STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 2 Parallel-Group, Randomized, Double-blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle [CHIKV-VLP], unadjuvanted or alum-adjuvanted)
Name of Test Drug:	PXVX0317 (CHIKV-VLP Vaccine)
Study Number:	PXVX-CX-317-001
Protocol Version:	4.0; Amendment
Protocol Date:	16 February 2018 (V 1.0); 31 January 2019 (V 2.0, Amendment 1); 24 July 2019 (V 3.0, Amendment 2) 22 May 2020 (V 4.0, Amendment 3)
Phase of Study:	Phase 2
Analysis Plan Version:	Final v3.0
Analysis Plan Date:	14-July-2020
Analysis Plan Author:	[redacted]

CONFIDENTIAL AND PROPRIETARY INFORMATION

Approvals:

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ab	Antibody
ADaM	Analysis Data Model
AE	Adverse Event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
CHIKV	Chikungunya Virus
CHIKV-VLP	Chikungunya Virus Virus-Like Particle
CI	Confidence Interval
CSR	Clinical Study Report
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
eTMF	Electronic Trial Master File
GMT	Geometric Mean Titer
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLT	High Level term
IEP	Immunogenicity Evaluable Population
IPD	Important Protocol Deviation
mITT	Modified Intent-to-Treat Population
MedDRA	Medical Dictionary for Regulatory Activities Terminology
N	Total Sample Size
PBMC	Peripheral Blood Mononuclear Cell
PT	Preferred Term
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SOC	System Organ Class
WHO	World Health Organization

1. INTRODUCTION

Chikungunya virus (CHIKV) is an arthropod-borne alphavirus of the family *Togaviridae*. As of April 22, 2016, excluding countries where only imported cases were documented, 103 countries or territories have documented cases of CHIKV infection (CDC 2017). Mosquitoes are the primary mode of transmission of CHIKV; however, blood-borne transmission via needles is possible. Maternal-fetal transmission has been documented during pregnancy (Staples 2017). Following an incubation period of 2 to 12 days, common clinical manifestations include high fever, rash, gastrointestinal complications, headache, muscle pain, nausea, fatigue, myalgia, and joint pain (Borgherini 2007, Taubitz 2007, Pialoux 2017). The most classic symptom of CHIKV is a debilitating polyarthralgia that is present in greater than 90% of cases. Diagnosis of CHIKV infection can be based on either serology or presence of the virus. There are currently no approved vaccines to prevent CHIKV infection or disease.

The current study builds on the findings of study VRC 311 and ongoing study VRC 704. VRC 311 is a Phase 1 open-label, dose-escalation trial and VRC 704 is a multi-center, randomized, placebo-controlled, double-blind Phase 2 study. The goal of VRC 704 is to evaluate the safety and immunogenicity of a regimen of CHKVLP059 20 mcg administered by IM injection at week 0 and week 4 in 400 healthy adults in CHIKV-endemic areas. In addition, this study will evaluate the safety and immunogenicity of booster doses of PXVX0317 administered at 78 weeks.

PXVX0317 is the Sponsor's research name for Chikungunya Virus Virus-Like Particle Vaccine (CHIKV-VLP vaccine). It is a field-formulated vaccine with three components: CHIKV-VLP, Diluent as required for each dose level, and Alhydrogen for the adjuvanted formulation. These three components are combined in prespecified volumes to achieve the formulations and doses indicated in the protocol. CHIKV-VLP is essentially identical to the VRC-CHKVLP059-00-VP used in the Nonclinical studies. Diluent is identical to the buffer of the CHIKV-VLP. Alhydrogel is a 2% (w/w) aqueous suspension of aluminum hydroxide. The primary goals of this Phase 2 parallel-group, randomized, double-blind study are to evaluate the safety and immunogenicity of PXVX0317 in healthy US adults.

1.1. Study Objectives

The goal of this trial is to evaluate the safety, tolerability and immunogenicity of 8 formulation/schedule combinations of PXVX0317 to select one or more formulation(s) for further development. One formulation and dosing schedule will be further evaluated in an open-label group, Group 9.

Immunogenicity Objectives

Primary

- To assess the induction of anti-chikungunya virus (CHIKV) neutralizing antibody responses by different formulations and schedules, as measured at 28 days after the last injection (Day 57).

Secondary

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

Exploratory

- To obtain high-titer serum to support non-clinical studies (including passive transfer studies) by using plasmapheresis at Day 57 to collect plasma from subjects immunized with one dose and regimen from Group 9 subjects.
- To obtain high-titer serum to support non-clinical studies (including passive transfer studies) by using plasmapheresis at Day 22 to collect plasma from Group 10 subjects immunized with a 40/300 mcg single adjuvant dose.
- To further assess anti-CHIKV neutralizing antibody, including isotypes(s) and epitope specificity, by using leukapheresis at Day 182 to collect large volumes of lymphocytes from consenting study subjects for testing.

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

Safety Objectives

To study the safety and tolerability of 8 different formulations and dosing schedule combinations of PXVX0317 in healthy adults.

The safety will be assessed by measuring the incidence of local and systemic solicited adverse events, unsolicited adverse events, and serious adverse events.

1.2. Study Design

This is a Phase 2 parallel-group, randomized, double-blind study in healthy adults 18-45 years of age. A least 430 subjects (400 double blinded, 20 open label in Group 9, and 10 open label in Group 10) are planned to be enrolled. The study has a screening period of 30 days, a treatment and observation period from Day 1 to Day 57, a follow-up period through Day 365 for all groups (Groups 1-8) and an additional follow-up period through Day 760 for some groups. All subjects will be unblinded after their Day 365 visit.

