANALYSIS 26

9.1 Extent of Exposure 27
9.2 Adverse Events 27
9.2.1 Overall Summary of Solicited and Unsolicited Adverse Events 27
9.2.2 Solicited Adverse Events from Day 1 until Day 8 28
9.2.3 Unsolicited Adverse Events from Day 1 through Day 29 28
9.3 Deaths, Other Serious Adverse Events and Other Significant Adverse Events 28
9.3.1 Deaths from Day 1 to Day 183 End of Study Visit 28
9.3.2 Serious Adverse Events from Day 1 to Day 183 End of Study Visit 29

9.3.3 Adverse Event of Special Interest from Day 1 to Day 183 End of Study Visit....29
9.3.4 Medically Attended Adverse Events....29
9.3.5 Adverse Events Leading to Study Discontinuation from Day 1 to Day 183 End of Study Visit....29
9.4 Clinical Laboratory Tests....29
9.4.1 Pregnancy Testing....29
9.4.2 Viral Serology Testing at Screening....29
9.5 Vital Signs, Physical Findings and Other Variables Related to Safety....29
9.5.1 Vital Signs....29
9.5.2 Physical Examinations....29
10 DATA MONITORING AND INTERIM ANALYSIS....30
10.1 Safety Monitoring Committee....30
10.2 Interim Analysis....30
11 REFERENCES....31

Appendices

APPENDIX I LIST OF TABLES, FIGURES AND LISTINGS....32
APPENDIX II TOXICITY GRADING SCALE....35
APPENDIX III STANDARD MEDDRA QUERY FOR ARTHRITIS....36

List of Figures

Figure 1: EBSI-CV-317-010 Schematic Diagram of Study Design.....10
Figure 2: Schedule of Events.....12

List of Abbreviations and Definition of Terms

AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomic Therapeutic Chemical classification
CI	Confidence Interval
CHIKV	Chikungunya Virus
CSR	Clinical Study Report
CTMS	Clinical Trial Management System
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ELISA	Enzyme-linked Immunosorbent Assay
ELISpot	Enzyme-linked Immunosorbent Spot
EOS	End of Study Visit
FDA	Food and Drug Administration
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intramuscular
IP	Investigational Product
LLOQ	Lower Limit of Quantitation
luc	Luciferase Assay
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
mL	milliliter(s)
mm	millimeter(s)
NT_{80}	80% Neutralizing Titer
PBMC	Peripheral Blood Mononuclear Cell
PT	Preferred Term
PXVX0317	Vaccine Investigational Product
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Standard Data Tabulation Model

SMC	Safety Monitoring Committee
SMQ	Standard MedDRA Query
SNA	Serum Neutralizing Antibody
SOC	System Organ Class
SR	Seroresponse Rate
TLF	Tables, Listings and Figures
US	United States
VLP	Virus-like Particle
WHO	World Health Organization

1 INTRODUCTION

This Statistical Analysis Plan (SAP) is based on Protocol EBSI-CV-317-010, "A Phase 2 Open-Label Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus-Like Particle Vaccine [CHIKV VLP], alum-adjuvanted) (Version 4.0 07-DEC-2021). This document specifies details of the definitions of the derived variables, analysis methods, assumptions, and data handling conventions. The document is accompanied by mock-up tables, listings, and figures (TLF shells). Some further details on the calculation of derived variables will be provided as programmer's notes in the TLF shells. The TLF shells serve only as a guide for programming the final TLF. They are working documents and can be updated as needed.

2 PROTOCOL SUMMARY

2.1 Study Objectives

2.1.1 Primary Objectives

- To assess the induction of 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 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.

2.1.2 Secondary Objective

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

2.1.3 Exploratory Objectives

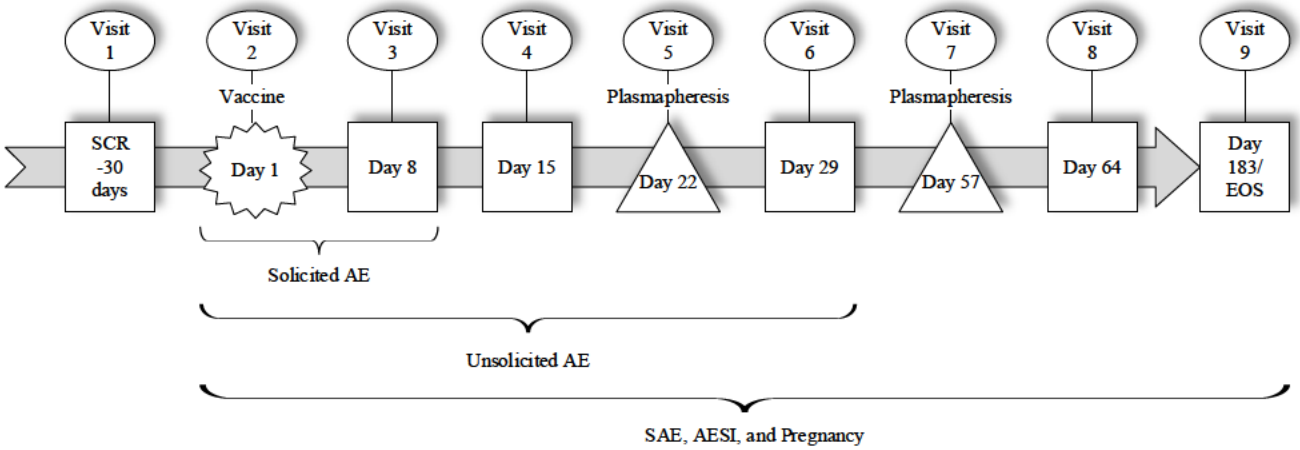
- To obtain plasma and sera at Days 22 and 57 from subjects immunized with a single dose of PXVX0317 to support nonclinical studies.
- To characterize the kinetics of CHIKV IgM, IgG, and neutralizing responses following a single dose of PXVX0317 as measured 7, 14, 21, and 56 days (Days 8, 15, 22, and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

2.2 Study Design and Conduct

Study EBSI-CV-317-010 is an open-label, single arm Phase 2 study in healthy adults 18 to 45 years of age. The sample size target is 25 subjects recruited at a single center in the U.S. The study has a screening period of up to 30 days, a treatment and observation period from

Day 1 to Day 22, and a follow-up period through Day 183 (see Figure 1). Subjects receive CHIKV VLP at a dose of 40 µg in combination with 300 µg Alhydrogel via intramuscular (IM) injection in the deltoid muscle 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 occurs on Day 29, followed by a second plasmapheresis procedure and collection of serum at Day 57. Final phone call follow-up visits to assess safety and concomitant medications occur on Day 64 and Day 183, the End of Study (EOS).

Figure 1: EBSI-CV-317-010 Schematic Diagram of Study Design



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

Refer to Figure 2, Schedule of Events, for a table of assessments at each visit. The per subject estimated total study duration is 7 months. Screening assessments include informed consent, review of eligibility criteria, medical history, prior and concomitant medications, demography, physical examination including height and weight for body mass index calculation, vital signs, urine pregnancy test for female subjects of child-bearing potential, viral markers for hepatitis B surface antigen (HBsAg), hepatitis C (HCV) and human immune deficiency virus 1 and 2 (HIV-1/HIV-2), and CHIKV IgG antibody enzyme-linked immunosorbent assay (ELISA). Detectable CHIKV IgG antibody by ELISA at screening is an exclusion criterion.

Prior to investigational product (IP) administration on Day 1 (baseline), eligibility, medical history and concomitant medications are reviewed, a negative urine pregnancy test result is required for female subjects of child-bearing potential, and baseline samples are drawn for CHIKV serum neutralizing antibodies (SNA), PBMCs, and CHIKV specific IgG and IgM antibody levels. A directed physical examination is performed if indicated at any scheduled visit. Vital signs including blood pressure, heart rate, respiratory rate and temperature are taken on Day 1 before and after IP administration. Following vaccination, subjects are observed in clinic for 30-60 minutes.

Local and systemic solicited adverse events (AEs) occurring within 7 days after injection are recorded by the subject using a memory aid (i.e., paper diary). Subjects are specifically asked to record local injection site events (pain, redness, swelling) and systemic events (oral

temperature ≥100.4ºF, chills, fatigue, headache, myalgia, joint pain, and nausea) each day for the collection period. Memory aids are reviewed at the Day 8 visit, and the Principal Investigator assesses AE severity and causality. Unsolicited AEs are monitored from Day 1 through Day 29; serious adverse events (SAEs) and adverse events of special interest (AESI; see Section 6.3 for definition) are monitored for the duration of the study. Any AEs occurring within 30 minutes of the plasmapheresis procedures at Days 22 and 57 are collected as well. Information on all concomitant medications used through Day 29 is recorded; after Day 29, only information on concomitant medications used for the treatment of an SAE or AESI is recorded. No safety laboratory samples are collected as part of the study.

After screening, blood is collected for immunogenicity assessments at Days 1 (before the vaccination), 8, 15, 22, and 57. SNA responses to CHIKV VLP are determined by a luciferase based CHIKV neutralization assay (CHIKV-luc). Titers are expressed as the reciprocal of the serum dilution achieving 80% neutralization (NT_{80}). CHIKV ELISA IgG and IgM titers are defined per commercial test kits (InBios) utilizing a sandwich method with horseradish peroxidase plus 3,3',5,5'-tetramethylbenzidine optical density quantitation. The provisional threshold for seroresponse is CHIKV SNA titer ≥40, should a definitive seroresponse threshold not be determined prior to final analysis; subjects meeting other CHIKV SNA titer thresholds (≥15, 40, 60, 80, 100, 160, 640, and 4-fold rise over baseline) will be summarized.

There is no Safety Monitoring Committee (SMC) for this study.

Figure 2: 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[7,10]	X[10]	X[10]	X[10]
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					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 exam, 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 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 7, Unsolicited AEs to Day 29; After Day 29, collect SAE and AESI only.

[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 protocol Appendix I.

2.3 Randomization and Blinding

2.3.1 Method of Randomization

Study EBSI-CV-317-010 is not a randomized study.

2.3.2 Randomization Errors

Not applicable.

2.3.3 Blinding and Unblinding

Study EBSI-CV-317-010 is not a blinded study.

3 STUDY ENDPOINTS

3.1 Primary Immunogenicity Endpoints

- CHIKV SNA seroresponse rate and associated 95% confidence interval (CI) at Day 22.
- CHIKV SNA geometric mean titer (GMT) and associated 95% CI at Day 22.
- CHIKV SNA seroresponse rate and CHIKV SNA GMTs with associated 95% CIs at Days 8, 15, and 57.
- 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 Days Days 8, 15, 22, and 57.
- GMFI in CHIKV ELISA IgG and IgM titer from Day 1 to Days Days 8, 15, 22, and 57.
- Numbers and percentages of subjects with an CHIKV SNA titer ≥15, 40, 60, 80, 100, 160, 640, and 4-fold rise over baseline thresholds.

Each endpoint is summarized overall and by sex and by race group (white and non-white).

3.2 Safety Endpoints

- Incidence of solicited AEs through Day 8.
- Incidence of unsolicited AEs through Day 29.
- Incidence of AESIs and SAEs through Day 183/EOS.
- Incidence of AEs within 30 minutes of plasmapheresis on Days 22 and 57.

Each endpoint is summarized overall and by sex and by race group (white and non-white).

3.3 Exploratory Immunogenicity Endpoints

- Kinetics of CHIKV immune responses (via SNA and ELISA; i.e., primary endpoints) over time.

- T and B cell responses from PBMC samples, including enzyme-linked immune absorbent spot (ELISpot) and flow cytometry results, at Days 1, 8, 15, 22, and 57.

4 POWER AND SAMPLE SIZE CONSIDERATIONS

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.

5 DATA CONSIDERATIONS

5.1 Protocol Deviations

A deviation occurs when site personnel or a subject does not adhere to the protocol's stipulated requirements, whether inadvertently or planned. All identified protocol deviations are documented (entered by the monitor in the Clinical Trial Management System [CTMS]) and classified by category and type (important/not important). In addition, programmatic checks for protocol deviations are run by the Sponsor. Programmatically identified protocol deviations are also entered to the CTMS and classified by category and type (important/not important).

5.1.1 Important Deviations

Important protocol deviations are defined by ICH E3 as those "that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a subject's rights, safety, or well-being" (2). Examples of important protocol deviations include:

- Subjects who were enrolled but did not meet study eligibility criteria.
- Subjects who received a prohibited prior or concomitant medication or vaccine.
- Subjects who previously received an investigational CHIKV vaccine/product, including subjects that are vaccinated more than once in the EBSI-CV-317-010 study.
- Other deviations from key study procedures such as subject noncompliance with assessment of primary outcome measures (e.g., missing samples, significantly out of window assessments).

5.1.2 Reporting of Protocol Deviations

All protocol deviations will be contained in the Study Data Tabulation Model (SDTM) datasets. Protocol deviations assessed as important are tabulated and listed by subject for the safety population (see Section 7.2).

5.2 Analysis Populations

Analysis is based on the following study populations:

Enrolled Population: All screened subjects who sign informed consent, are entered in the electronic data collection (EDC) database, and meet all eligibility criteria.

Exposed Population: All subjects who receive IP.

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

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

5.3 Analysis Groups

Tables are displayed with a single column for this open-label, single arm study.