- Subjects in Groups 2, 3, 5, 6, and 7 will complete the study after the Day 365 visit.
- Subjects in Groups 1 and 8 will continue to Day 760 without receiving a boost dose at Day 547.
- Subjects in Group 4 will receive a boost dose of 40/300 mcg at Day 547 and will continue follow-up to Day 760.
- Subjects in Group 9, after a treatment and observation period from Day 1 through Day 57, will complete the study after the Day 182 visit.
- Subjects in Group 10, after a treatment and observation period from Day 1 through Day 22, will complete the study after the Day 29 visit.

Study Duration

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

Inclusion Criteria

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

Exclusion Criteria:

1. Current acute febrile illness.
2. Clinically significant cardiac, respiratory, or rheumatolgic disease, determined by the Investigator.
3. Pregnant or breast-feeding.
4. Laboratory evidence of infection with Hepatitis B/C or HIV.
5. History of chikungunya virus infection.
6. Travel to a WHO-designated chikungunya-endemic region within 30 days prior to Day 1.
7. History of allergic reaction to any component of CHIKV-VLP vaccine, Diluent, or Alhydrogel®.

8. Inability to discontinue systemic immunomodulatory or immunosuppressive medications 30 days prior to Day 1.
9. Received or plans to receive any licensed vaccine from 30 days prior to Day 1 through Day 57.
10. Received or plans to receive an investigational agent from 30 days prior to Day 1 through the duration of study participation.
11. Any other condition that, in the opinion of the Investigator, creates an unacceptable risk to the subject.
12. Any other condition that, in the opinion of the Investigator, may interfere with the conduct of the study or the validity of the data.

    Any other condition that, in the opinion of the Investigator, creates an unacceptable safety risk for apheresis (Group 9 and 10 subjects only).
13. Restricted venous access that would prevent the collection of PMBCs, plasma, and lymphocytes necessary for participation (Group 9 and 10 subjects only).
14. Weight < 110 pounds (Group 9 and 10 subjects only)

Randomization

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

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

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

Groups 9 and 10 enrollments will be open label and thus no blinding nor treatment randomization will be required.

Treatment groups are shown in Table 1. Group 1 (the "Reference Group") receives a dose of 20 mcg CHIKV-VLP (unadjuvanted). Groups 2 through 8 receive CHIKV-VLP doses of 6, 10, 20, or 40 mcg in combination with Alhydrogel® 300 mcg. Groups 1 through 8 receive intramuscular (IM) injections at Day 1, 15, and 29. Groups 1 through 4 receive PXVX0317 at Days 1 and 29 (placebo at Day 15); Groups 5 through 7 receive PXVX0317 at Days 15 and 29

(placebo at Day 1); and Group 8 receives PXVX0317 only at Day 29 (placebo at Days 1 and 15). Group 9 receives PXVX0317 at Days 1 and 29 and must participate in plasmapheresis collection at Day 57 (mandatory) and may participate in leukapheresis collection at Day 182 (optional). Group 10 receives PXVX0317 only at Day 1 and must participate in plasmapheresis collection at Day 22 (mandatory). The boost administered at Day 547 at group 4 will be CHIKV-VLP dose of 40 mcg in combination with Alhydrogel 300 mcg. The 10 treatment dose groups are summarized in Table 1 below:

Table 1: Treatment Dose Groups

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

After signing the informed consent form, subjects will undergo screening procedures up to 30 days before the first injection. Except for the open-label Groups 9 and 10, eligible subjects will be enrolled into a treatment group according to a randomization schedule in a 1:1:1:1:1:1:1:1 ratio. Group 9 will receive the same dosing schedule as subjects in Group 4, except no placebo at Day 15. Group 10 will receive CHIKV-VLP dose 40/300 mcg only at Day 1. A total of 400 subjects are planned to be randomized, 50 subjects to each group for Groups 1 through 8.

For Groups 1 through 8, follow-up visits will also be performed and blood collected at Days 182, and 365. All blinded subjects will be unblinded after their Day 365 visit.

- Subjects in Groups 2, 3, 5, 6, and 7 will complete the study after the Day 365 visit.
- Subjects in Groups 1 and 8 will continue to Day 760 without receiving a boost dose at Day 547. Subjects in Group 4 only will receive a boost dose of 40/300 mcg at Day 547 and will continue follow-up to Day 760. Follow-up visits and blood collection for safety and immunogenicity will be performed at Days 575 (Group 4 only) and 760 for Groups 1, 4 and 8.

Study Procedures

Subjects will be observed in clinic for 30 minutes after each injection and vitals obtained at least 30 minutes but no more than 60 minutes after injection. The subjects for Group 9 will be

monitored for 30 minutes following the plasmapheresis and leukapheresis procedures and Group 10 following the plasmapheresis procedure only. Local and systemic solicited events occurring on the day of and within 7 days after each injection will be recorded by the subject using a memory aid. Subjects will be specifically asked to record local injection site events (pain, redness, swelling) and systemic solicited events (oral temperature ≥100.4° F, chills, malaise, fatigue, headache, myalgia, joint pain, and nausea). Any other adverse events and medications used through Day 57 will also be recorded. Blood will be collected at Day 1 (before the first injection) and Days 8, 15, 22, 29, 36, and 57 (Group 9 does not have a Day 15 or Day 22 visit and Group 10 does not have a Day 29,Day 36 or Day 57). For Groups 1 through 8, blood will also be collected at later points (Days 182 and 365).

Antibody (Ab) responses to CHIKV-VLP will be determined by a luciferase-based anti-CHIKV neutralization assay. Titers are expressed as the reciprocal of the serum dilution achieving 80% neutralization (NT80). Persistence of the induced response will be assessed by comparing anti-CHIKV neutralization responses at later time points with those obtained earlier. IgG/IgM Ab responses will also be assessed by a commercial anti-CHIKV ELISA at Baseline and Day 57.

Sample Size and Power

[redacted]

2. TYPE OF PLANNED ANALYSIS

The analysis plan describes the methods by which the data from this study will be analyzed.