5.4 Analysis Time Points

For immunogenicity analyses, CHIKV SNA samples are collected at Day 1 (baseline), Day 8 (+3 days), Day 15 (±2 days), Day 22 (+5 day) and Day 57 (+5 days). For all time points, all available data (including out of window data) are included in the summaries according to the analysis set defined for a subject's inclusion.

Data collected at the early discontinuation visit or at unscheduled visits are not presented in the by-visit summary analyses but are included in data listings by subject.

5.5 Definition of Baseline

For all analyses, the baseline value for each measure is defined as the last non-missing value prior to IP administration.

5.6 Multiple Records in an Analysis Window

If more than one sample is collected for CHIKV SNA NT_{80} assessment for a given time point, the sample closest to the nominal time point is used for analysis.

5.7 Coding Dictionaries

Medical history and AEs will be coded to system organ class (SOC) and preferred term (PT) based on the Medical Dictionary for Regulatory Activities (MedDRA) dictionary version 20.1.

Prior and concomitant medications and vaccines will be coded according to the World Health Organization's (WHO) WHO-Drug Global Dictionary version B3Sep2017 to Anatomical Therapeutic Chemical (ATC) classification and preferred drug name.

5.8 Adverse Event Toxicity/Severity Grading

With the exception of redness and swelling, all solicited AEs are graded by the Investigator according to severity grading scales from "mild" to "potentially life-threatening." The

severity of redness and swelling, recorded as a diameter (mm) in the solicited adverse event memory aid, is summarized according to categories based on the largest-diameter linear measurement when a local reaction is present:

- Grade 0/absent = 0-24 mm.
- Grade 1/mild= 25-50 mm.
- Grade 2/moderate= 51-100 mm.
- Grade 3/severe= >100 mm.
- Grade 4/potentially life threatening = necrosis or exfoliative dermatitis.

Events reported as not present (0 mm is entered) are reported as Grade 0. Similarly, body temperatures recorded by the subject that are below 100.4° result in fever reported as Grade 0/absent.

Unsolicited AEs and solicited AEs other than redness and swelling are graded by the Investigator for severity. The severity grading scales (Grade 1 = Mild, Grade 2 = Moderate, Grade 3 = Severe, Grade 4 = Potentially Life-Threatening) that are used for safety assessment in this study are provided in Appendix II of this document and Section 7.1.7 and Appendix II of the Protocol, based on the *FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials* (1). Note that AEs that result in death are classified as Grade 4 and indicated as resulting in death.

6 STATISTICAL CONSIDERATIONS

6.1 Statistical Considerations

Data summaries are tabulated for selected analysis populations defined in Section 5.2.

Continuous endpoints are summarized by descriptive statistics including number of non-missing observations (n), mean, standard deviation (SD), median, minimum and maximum. In the analysis of the CHIKV-luc assay data and ELISA IgG and IgM as continuous variables, values are logarithmically transformed (base10), and the GMTs and associated 95% CI for each treatment group are computed by exponentiating the corresponding log-transformed means and two-sided 95% CIs. Categorical variables are summarized by frequency counts (n) and percentages of subjects (%) in each category, including missing or unknown when appropriate. Unless otherwise specified, CIs and hypothesis tests are two-sided with 95% confidence.

The reporting conventions to be applied to all tables, listings and figures are included in a living document that accompanies the table and listing shells. All derivations, statistical analyses, summaries and listings are generated using SAS Version 9.4 or higher (SAS Institute, Inc., Cary, North Carolina, United States).

6.1.1 Seroresponse Rates

The immune response to PXVX0317 vaccine will be demonstrated at Day 22 using seroresponse rate (the proportion of subjects with a CHIK-luc assay CHIKV SNA NT_{80} ≥provisional threshold of 40, should a definitive seroresponse threshold not be determined prior to final analysis). Proportions of subjects with CHIKV-luc assay CHIKV SNA NT_{80} ≥provisional threshold of 40 will be reported with associated two-sided 95% Wilson score method CIs (keyword /BINOMIAL (WILSON) on tables statement).

In addition, proportions of subjects meeting other CHIKV SNA titer thresholds (≥15, 40, 60, 80, 100, 160, 640, and 4-fold rise over baseline) will be summarized. Proportions of subjects meeting other CHIKV SNA titer thresholds will be reported with associated two-sided 95% Wilson score method CIs. See Section 8.1.7 for analysis outputs.

Identical methods will be used for analysis of seroresponse rates at Days 8, 15, and 57.

6.1.2 Geometric Mean Titers and Geometric Mean Fold Increase

The immunogenicity of PXVX0317 vaccine will be explored further using Day 22 GMTs. The mean and two-sided 95% CIs of the log_{10}-transformed CHIKV SNA titers (log_{10}) will be calculated and then back transformed and reported as the group GMT value with associated CI. Identical methods will be used for CHIKV ELISA IgG and IgM GMTs, as well analysis of GMTs at Days 8, 15, and 57. See Section 8.1.4 for analysis outputs.

Geometric mean fold increase (GMFI) from baseline (Day 1) to Days 8, 15, 22 and 57 will be calculated using the difference from Day 1 in log_{10}-transformed titers for both CHIKV SNA titers and CHIKV ELISA IgG and IgM titers with the geometric mean and 95% CI for fold increase calculated as mentioned above for GMT. The median, minimum and maximum titers and fold-increases will be based on the non-transformed scale. See Section 8.1.6 for analysis outputs.

6.2 Units and Precision

No intermediate rounding will be performed in SDTM datasets; only final values for TLFs will be rounded as follows. Immunogenicity titers will be reported with one decimal place or two significant digits (e.g., 0.032, 18.0).

Vital signs data will be reported using standard international units. Vital signs variables including derivations thereof will be reported to the same precision as the source data. Parameters derived directly from source data (e.g., minimum, maximum) or first-order statistics (e.g., mean) will be reported and analyzed with the same precision as the source data. Second-order statistics (e.g., SD) will be reported with one more significant digit than the source data. Percentages will be reported to one decimal place.

6.3 Derived Variables

This section provides definitions of the derived variables. In some cases, the definitions are provided in the relevant sections as noted.

AE, causing study discontinuation, is defined as a Yes response to the corresponding question on the AE eCRF.

AE, medically attended (MAAE), is defined as a Yes response to the corresponding question on the AE eCRF.

AE, related, is defined as one that is "probably" or "possibly" related to IP per Investigator assessment on the eCRF. If the relationship is missing for one or more occurrences of an AE for a given subject, the closest relationship of the remaining occurrences of the AE for that subject will be used; if the relationship is missing for the only occurrence of an AE for a given subject, then that event will be assumed to be related in order to be conservative.

AE, resulting in death, is defined as a Yes response to the corresponding question on the AE eCRF and/or a fatal outcome.

AE, serious, is defined per the derived eCRF field for AE seriousness, based on the individual criteria that make an AE qualify as serious (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, or any important medical event such as an event that may jeopardize the subject or require medical or surgical intervention to prevent one of the above outcomes).

AESI is defined in this study as new onset or worsening arthralgia that is medically attended, as noted by an AE coded to any of the narrow or broad preferred terms included in the MedDRA Standard Query (SMQ) for arthritis (see Appendix III) or having a Yes response to the corresponding AESI question on the AE eCRF.

AE, severity, is graded Grade 1 (mild) to Grade 4 (potentially life-threatening) as described in Section 5.8. If the severity is missing for one or more occurrences of an AE for a given subject, the maximum severity of the remaining occurrences of the AE for that subject will be used; if the severity is missing for the only occurrence of an AE for a given subject, then that event will be assumed to be severe in order to be conservative.

AEs, solicited, are defined as those collected in the memory aid. Solicited AEs include local (i.e., pain, redness, and swelling) and systemic reactions (i.e., fever, chills, fatigue, headache, myalgia, arthralgia, and nausea).

AE, treatment emergent. AE collection begins at Day 1 (baseline), the day of IP administration. AEs occurring after a subject has given informed consent, but before vaccination, will be excluded from summaries. Only treatment-emergent AEs will be summarized.

AEs, unsolicited, are defined as those AEs not listed in the memory aid but collected in the EDC.

Age at screening visit is automatically calculated by EDC based on date of birth versus date of informed consent. Age at screening will be summarized in all applicable outputs.

Analysis visits are defined in Section 5.4.

Analysis populations are defined in Section 5.2.

Baseline is defined in Section 5.5. Change from baseline is defined as (value at post-baseline assessment – value at baseline).

Baseline seropositive rate is defined as the percentage of subjects with CHIKV-luc NT_{80} ≥lower limit of quantitation (LLOQ).

Body mass index at screening visit is automatically calculated by EDC as a subject's weight in kg divided by height in m^2.

Chikungunya virus ELISA IgG and IgM titers are defined per commercial test kits (InBios) utilizing a sandwich method with horseradish peroxidase plus 3,3',5,5'-tetramethylbenzidine optical density quantitation. CHIKV ELISA IgG and IgM values below the LLOQs for each will be replaced by LLOQ/2 in the immunogenicity analyses including GMT and GMFI.

Chikungunya virus luciferase neutralization assay (CHIKV-luc) SNA NT_{80} (80% neutralizing titer) is defined as the dilution factor that corresponds to an 80% reduction of luciferase activity compared to virus-only control. CHIKV-luc assay CHIKV SNA NT_{80} values below the LLOQ will be replaced by LLOQ/2 in the immunogenicity analyses including GMT and GMFI. For the 4-fold rise over Day 1 (baseline), values below the LLOQ will be replaced with the LLOQ.

Completion of study for an individual subject is defined as completion of the Day 183/EOS visit and any required safety follow-up (i.e., a disposition status of completed).

Early discontinuation for an individual subject is defined as completion of the Early Discontinuation Visit (i.e., a disposition status other than completed).

Medical history items entered on the medical history eCRF will be considered to predate the study regardless of onset date.

Medication, concomitant, is defined as one taken after start of IP administration on Day 1 (baseline) through the end of study. For analysis purposes, any medication which is ongoing or with a stop date that is on or after the study medication administration date will be categorized as a concomitant medication. Partial or missing concomitant medication start and/or end date will be imputed according to Section 6.5.1.

Medication, prior, is defined as one with a stop date within 30 days prior to the screening visit (90 days for blood products and within 6 months for immunosuppressive/ immunomodulatory medications) through the start of IP administration on Day 1 (baseline).

Protocol deviations are defined by inclusion in the CTMS protocol deviation module (subject-level only, not site-level), including monitor-identified deviations as well as Sponsor-identified programmatic deviations. Protocol deviations will be classified by category and type (important/not important).

Rounding of values is described in Section 6.2.

Seroresponse rate is defined as the percentage of subjects who achieve a CHIKV-luciferase (luc) assay 80% neutralizing titer (NT_{80}) ≥provisional threshold of 40, should a definitive seroresponse threshold not be determined prior to final analysis.

Study Day 1 is defined as the day of IP administration on Day 1 (baseline). The day prior to Day 1 is Day -1. There is no Day 0. If the subject is not treated, then the Day 1 (baseline) is defined as the day of planned treatment.

Study day relative to Day 1 will be calculated as:

- **Study Day** = (assessment date – date of Day 1 + 1) if the assessment is on or after Day 1.
- **Study Day** = (assessment date – date of Day 1) if the assessment is before Day 1.

For conversion of durations, 1 year = 365.25 days and 1 month = 365.25/12 = 30.4375 days.

Subgroups for sex (male and female) and race (white and non-white) are defined based on the corresponding demographic questions. Subjects missing sex or race will be omitted from analyses by subgroup.

Summary statistics are described in Section 6.1.

Time duration (in days) between event A and event B is (date of event B – date of event A + 1). For conversion of durations, 1 year = 365.25 days and 1 month = 365.25/12 = 30.4375 days.

Treatment, actual, is defined as the treatment administered to the subject by the site (i.e., PXVX0317).

6.4 Statistical Hypotheses

There is no formal statistical hypothesis tested because there is no comparator group in this study. No p-values will be reported.

6.5 Handling of Missing Data and Other Data Issues

Please see Section 6.3 for assumptions for AEs with missing relatedness and/or severity.

6.5.1 Missing or Partial Dates

AE collection begins at Day 1 (baseline), the day of IP administration. Consequently, all AEs will be considered treatment-emergent regardless of onset date.

For missing or partial dates for prior or concomitant medication/therapy, the following conventions will be used for the purpose of determining whether the medication/therapy is concomitant or not. Original values will be provided in the listings as is, without imputation.

- For start date missing completely or missing the year, impute the date to the date of first exposure to any study treatment.
- For start date missing both the month and the day, if the year is the same as the date of first exposure to any study treatment, impute the date to the date of first exposure to any study treatment, otherwise, impute the date to January 1st.
- For start date missing the day only, if the year and the month are the same as the date of first exposure to any study treatment, impute the date to the date of first exposure to any study treatment, otherwise, impute the date to the first of the month.

- For end date missing completely or missing the year, impute the date to the date of last contact.
- For end date missing both the month and the day, if the year is the same as the date of last contact, impute the date to the date of last contact, otherwise, impute the date to December 31st.
- For start date missing the day only, if the year and the month are the same as the date of last contact, impute the date to the date of last contact, otherwise, impute the date to the last day of the month.

Note that when the dates are parts of an outcome measure (e.g., the date of death, date of hospital discharge), the imputation rules in this section do not apply.

6.5.2 Missing Outcome and Covariates

Subjects will be included in the analyses to the extent of their available data; missing immunogenicity data will not be imputed. See Section 5.4 and definition of CHIKV-luc SNA NT_{80} in Section 6.3.