2.1. Interim Analyses

There will be a safety and immunogenicity interim analysis for the selection of a formulation and schedule based on the data collected for Groups 1 through 8 through Day 57. Results will be reported by treatment group, thus preserving the double-blind status on the subject level.

The Table of Contents and mocks for the interim analysis outline which tables will be included in the interim. Separate interim mock tables will be developed in order to limit the statistics shown to maintain the blind at the subject level. Output that could unblind at the subject level (e.g., minimum, maximums) will be excluded from the displays. Similarly, events, solicited or unsolicited, will only be shown in aggregate if the counts could unblind a subject (e.g., only one subject had a specific AE). After the interim analysis, one existing or new group will be considered for a plasmapheresis sub-study.

2.2. Final Analyses

The end of EDC data collection for the study will be declared when the following conditions are met:

- Completion of the Day 760 visit for Groups 1, 4, and 8;
- Completion of Day 365 for Groups 2, 3, 5, 6, and 7;
- Completion of Day 182 for Group 9;
- Completion of Day 29 for Group 10;
- Completion of any required safety follow-up for the final subjects.

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.

3. GENERAL CONSIDERATIONS FOR DATA ANALYSES

Continuous variables will be summarized by sample size, mean, standard deviation, median, minimum and maximum. The geometric mean and 95% confidence interval (CI) will be presented as appropriate. Calculation of the geometric mean will be performed by taking the mean of the $\log_{10}$-transformed data and then exponentiating the mean log to convert back to the original scale. Similarly, the corresponding 95% CI will be calculated. Categorical variables will be summarized using frequency counts and percentages. In general, data will be summarized by treatment group. Unless otherwise noted, the denominator for the percentages will include all subjects in the respective treatment group.

The minimum and maximum values will be presented with the same number of decimal places as the raw data collected. The mean, median, and standard deviation will be presented using one additional decimal place. The standard error will be presented using two additional decimal place.

For subject counts/frequencies, percentages will generally be shown to one decimal place. The denominator will be the total size of the sample, N, unless otherwise noted. Counts of 0 will be shown but 0% will be shown as blank. A percentage of 100% will be reported as 100%.

3.1. Analysis Populations

Analysis populations define which subjects and records are included in an analysis.

Subjects' protocol deviations through Day 365 and exclusions from the analysis populations will be finalized prior to the subject unblinding after Day 365 and will be stored as a locked excel file and signed off in the Sponsor's eTMF. These data will be transferred to Accenture prior to the final SDTM and ADaM creation as an external file. A summary of the number and percent of subjects in each analysis population will be provided by treatment group and in total.

3.1.1. Randomized Population

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

Analyses based on the randomized population will be performed according to the treatment group to which a subject was randomized or assigned.

3.1.2. Exposed Population

The exposed population includes all randomized subjects who receive at least one study vaccination. Analyses based on the exposed population will be performed according to the treatment group to which a subject was actually treated.

3.1.3. Safety Population

The safety population will include 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 a significant AE. Subjects will be analyzed according to actual treatment received.

3.1.4. Modified Intent-to-Treat (mITT) Population

The modified intent-to-treat (mITT) population includes all subjects in the randomized population who were treated and have evaluable immunogenicity results for baseline (Day 1) and at least 1 later on-study sample. The mITT population will be used in the interim analysis.

3.1.5. Immunogenicity Evaluable Population (IEP)

The immunogenicity evaluable population (IEP) includes subjects in the exposed population who:

- Have no major protocol deviation or other reason to be excluded as defined prior to unblinding or analysis.
- Received all three scheduled vaccinations through Day 29.
- Have not received a prohibited medication.
- Provide evaluable serum sample results for baseline, the relevant post-vaccination time points, and within the required time frames (Groups 1-8 only):
  - Baseline: Day 1 or within 30 days before first study vaccine administration
  - Day 57: Day 54 through Day 62, inclusive

3.2. Missing Data and Outliers

Missing Data

A missing datum for a given study visit may be due to the fact that:

1. data were not collected for the visit or were unusable, or
2. a subject permanently discontinued from the study before reaching the assessment.

There are no plans to impute values for missing data points except for imputing missing relationship to study drug for AEs as related.

3.3. Data Handling Conventions and Transformations

By-subject listings will be presented for all randomized subjects sorted by subject ID number, treatment group, visit date, and time (if applicable). Data collected on log forms, such as AEs, will be presented in chronological order within subject. Subjects will be listed according to the actual treatment received. If not treated as randomized, the treatment to which the subject was randomized will also be presented.

Baseline is defined as the Day 1 value. If the Day 1 value is missing, then the last non-missing value prior to Day 1 will be used as the baseline value.

Data that are less than the lower limit of quantification (LLOQ) or above the upper limit of quantification (ULOQ) will be imputed as follows:

- Anti-CHIKV neutralizing antibody assay results that are reported as less than the LLOQ will be imputed as the LLOQ/2 when calculating geometric mean titer (GMT) and geometric

mean ratio (GMR). For example, if the LLOQ is 10 and a result is noted as "<10", a titer of 10/2 (5) will be imputed.

- For fold rise over baseline in anti-CHIKV titer, if a value is < LLOQ, it will be imputed as the LLOQ. For example, if the LLOQ is 10 and a result is noted as "<10", a titer of 10 will be imputed.
- A value that is one unit above the upper limit of quantitation will be used for calculation of descriptive statistics if the datum is reported in the form of "> x" (where x is considered the upper limit of quantitation). Values with decimal points will follow the same logic as above.

The limit of quantitation will be used for calculation of descriptive statistics if the datum is reported in the form of "≤ x" or "≥ x" (where x is considered the limit of quantitation).