Subjects with missing categorical data are counted in missing or unknown categories when appropriate. Subjects with missing numeric data are treated as missing completely at random when calculating summary statistics. For likelihood-based analyses (e.g., regression), missing at random is assumed.

6.5.3 Non-Quantifiable Laboratory Data

Not applicable.

6.5.4 Implausible Subject Reported Outcomes

Values measured and reported by the subject directly (e.g., via the memory aid) will be corrected in a reconciliation process with the Primary Investigator, to the extent possible. In cases where the reported values are clearly biologically implausible, the reported values and associated toxicity grades will be set to missing and excluded from analysis. These reported values will be included in listings.

Example: Body (oral) temperatures less than ≤33°C or ≥42°C.

6.6 Adjustment for Covariates

No adjustment for covariates will be performed.

6.7 Multicenter Study

This is a single center study.

6.8 Subgroup Analysis

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

6.9 Multiplicity Adjustment

Since no formal statistical hypothesis is being tested, no multiplicity adjustment is needed.

7 STUDY POPULATION CHARACTERISTICS

7.1 Subject Disposition

Subject disposition over the course of the study will be summarized for all enrolled subjects, including number and percentages of subjects still in the study at each scheduled visit, completing the study and reason for not completing the study. Screen failure reasons will be listed by subject. Subject participation in plasmapheresis at Days 22 and 57 will be summarized.

Subjects not treated or those receiving an incorrect dose will be listed. Subjects who were enrolled despite not meeting entry criteria and those who received an excluded concomitant medication or vaccination will be listed.

7.2 Protocol Deviations

Protocol deviations defined in Section 5.1 will be categorized as important or not important. Important protocol deviations will be tabulated by category for the exposed population as well as listed by subject.

7.3 Populations Analyzed

The number and percentage of participants in each analysis population (enrolled, exposed, safety, mITT) will be summarized for all subjects. Reasons for exclusion from analysis populations will be summarized for all exposed subjects and listed by subject.

7.4 Demographics and Baseline Characteristics

7.4.1 Demographics

Demographic and baseline characteristics including age, sex, race, ethnicity, baseline height, weight and body mass index will be tabulated for the exposed and mITT populations.

7.4.2 Baseline Disease Characteristics

This study will enroll healthy US adolescent and adult subjects 18 to 45 years of age, so no baseline disease characteristics will be collected.

7.5 Medical History

Medical history will be coded to the MedDRA dictionary SOC and PT (see Section 5.7). A summary table and a listing of medical history will be supplied for the exposed population.

7.6 Prior and Concomitant Medications and Vaccines

Prior medications are defined as those taken within 30 days prior to the screening visit (90 days for blood products and within 6 months for immunosuppressive/ immunomodulatory medications) through the start of IP administration on Day 1 (baseline). Concomitant medications are defined as those taken after start of IP administration on Day 1 (baseline) through Day 183/EOS. For analysis purposes, any medication/procedure with a stop date between 30 days before date of screening and prior to the start date of the first study treatment will be categorized as prior; any medication/procedure which is ongoing or with a stop date that is on or after the start date of the study medication administration date will be categorized as a concomitant medication/procedure. Partial dates will be imputed according to Section 6.5.1.

Prior and concomitant medications and vaccines will be coded to the WHO-Drug Global Dictionary ATC classification and preferred drug name (see Section 5.7). All prior and concomitant medications and vaccines will be tabulated together by ATC classification and preferred drug name for the safety population. A subject data listing of all prior and concomitant medications will be generated.

7.7 Treatment Compliance

As the IP administration is a single 0.8 mL intramuscular injection performed by site personnel, measurements of treatment compliance are not applicable. A vaccine administration summary will be provided and details listed (see Section 9.1).

8 IMMUNOGENICITY ANALYSIS

The analysis of all immunogenicity endpoints will utilize the mITT population. Summary statistics for immunogenicity results by scheduled visit will be provided for the mITT population. CHIKV SNA seroresponse rates and GMTs will be tabulated by visit, as will CHIKV ELISA IgG and IgM GMTs. Geometric mean fold increase as well as number and percentage of subjects meeting various CHIKV SNA titer thresholds will also be displayed by post-vaccination scheduled visit. Reverse cumulative distribution plots of CHIKV-luc assay CHIKV SNA NT_{80} versus proportion of subjects will be generated by scheduled visit. CHIKV SNA and CHIKV ELISA IgG and IgM GMTs will also be plotted over time.

The primary immunogenicity endpoint analyses will be repeated by sex and by race group (white and non-white).

8.1 Primary Immunogenicity Endpoints

8.1.1 CHIKV SNA Seroresponse Rate and Associated 95% CI at Day 22

The immunogenicity of PXVX0317 vaccine will be illustrated at Day 22 by estimating the seroresponse rate and associated 95% CI. See Section 6.1 for statistical details.

This endpoint will be summarized for the mITT population, as well as by sex and by race group (white and non-white).

8.1.2 CHIKV SNA GMT and Associated 95% CI at Day 22

Day 22 CHIKV SNA GMTs and associated 95% CIs will be estimated to demonstrate immunogenicity of PXVX0317 vaccine. See Section 6.1 for statistical details.

This endpoint will be summarized for the mITT population, as well as by sex and by race group (white and non-white). A graph of CHIKV SNA GMTs by visit will be presented for the mITT population. As well, a reverse cumulative distribution plot of titers by visit will be displayed for the mITT population.

8.1.3 CHIKV SNA Seroresponse Rates and GMTs with Associated 95% CIs at Days 8, 15, and 57

Day 8, Day 15, and Day 57 CHIKV SNA seroresponse rates and GMTs with associated 95% CIs will be reported in the same manner as for Day 22 above.

This endpoint will be summarized for the mITT population, as well as by sex and by race group (white and non-white).

8.1.4 CHIKV ELISA IgG and IgM GMTs and Associated 95% CIs at Days 8, 15, 22, and 57

CHIKV ELISA IgG and IgM GMTs and associated 95% CIs will be estimated at Days 8, 15, 22, and 57. See Section 6.1 for statistical details.

These endpoints will be summarized for the mITT population, as well as by sex and by race group (white and non-white). Graphs of CHIKV ELISA IgG and IgM GMTs by visit will be presented for the mITT population.

8.1.5 Geometric Mean Fold Increase in CHIKV SNA Titers from Day 1 to Days 8, 15, 22, and 57

GMFIs for increase over Day 1 (baseline) titer will be analyzed as described for GMTs for each post-vaccination time point (Days 8, 15, 22, and 57). See Section 6.1 for statistical details and Section 6.3 for handling of CHIKV SNA NT_{80} values below LLOQ.