3.4. Visit Windows

For determination of Baseline visit and all other visits, an analysis visit will be derived to summarize the data by the nominal visit assignment. Please refer to the protocol APPENDIX A1 for Groups 2, 3, 5, 6, and 7, the protocol APPENDIX A2 for Groups 1, 4 and 8, APPENDIX B1 for Group 9 and APPENDIX B2 for Group 10: SCHEDULE OF EVENTS (GROUPS 2, 3, 5, 6, AND 7), SCHEDULE OF EVENTS (GROUPS 1, 4, AND 8), SCHEDULE OF EVENTS (GROUP 9 ONLY), and SCHEDULE OF EVENTS (GROUP 10 ONLY). All the visits will be based on the nominal time points, but the visit windows at Visit 57 will be followed.

The following algorithm will be used for the study day determination:

- Day 1 Day of the First Vaccination;
- If Date of Assessment/Visit > Date of the First Vaccination then Study Day (Date of Assessment/Visit - Date of the First Vaccination) + 1;
- If Date of Assessment/Visit < Date of the First Vaccination then Study Day (Date of Assessment/Visit - Date of the First Vaccination).

4. SUBJECT DISPOSITION

4.1. Subject Enrollment

The number of subjects randomized or assigned will be summarized by site and by treatment group.

4.2. Disposition of Subjects

A summary of subject disposition will be tabulated by treatment group and overall. This summary will present the number of subjects who completed through:

- Day 365 for Groups 2, 3, 5, 6, and 7;
- Day 760 for Groups 1, 4, and 8;
- Day 182 for Group 9;
- Day 29 for Group 10,

and subjects who discontinued from the study early.

The table will also display the primary reason for subjects who discontinued the study early. No inferential comparisons (p-values) between groups will be performed.

A data listing of reasons for early study discontinuation for the randomized population will be provided as well as a listing of subjects who were screen failures and the reasons for their screen failure.

5. BASELINE DATA

5.1. Demographics

Subject demographic data (e.g., age, sex, weight, height, body mass index (BMI), race, and ethnicity) will be summarized by treatment group and overall using descriptive statistics for continuous data and by the number and percent of subjects for categorical data. Age is calculated in years at the time of Day 1 vaccination. No inferential comparisons (p-values) between groups will be performed.

The summary will be provided for the randomized, safety, and immunogenicity evaluable populations. A listing of demographic data will be provided for the randomized population.

5.2. Medical History

Medical history will be coded using version 20.1 the Medical Dictionary for Regulatory Activities (MedDRA). Medical history will be summarized by system organ class (SOC), preferred term, and by treatment group and overall for the randomized population and subjects are counted only once per unique preferred term. Medical history will also be listed for the randomized population.

6. PRIMARY AND SECONDARY ANALYSES

This study has the following period per groups:

- Treatment and observation period for all Groups 1-9 (Group 9 without the injection on Day 15) from Day 1 to Day 57;
- Treatment and observation period for Group 10 from Day 1 to Day 29 (Group 10 injection on Day 1 only);
- Follow-up period for Groups 2, 3, 5, 6, and 7 through Day 365;
- Follow-up period for Groups 1, 4, and 8 through Day 760;
- Booster period for Group 4 only from Day 547 to Day 575;
- Follow-up period for Group 9 only through Day 182;

The primary and secondary analyses will be performed after the last subject has completed the study and the immunogenicity and safety data have been cleaned and locked.

The primary and secondary endpoints in immunogenicity will be analyzed based on the immunogenicity evaluable population. The subset of immunogenicity analyses included in the interim analysis will be based on the mITT population.

6.1. Primary Endpoints

The primary endpoint is the geometric mean titer (GMT) of anti-CHIKV neutralizing antibody determined by luciferase-based assay at Day 57.

6.1.1. Analysis Methods for Primary Endpoints

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

The GMTs associated with the primary objective will be analyzed via linear model.

The primary model is an analysis of variance (ANOVA), with logarithmically-transformed anti-CHIKV titers (log_{10}) as the dependent variable and treatment group and study site as the fixed effects in the model. The results included in the main tables of the report will be based on this basic ANOVA model. As a secondary analysis, in order to remove the effect of baseline concentration on the GMTs, an analysis of covariance (ANCOVA) will also be performed. The ANCOVA includes treatment group and study site as the fixed effects and log_{10} baseline anti-CHIKV titer as the covariate in the model. The rationale for including baseline values as a covariate is that prior data have shown vaccine response is correlated with baseline antibody levels. Should a large majority of the subjects (e.g., > 90%) have titers below the LLOQ at baseline, the ANCOVA analysis will not be performed.

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

the case of the ANCOVA). All the analysis output and details from the ANOVA/ANCOVA model will be presented in a separate table.

Comparisons between relevant groups will be based on the estimated adjusted GMTs measured at Day 57 and mean square error calculated from the ANOVA/ANCOVA models using contrast statements. The analysis of GMR relative to Day 1 will also be computed using the ANOVA model specified above but substituting the difference in $\log_{10}$-transformed titers (on-study minus baseline) as the dependent variable in the model. The analysis of the boost GMR will be relative to Day 547. Specifically, testing will be done of the null hypothesis:

H_0: μ_i μ_j

against the alternative hypothesis of inequality:

H_0: $\mu_i \neq \mu_j$

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

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

6.2. Secondary Endpoints

Secondary endpoints include:

- The geometric mean titer (GMT) of anti-CHIKV neutralizing antibody determined by luciferase-based assay at all time points (For Groups 1 through 8: Days 1, 8, 15, 22, 29, and 36; for Group 9: Days 1, 8, 29, and 36; for Group 10: Days 1, 8, 15, and 22, but no Day 57) before Day 57.
- Geometric Mean Ratio (GMR) of anti-CHIKV neutralizing antibody-determined by luciferase-based fold increase at all time points (For Groups 1 through 8: Days 8, 15, 22, 29, 36, and 57; for Group 9: Days 8, 29, and 36; for Group 10: Days 8, 15, and 22) (relative to Day 1).
- The GMTs and GMRs (relative to Day 547 for Group 4 only) based on antibody titers measured at all other protocol-specified time points from before to after Boost Dose.
- Proportion (percentage) of subjects with anti-CHIKV titer exceeding the following defined cut-off values: ≥ 15, 40, 160, 640, and 4-fold rise over baseline.

6.2.1. Analysis Methods for Secondary Endpoints

The GMTs and GMRs (relative to Day 1 or 547, as applicable) based on antibody titers measured at all other protocol-specified time points will be analyzed as described above for Day

57. The difference in persistence of the antibody response induced by different treatment groups after first vaccination at each time point will be visually assessed.

The percentage of subjects achieving specified thresholds (e.g., ≥ 15, 40, 160, 640, and 4-fold rise over baseline), and associated Wilson 95% CI [Agresti, 1998], will be calculated for the Day 57 visit data for each treatment group. The significance of the differences between pair-wise treatment groups will be determined via Fisher's Exact test.

The cumulative percentage of subjects with a response through a visit is defined as the number of subjects who meet the specified thresholds at or prior to that visit. The denominator for calculating the cumulative percentage at a visit is the number of subjects with analyzable sample available at or before the indicated visit in the treatment group.

Baseline value will be defined as the last available value collected prior to the first dose of treatment. The geometric mean fold-increase in titer over baseline (GMR) will also be presented at each visit. To calculate the geometric mean fold-increase and 95% CI, the difference from Day 1 (or Day 547, as applicable) in the $\log_{10}$-transformed titers will be the dependent variable in the ANOVA model. The least square means, and their 95% CIs calculated based on the ANOVA, will be back transformed and reported as the group GMR values. The median, minimum, and maximum titers and fold-increases will be based on the non-transformed scale. The luciferase-based CHIKV assay titers and CHIKV ELISA assay data will be analyzed using identical methods applicable to the time points of the data collection.

The interim analysis will include GMT and GMR analyses based only on samples collected up to Day 57.

6.3. Exploratory Endpoints

Exploratory endpoints include:

- The anti-CHIKV neutralizing antibody response further in terms of isotype(s) and epitope specificity by using leukapheresis at Day 182 (Group 9 subjects) and from PBMC sample at Days 1, 8, 15 and 22 (Group 10 subjects).
- The anti-CHIKV cellular immune response by collecting PBMCs at Days 1, 29, 57, and 182 from Group 9 subjects and at Days 1, 8, 15 and 22 for Group 10 subjects.

6.3.1. Analysis Methods for Exploratory Endpoints

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

7. Safety Analyses

7.1. Adverse Events

An adverse event (AE) is any untoward medical occurrence in a study participant, regardless of the suspected causal relationship with study vaccine. An AE can therefore be a new onset of a symptom or disease, an exacerbation of a pre-existing symptom or disease, a new onset laboratory abnormality considered by the Investigator to be clinically significant, or a new onset symptom or disease that occurs as a result of a protocol-specified procedure.

A solicited adverse event (solicited AE) is a protocol-specified AE that the Investigator or designee proactively asks the subjects about during a protocol-specified time period. Solicited adverse events for this study are local events of pain, redness, swelling at the injection site, systemic events of fever (defined as oral temperature ≥ 100.4 F), chills, malaise, fatigue, headache, myalgia, joint pain, and nausea. An unsolicited adverse event (unsolicited AE) is an AE that is spontaneously reported by the subject or discovered by the Investigator.

The reporting period for a serious adverse event (SAE) begins at the time of informed consent and continues for the duration of study participation. The reporting period for solicited AEs begins immediately after each injection and continues for 7 days after each injection, including booster injection (for Group 4 only). The two reporting periods for unsolicited AE are immediately after study vaccine administration on Day 1 through Day 57 and immediately after booster injection on Day 547 through Day 575 (booster injection is applied to Group 4 only). AEs that correspond to solicited AE terms but occur outside of (or continue past) the solicited AE collection periods are collected as an unsolicited AE.