This endpoint will be summarized for the mITT population, as well as by sex and by race group (white and non-white). GMFI will be plotted by visit for the mITT population, and a reverse cumulative distribution of fold-rise from baseline by visit will also be presented for the mITT population.

8.1.6 Geometric Mean Fold Increase in CHIKV ELISA IgG and IgM Titers from Day 1 to Days 8, 15, 22, and 57

GMFIs for increase over Day 1 (baseline) titer will be analyzed as described for GMTs for each post-vaccination time point (Days 8, 15, 22, and 57). See Section 6.1 for statistical details and Section 6.3 for handling of CHIKV ELISA IgG and IgM values below LLOQs.

These endpoints will be summarized for the mITT population, as well as by sex and by race group (white and non-white). GMFIs will be plotted by visit for the mITT population for each.

8.1.7 Subjects with CHIKV SNA Titer ≥15, 40, 60, 80, 100, 160, 640, and 4-fold Rise over Baseline Thresholds at Days 8, 15, 22, and 57

Number and percentage of subjects with an CHIKV SNA NT_{80} titer ≥15, 40, 60, 80, 100, 160, 640, and 4-fold rise over Day 1 (baseline) thresholds at Days 8, 15, 22 and 57 will be analyzed as described for the Day 22 threshold. See Section 6.1 for statistical details.

This endpoint will be summarized for the mITT population, as well as by sex and by race group (white and non-white).

8.2 Secondary Immunogenicity Endpoints

There are no secondary immunogenicity endpoints in this study.

8.3 Exploratory Immunogenicity Endpoints

8.3.1 Kinetics of CHIKV Immune Responses over Time

As mentioned above, SNA and ELISA IgG and IgM GMTs will be plotted by visit for the mITT population. If data permit, regression modeling may be performed to explore the shape of the time-response curve for each analyte using a linear or non-linear model, as appropriate, with time as the covariate.

8.3.2 PBMC T and B Cell Responses at Days 1, 8, 15, 22, and 57

T and B cell responses from PBMC samples will be analyzed via enzyme-linked immune absorbent spot (ELISpot) assay and flow cytometry at Days 1 (baseline), 8, 15, 22, and 57. Results will be summarized by visit for the mITT population.

8.4 Pharmacokinetic and Pharmacodynamic Analysis

Not applicable.

9 SAFETY ANALYSIS

All safety data will be presented in the form of tabulations and subject listings, based on the safety population.

9.1 Extent of Exposure

The frequencies and percentages of subjects receiving IP administration will be summarized for the exposed population, along with whether or not the dose was administered per protocol (yes/no).

9.2 Adverse Events

The safety of PXVX0317 in healthy adult and adolescent subjects 18 to 45 years of age will be evaluated using solicited AEs occurring from IP administration on Day 1 through Day 8, unsolicited AEs through Day 29 and AESI and SAEs though Day 183/EOS. Incidence of AEs occurring within 30 minutes of plasmapheresis on Days 22 and 57 will also be assessed.

AEs will be coded to system organ class (SOC) and preferred term (PT) according to the MedDRA dictionary per Section 5.7. They will be coded for severity according to Section 5.8. Only treatment-emergent AEs will be summarized (i.e., excluding those after a subject has given informed consent, but before vaccination). See Section 6.3 for definitions.

Solicited AEs, unsolicited AEs, AESI, MAAEs, and SAEs will be summarized separately and maximum severity for the safety population. Separate subject data listings of solicited and unsolicited AEs sorted by subject identifier and AE start date will be generated.

9.2.1 Overall Summary of Solicited and Unsolicited Adverse Events

A high-level summary of solicited and unsolicited AEs combined will review the frequency counts and percentages of subjects reporting AEs in each of the following categories:

- Any AE and any AE ≥Grade 3
- Any solicited AE and any solicited AE ≥Grade 3
- Any treatment-related solicited AE and any treatment-related solicited AE ≥Grade 3
- Any unsolicited AE and any unsolicited AE ≥Grade 3
- Any treatment-related unsolicited AE and any treatment-related unsolicited AE ≥Grade 3
- Any SAE
- Any treatment-related SAE
- Any AE leading to study discontinuation
- Any fatal AE
- Any AESI (see definition in Section 6.3)
- Any MAAE and any treatment-related MAAE

In addition, summaries of combined solicited and unsolicited AEs by SOC, PT and highest reported severity will be provided for the following populations:

- Safety population by sex

- Safety population by race group

9.2.2 Solicited Adverse Events from Day 1 until Day 8

Solicited AEs recorded daily in the memory aid until Day 8 include local (i.e., pain, redness, and swelling) and systemic reactions (i.e., fever, chills, fatigue, headache, myalgia, arthralgia, and nausea). In addition, solicited AEs ongoing after seven days post-injection (Day 8) will be also recorded as unsolicited AEs. Severity of solicited AEs will be assessed by the Investigator in the same manner as unsolicited AEs.

Frequencies and percentages of subjects experiencing each solicited AE will be presented by maximum severity. The following summaries of solicited AEs will be produced:

- Solicited AEs by maximum event severity, for each event and for any event
- Solicited AEs of severe (Grade 3) or higher severity, for each event and for any event
- Treatment-related solicited AEs by maximum event severity, for each event and for any event
- Day of first onset of solicited AEs, for each event and any event
- Duration of solicited AEs, for each event and any event
- Solicited AEs by day post-injection for each event and for any event

Occurrence of at least one AE by category (local, systemic) for solicited AEs 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.

9.2.3 Unsolicited Adverse Events from Day 1 through Day 29

All unsolicited AEs occurring during the prescribed collection period in the study will be recorded, regardless of their assessment of relatedness by the Investigator. Unsolicited AEs will include those solicited AEs ongoing after seven days post-injection (Day 8).

All reported unsolicited AEs will be summarized according to MedDRA SOC, PT and highest reported severity. When an unsolicited AE occurs more than once for a subject, the maximum severity and closest relationship to treatment will be counted. The unsolicited AE analysis will include unsolicited AEs judged by the Investigator as at least possibly related to IP.

9.3 Deaths, Other Serious Adverse Events and Other Significant Adverse Events

9.3.1 Deaths from Day 1 to Day 183 End of Study Visit

Deaths are not expected in this study with healthy participants. A tabulation and subject data listing of AEs leading to death will be provided.

9.3.2 Serious Adverse Events from Day 1 to Day 183 End of Study Visit

SAEs will be tabulated according to MedDRA SOC, PT and highest reported severity. A separate tabulation will be limited to treatment-related SAEs only. A subject data listing of all SAEs will be provided.

9.3.3 Adverse Event of Special Interest from Day 1 to Day 183 End of Study Visit

AESI (see definition in Section 6.3) will be tabulated according to MedDRA SOC, PT and highest reported severity.