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

Figure 1: **Safety Events Reporting Periods (Groups 2, 3, 5, 6, and 7)**

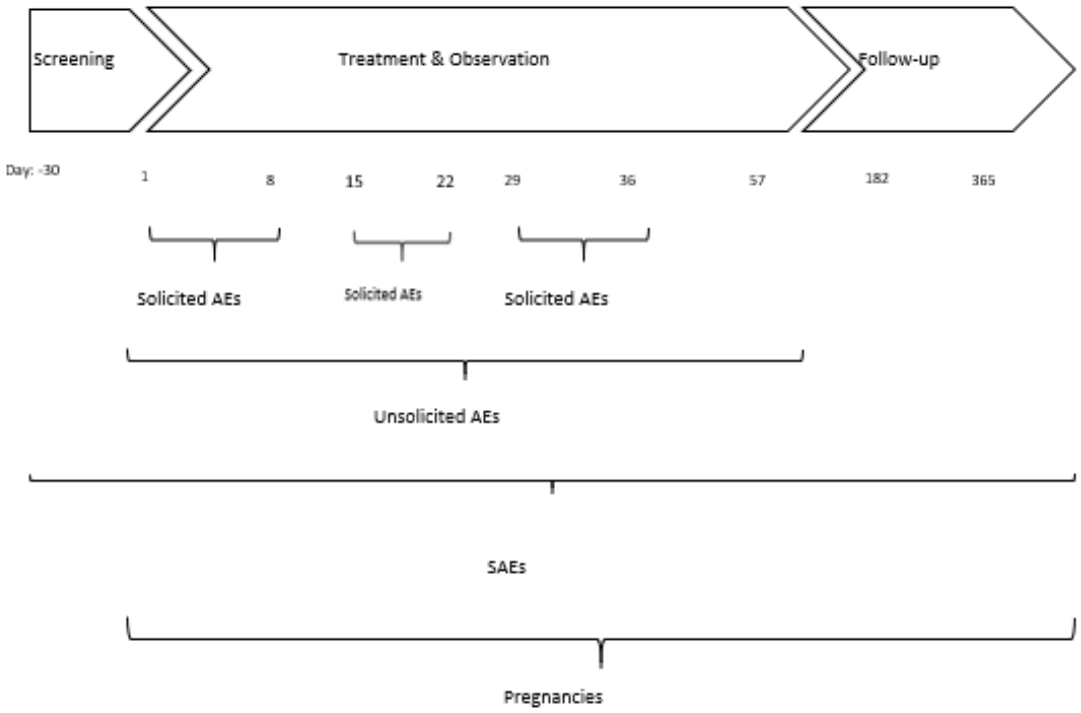
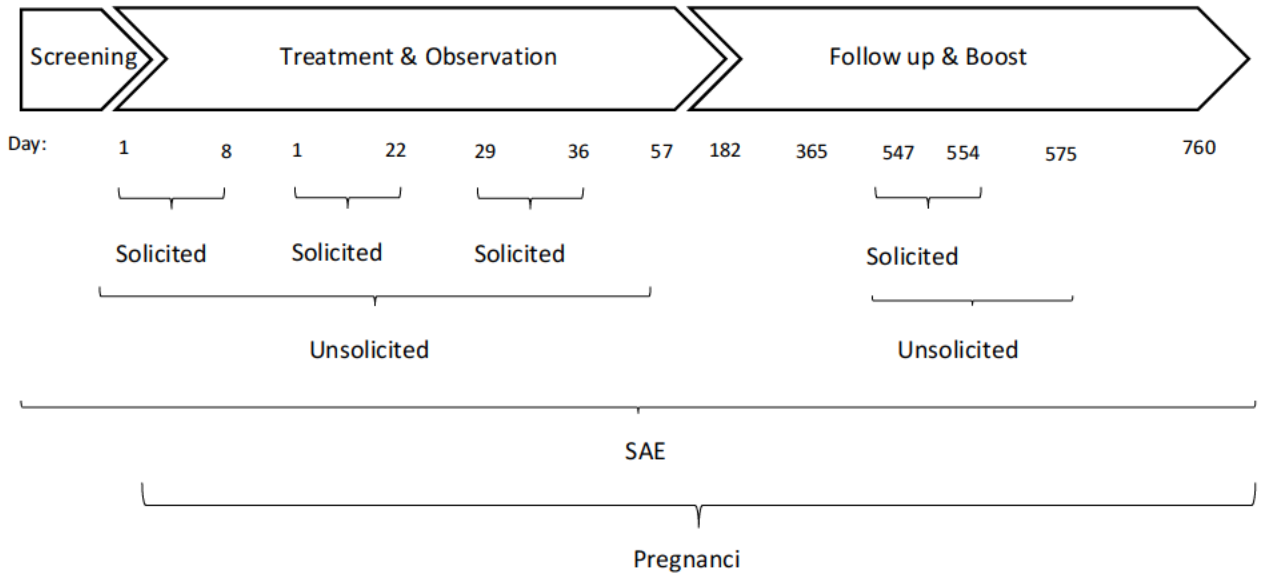


Figure 2: Safety Events Reporting Periods (Groups 1, 4, and 8 only)

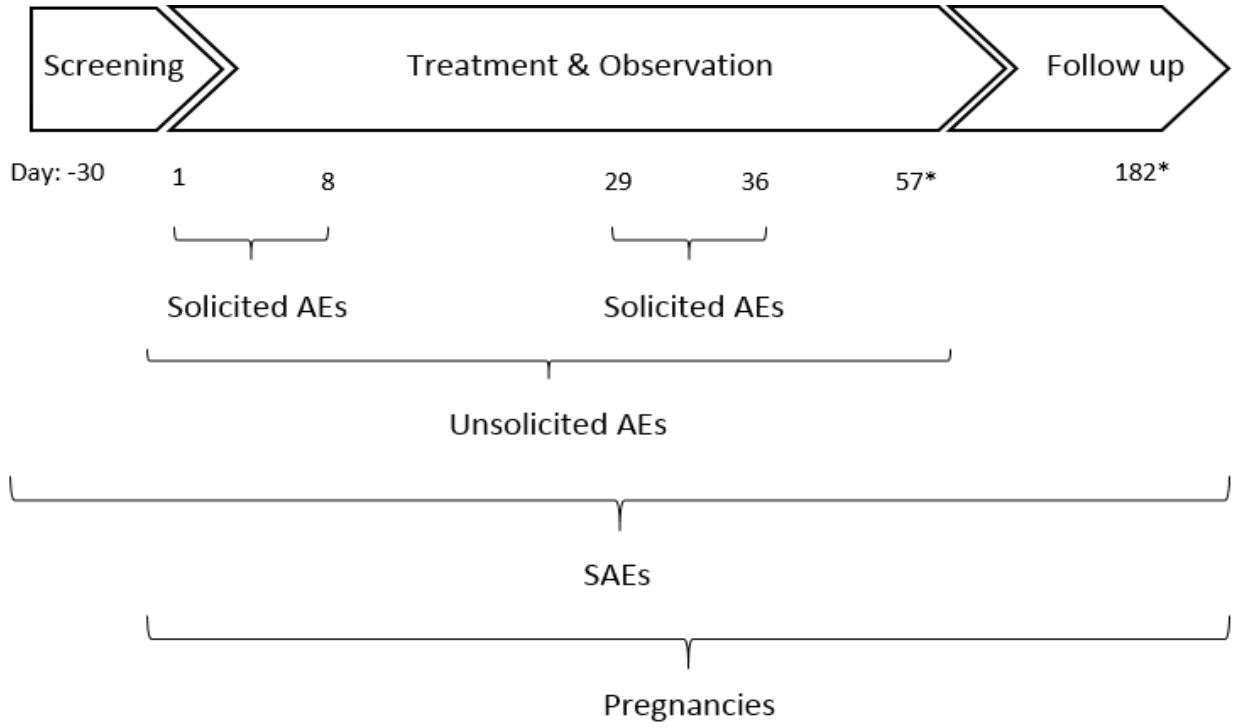


* Only Group 4 will receive the boost at Day 547 along with the memory aid.

~ Follow-up phone call for Group 4 only

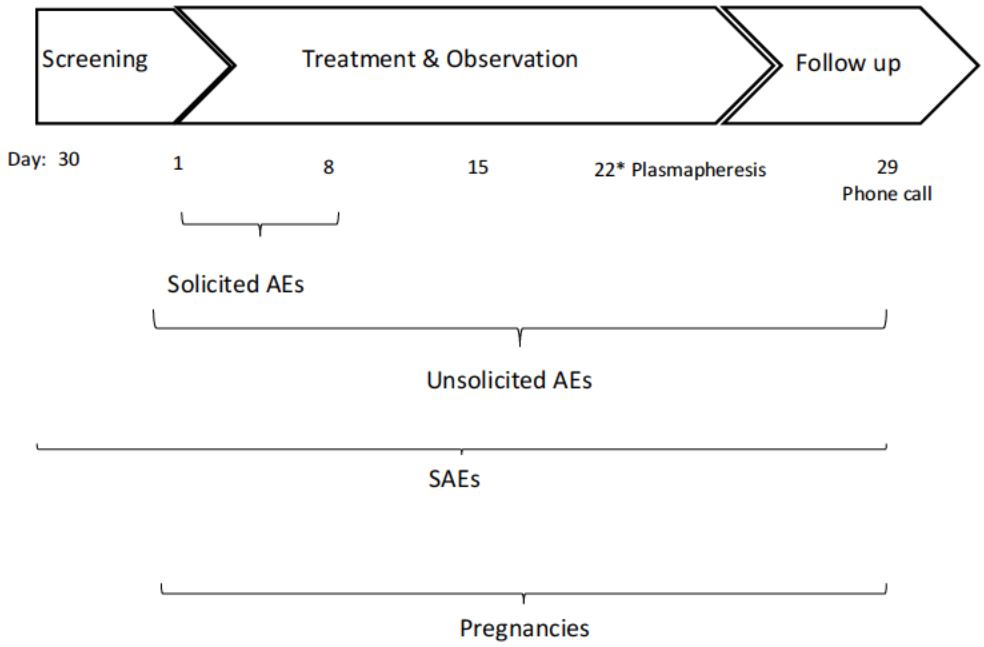
^ Day 575 is for Group 4 only (collection and recording of solicited AEs)

Figure 3: Safety Events Reporting Periods (Group 9 only)



*Specifies apheresis procedure-related AEs.

Figure 4: Safety Events Reporting Periods (Group 10 only)



*Specifies apheresis procedure-related AEs.

Adverse Event Dictionary

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.1. System Organ Class (SOC), High Level Group Term (HLGT), High Level Term (HLT), Preferred Term (PT), and Lowest Level Term (LLT) will be attached to the clinical database.

With the exception of redness and swelling, all solicited AEs will be summarized according to severity grading scales defined in Section 7.2 of the protocol; these range from "mild" to "potentially life-threatening."

The analyses of solicited AEs (any event, after any injection, and after each injection) will be tabulated by maximum severity and by treatment group. In addition, solicited AEs ongoing after 7 days post- injection will be also recorded as unsolicited AEs.

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

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

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

The following classifications are used in the summaries: Grade 0 (0-24 mm), Any (> 24-50 mm, > 50-100 mm, > 100 mm).

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

All the unsolicited AEs occurring during the study that occur during reporting periods will be recorded, regardless of their assessment of relatedness by the Investigator.

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

Only treatment-emergent AEs will be summarized, i.e., excluding those after a subject has given informed consent, but before vaccination. The selection of unsolicited AEs and their assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

7.1.1. Adverse Event Severity

AEs (inclusive of all solicited and unsolicited adverse events) are graded by the Investigator or designee as Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe) or Grade 4 (potentially life-threatening) according to toxicity criteria specified in the study protocol (see Appendix C of the protocol). The severity grade of events for which the Investigator did not record severity will be categorized as "missing" for tabular summaries and data listings, and it will be considered the least severe for the purposes of sorting for data presentation (will sort to the top).

7.1.2. Relationship of Adverse Events to Study Drug

AEs and SAEs are determined to be related or unrelated to study product by study Investigators. The Investigators will evaluate the relatedness of an AE to vaccine treatment using three categories: Not Related, Possibly Related, and Probably Related. Related AEs are those for which the Investigator answers "Possibly Related" or "Probably Related". Events for which the Investigator did not record relationship to study drug will be considered related to study drug for the purposes of analysis. Data listings will show relationship as missing in this case.

7.1.3. Serious Adverse Events

Serious adverse events (SAEs) are those identified in the clinical database as serious by the Principal Investigator or designee. Further information on the definition of a SAE is provided in Section 7.1.4 of the study protocol.

7.1.4. Summaries of Adverse Events and Deaths

A single summary table of AEs will tabulate, by treatment group, the number and percentage of subjects who had any

1. solicited or unsolicited AE;
2. solicited AE;
3. unsolicited AE;
4. treatment-related solicited or unsolicited AE;
5. solicited treatment-related AE;
6. unsolicited treatment-related AE;
7. SAE;
8. treatment-related SAE;
9. solicited or unsolicited AE leading to permanent discontinuation from the study;
10. death during study.