9.3.4 Medically Attended Adverse Events

MAAE (see definition in Section 6.3) will be tabulated according to MedDRA SOC, PT and highest reported severity.

9.3.5 Adverse Events Leading to Study Discontinuation from Day 1 to Day 183 End of Study Visit

A subject data listing of all AEs leading to study discontinuation (see definition in Section 6.3) will be provided.

9.4 Clinical Laboratory Tests

Not applicable.

9.4.1 Pregnancy Testing

Subject listings of urine pregnancy test results will be created. A listing of pregnancy outcomes, if applicable, will be provided.

9.4.2 Viral Serology Testing at Screening

Viral serology testing results will be supplied in a subject listing.

9.5 Vital Signs, Physical Findings and Other Variables Related to Safety

9.5.1 Vital Signs

Observed values in vital signs include temperature, blood pressure, respiratory rate, and heart rate at the screening visit and pre- and post-vaccination on Day 1. All vital sign records will be displayed in the subject data listings.

9.5.2 Physical Examinations

Physical examinations by body system include a complete examination at screening and directed examinations at Days 1, 8, 15, 22 and 57 only if indicated in order to determine if an AE needs to be recorded. Physical examination findings will be listed by subject.

10 DATA MONITORING AND INTERIM ANALYSIS

10.1 Safety Monitoring Committee

There is no SMC for this study.

10.2 Interim Analysis

No interim analysis is planned for this study.

11 REFERENCES

1. CBER (2007) *Guidance for Industry: Toxicity Grading Scale for Health Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*.
2. ICH E3 (1996) *Guidance for Industry: Structure and Content of Clinical Study Reports*.

APPENDIX I LIST OF TABLES, FIGURES AND LISTINGS

Note the list of TLFs is a living document and subject to changes during the study.

Table	Title
Baseline	
14.1.1	Analysis Populations – Enrolled Population
14.1.2	Subject Disposition – Enrolled Population
14.1.3.1	Demography – Exposed Population
14.1.3.2	Demography – mITT Population
14.1.4	Medical History – Exposed Population
14.1.5	Timing of Blood Sample Collection – Exposed Population
14.1.6.1	Important Protocol Deviations – Exposed Population
14.1.6.2	Reasons for Exclusion from Analysis Populations – Exposed Population
14.1.7	Prior and Concomitant Medications by Anatomic Therapeutic Class and Drug Name – Safety Population
Immunogenicity	
14.2.1.1	CHIKV-luc SNA Seroresponse Rate by Visit – mITT Population
14.2.1.2	CHIKV-luc SNA Seroresponse Rate by Visit – mITT Population by Sex Subgroups
14.2.1.3	CHIKV-luc SNA Seroresponse Rate by Visit – mITT Population by Race Subgroups
14.2.2.1	CHIKV-luc SNA GMT by Visit – mITT Population
14.2.2.2	CHIKV-luc SNA GMT by Visit – mITT Population by Sex Subgroups
14.2.2.3	CHIKV-luc SNA GMT by Visit – mITT Population by Race Subgroups
14.2.3.1	CHIKV ELISA IgG and IgM GMTs by Visit – mITT Population
14.2.3.2	CHIKV ELISA IgG and IgM GMTs by Visit – mITT Population by Sex Subgroups
14.2.3.3	CHIKV ELISA IgG and IgM GMTs by Visit – mITT Population by Race Subgroups
14.2.4.1	Geometric Mean Fold Increase from Baseline in CHIKV-luc SNA Titer from Day 1 to each Visit – mITT Population
14.2.4.2	Geometric Mean Fold Increase from Baseline in CHIKV-luc SNA Titer from Day 1 to each Visit – mITT Population by Sex Subgroups
14.2.4.3	Geometric Mean Fold Increase from Baseline in CHIKV-luc SNA Titer from Day 1 to each Visit – mITT Population by Race Subgroups
14.2.5.1	Geometric Mean Fold Increase from Baseline in CHIKV ELISA IgG and IgM Titers from Day 1 to each Visit – mITT Population
14.2.5.2	Geometric Mean Fold Increase from Baseline in CHIKV ELISA IgG and IgM Titers from Day 1 to each Visit – mITT Population by Sex Subgroups
14.2.5.3	Geometric Mean Fold Increase from Baseline in CHIKV ELISA IgG and IgM Titers from Day 1 to each Visit – mITT Population by Race Subgroups
14.2.6.1	Subjects with CHIKV-luc SNA Titer at or above Selected Thresholds by Visit – mITT Population
14.2.6.2	Subjects with CHIKV-luc SNA Titer at or above Selected Thresholds by Visit – mITT Population by Sex Subgroups
14.2.6.3	Subjects with CHIKV-luc SNA Titer at or above Selected Thresholds by Visit – mITT Population by Race Subgroups
14.2.7.1	PBMC T and B Cell Responses via ELISpot Assay by Visit – mITT Population

Table	Title
14.2.7.2	PBMC T and B Cell Responses via Flow Cytometry by Visit – mITT Population
Safety	
14.3.1	Vaccine Administration – Exposed Population
14.3.2.1	High Level Summary of Solicited and Unsolicited Adverse Events – Safety Population
14.3.2.2	Solicited Adverse Events by Highest Reported Severity – Safety Population
14.3.2.3	Solicited Events of Severe (Grade 3) or Higher Severity by Highest Reported Severity – Safety Population
14.3.2.4	Treatment-related Solicited Adverse Events by Highest Reported Severity – Safety Population
14.3.2.5	Day of Onset for Subjects who Reported Solicited Adverse Events – Safety Population
14.3.2.6	Number of Days with Symptoms for Subjects who Reported Solicited Adverse Events – Safety Population
14.3.2.7	Percentage of Subjects with Solicited Adverse Events by Day on Study – Safety Population
14.3.3.1	Solicited and Unsolicited Adverse Events by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population
14.3.3.2	Solicited and Unsolicited Adverse Events by Highest Reported Severity – Safety Population by Sex Subgroups
14.3.3.3	Solicited and Unsolicited Adverse Events by Highest Reported Severity – Safety Population by Race Subgroups
14.3.4.1	Listing of Serious Adverse Events
14.3.4.2	Listing of Adverse Events Leading to Study Discontinuation
14.3.4.3	Listing of Adverse Events Leading to Death
14.3.5.1	Unsolicited Adverse Events by Preferred Term (Descending Frequency) – Safety Population
14.3.5.2	Unsolicited Adverse Events by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population
14.3.5.3	Treatment-related Unsolicited Adverse Events by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population
14.3.5.4	Serious Adverse Events by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population
14.3.5.5	Treatment-related Serious Adverse Events by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population
14.3.5.6	Adverse Events of Special Interest by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population
14.3.5.8	Medically Attended Adverse Events by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population
14.3.5.9	Treatment-related Medically Attended Adverse Events by System Organ Class, Preferred Term and Highest Reported Severity – Safety Population

Figure	Title
14.4.2.1	Geometric Mean CHIKV-luc SNA Titers by Visit – mITT Population
14.4.2.2	Geometric Mean CHIKV ELISA IgG and IgM Titers by Visit – mITT Population
14.4.2.3	Reverse Cumulative Distribution of CHIKV-luc SNA Titers by Visit – mITT Population
14.4.2.4	Geometric Mean Fold Increase in CHIKV-luc SNA Titers by Visit – mITT Population

Figure	Title
14.4.2.1	Geometric Mean CHIKV-luc SNA Titers by Visit – mITT Population
14.4.2.2	Geometric Mean CHIKV ELISA IgG and IgM Titers by Visit – mITT Population
14.4.2.5	Geometric Mean Fold Increase in CHIKV ELISA IgG and IgM Titers by Visit – mITT Population

Listing	Title
16.2.1.1	Subject Disposition
16.2.1.2	Reasons for Screen Failure
16.2.2.1	Subjects Enrolled who Did Not Satisfy Entry Criteria
16.2.2.2	Subjects who Received an Incorrect Vaccination Dose
16.2.2.3	Subjects who Received an Excluded Concomitant Medication
16.2.2.4	Important Protocol Deviations
16.2.3	Analysis Populations
16.2.4.1	Demography
16.2.4.2	Medical History
16.2.4.3	Prior and Concomitant Medications and Vaccines
16.2.5.1	Luciferase Assay CHIKV SNA NT_{80} Titers
16.2.5.2	CHIKV ELISA IgG and IgM Titers
16.2.6	Study Vaccine Administration
16.2.7.1	Solicited Adverse Events
16.2.7.2	Unsolicited Adverse Events
16.2.8.1	Vital Sign Results
16.2.8.2	Physical Examination Results
16.2.8.3.1	Pregnancy Test Results
16.2.8.3.2	Pregnancy Outcomes
16.2.8.4	Viral Screening Results
16.2.9	Health Care Encounter Details

APPENDIX II TOXICITY GRADING SCALE

EVENT	MILD (Grade 1)	MODERATE (Grade 2)	SEVERE (Grade 3)	POTENTIALLY LIFE THREATENING (Grade 4)
Fever	≥100.4-101.1°F (≥38.0-38.4°C)	≥101.2-102°F (≥38.5-<39°C)	≥102.1°F-104°F (≥39°C-40°C)	>104°F (>40°C)
Headache	No interference with activity	Some interference with activity, may require repeated use of non-narcotic pain reliever for more than 24 hours	Significant, prevents daily activity, any use of narcotic pain reliever	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Nausea	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization for hypotensive shock
Vomiting	1–2 episodes/24 hours	>2 episodes/24 hours	Requires IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400 g/24 hours	4-5 stools or 400-800 g/24 hours	6 or more watery stools or >800 g/24 hours or requires outpatient IV hydration	ER visit or hospitalization
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	51 mm-100 mm	>100 mm	Necrosis or exfoliative dermatitis
Injection site induration/swelling	25-50 mm and does not interfere with activity	51 mm-100 mm or interferes with activity	>100 mm or prevents daily activity	Necrosis

APPENDIX III STANDARD MEDDRA QUERY FOR ARTHRITIS

The following preferred terms include all narrow and broad terms for the Arthritis SMQ for MedDRA version 20.1.

Ankylosing spondylitis
Arthritis
Arthritis allergic
Arthritis bacterial
Arthritis climacteric
Arthritis enteropathic
Arthritis fungal
Arthritis gonococcal
Arthritis helminthic
Arthritis infective
Arthritis reactive
Arthritis rubella
Arthritis salmonella
Arthritis viral
Autoimmune arthritis
Axial spondyloarthritis
Caplan's syndrome
Carcinomatous polyarthritis
Chondrocalcinosis
Chondrocalcinosis pyrophosphate
Chondromalacia
Diffuse idiopathic skeletal hyperostosis
Enteropathic spondylitis
Epidemic polyarthritis
Facet joint syndrome
Felty's syndrome
Gout
Gouty arthritis
Gouty tophus
Haemophilic arthropathy
Infusion site joint infection
Infusion site joint inflammation
Injection site joint infection
Injection site joint inflammation
Juvenile idiopathic arthritis
Juvenile psoriatic arthritis
Juvenile spondyloarthritis

Laryngeal rheumatoid arthritis
Medical device site joint infection
Nodal osteoarthritis
Osteoarthritis
Palindromic rheumatism
Paraneoplastic arthritis
Patellofemoral pain syndrome
Periarthritis
Periarthritis calcarea
Plica syndrome
Polyarthritis
Pyogenic sterile arthritis pyoderma gangrenosum and acne syndrome
Rapidly progressive osteoarthritis
Reiter's syndrome
Rheumatic disorder
Rheumatic fever
Rheumatoid arthritis
Sacroiliitis
Septic arthritis haemophilus
Septic arthritis neisserial
Septic arthritis staphylococcal
Septic arthritis streptobacillus
Septic arthritis streptococcal
Seronegative arthritis
SLE arthritis
Spinal osteoarthritis
Spondylitis
Still's disease
Synovitis
Temporomandibular joint syndrome
Traumatic arthritis
Vaccination site joint infection
Amyloid arthropathy
Ankle arthroplasty
Arthralgia
Arthrodesis
Arthropathy
Arthroscopy abnormal
Arthrotoxicity
Articular calcification

Aspiration joint abnormal
Crystal arthropathy
Destructive spondyloarthropathy
Hip arthroplasty
Infusion site joint effusion
Infusion site joint erythema
Infusion site joint movement impairment
Infusion site joint pain
Infusion site joint swelling
Infusion site joint warmth
Injection site joint effusion
Injection site joint erythema
Injection site joint movement impairment
Injection site joint pain
Injection site joint swelling
Injection site joint warmth
Intervertebral discitis
Joint abscess
Joint adhesion
Joint arthroplasty
Joint contracture
Joint crepitation
Joint debridement
Joint destruction
Joint effusion
Joint fluid drainage
Joint range of motion decreased
Joint stiffness
Joint swelling
Joint warmth
Knee arthroplasty
Musculoskeletal stiffness
Neck pain
Neuropathic arthropathy
Osteoarthropathy
Periarticular disorder
Psoriatic arthropathy
Rheumatoid nodule removal
Shoulder arthroplasty
Spinal pain

Spondyloarthropathy
Swollen joint count
Swollen joint count decreased
Swollen joint count increased
Synovectomy
Synovial fluid analysis abnormal
Synovial fluid crystal present
Synovial fluid protein present
Synovial fluid red blood cells positive
Synovial fluid white blood cells positive
Synoviorthesis
Tender joint count
Tender joint count decreased
Tender joint count increased
Traumatic arthropathy