Summaries (number and percent of subjects) of AEs will be provided by SOC and PT and treatment group as follows:

- All AEs (inclusive of all solicited and unsolicited AEs)
- All unsolicited AEs

- Treatment-related unsolicited AEs
- AEs that caused permanent discontinuation from study,
- Unsolicited AEs by maximum severity grade,
- Treatment-related unsolicited AEs by maximum severity grade.

For subjects with multiple events, only one event will be counted in each summary. For data presentation, SOCs will be ordered alphabetically, with PT sorted by decreasing total frequency. Solicited AEs will be presented by decreasing total frequency. For summaries by maximum severity grade, only the event with the highest severity will be presented. For summaries by relatedness, only one event per relatedness category will be presented. Summaries will be provided by treatment group for the Safety Population.

In addition to the by-treatment summaries, data listings will be provided for the following:

- All unsolicited AEs
- All solicited events
- AEs leading to discontinuation from study
- SAEs
- Deaths

7.2. Safety Endpoints

Safety endpoints include:

- Incidence and severity of solicited events for 7 days after each injection, including booster injection (for Group 4 only)
    - Solicited adverse events include: pain, redness, swelling at the injection site, systemic events of fever (defined as oral temperature ≥ 100.4 F), chills, malaise, fatigue, headache, myalgia, joint pain, and nausea.
- Incidence and severity of unsolicited adverse events. The two reporting periods for unsolicited nonserious AE are immediately after study vaccine administration on Day 1 through Day 57 and immediately after booster injection on Day 547 through Day 575 (for Group 4 only). Serious AEs are reported from the time of informed consent.
- Incidence of serious adverse events (SAE) through Day 365 for Groups 2, 3, 5, 6, and 7; through Day 760 for Groups 1, 4, and 8; through Day 182 for Group 9 only; through Day 29 for Group 10 only.

7.2.1. Analysis Methods for Safety Endpoints

The number and percentage of subjects who experience a solicited AE (any event, after any injection, and after each injection) recorded daily until 7 days post-injection will be summarized according to maximum severity grade by treatment group and decreasing total frequency. For subjects with multiple events of the same type, only the event of the highest severity will be counted in each summary. A Fisher's Exact test on the number and percentage of subjects with

the event will be used to compare each treatment group and Group 1. Events with missing severity grades will be handled according to Section 7.1.1. This summary will be repeated for treatment-related solicited AEs and solicited AEs at any time within 7 days following vaccination of at least severe (Grade 3) or higher severity.

Day of onset post each injection will be summarized with descriptive statistics by treatment group. The median and 95% CI will be estimated by Kaplan-Meier analysis. The log-rank test will be used to compare day of onset between a treatment group and group 1, respectively. This summary will be repeated for treatment-related solicited AEs. Also included will be a summary of the number and percentage of subjects reporting each solicited event at any time within 7 days following each and any vaccination.

The number of days a subject experience each solicited AE within 7 days post injection will be summarized by descriptive statistics (number of subjects with each solicited AE, mean, standard deviation, median, minimum and maximum) by treatment group. The days may not be consecutive. The mean and its 95% CIs will be based on t-statistics assuming normal distribution. The median and 95% CIs will be distribution free estimates. A Wilcoxon rank sum test on the number of days of symptoms will be used to compare each treatment group and group 1. This summary will be repeated for treatment-related solicited AEs.

A summary of the number and percentage of subjects who experience an unsolicited AE during Days 1 through 57 (for all Groups except for Group 10, during Days 1 through 29 for Group 10) and Days 547 through 575 (for Group 4 only), by SOC and PT will be presented separately. Similarly, a summary of the number and percentage of subjects who experience a treatment-related AE during Days 1 through 57 (for all Groups except for Group 10, during Days 1 through 29 for Group 10) and Days 547 through 575 (for Group 4 only) by SOC and PT will be provided separately.

In addition, a summary of the number and percentage of subjects who experience an SAE during Days 1 through 760 (for Groups 2, 3, 5, 6, and 7, Days 1 through 365; for Group 9, Days 1 through 182; for Group 10, Days 1 through 29) by SOC and PT will be presented. Similarly, a summary of the number and percentage of subjects who experience a treatment-related SAE during Days 1 through 760 (for Groups 2, 3, 5, 6, and 7, Days 1 through 365; for Group 9, Days 1 through 182; for Group 10, Days 1 through 29) by SOC and PT will be provided.

7.3. Prior and Concomitant Medications

Medications taken from 30 days prior to Day 1, all medications from Day 1 through Day 57 visit (for Groups 1-9), and again from Day 547 through Day 575 visit (for Group 4 only), and from Day 1 through Day 29 visit (for Group 10) and medications associated with a SAE through the end of the study, will be recorded. Additionally, any prohibited medication used during the study will also be recorded.

Any medications started and stopped prior to or on the date of first vaccination will be considered prior medications. If a partial stop date is entered and the month and year (if day is missing) or year (if day and month are missing) of the stop date are before the date of first vaccination, the medication will be considered a prior medication.

Any medications started prior to or on the date of first vaccination and continued to be taken after the date of first vaccination, or started after the date of first vaccination will be considered a concomitant medication. If a partial stop date is entered and the month and year (if day is missing) or year (if day and month are missing) of the stop date are after the date of first vaccination, the medication will be considered a concomitant medication.

Concomitant medications (i.e., medications other than study vaccine that are taken while receiving study vaccine) and prior medications (medications started and ended before receiving study vaccine) will be coded using the World Health Organization (WHO) Drug Dictionary version September 2017. The WHO preferred name and drug code will be attached to the clinical database.

Use of concomitant and prior medications will be summarized (number and percentage of subjects) by treatment group, WHO drug class (ATC level 2), and WHO generic name. Multiple drug use (by preferred name) will be counted once only per subject. The summary will be sorted alphabetically by drug class and then by decreasing total frequency within a class. Concomitant vaccines will also be reported for all subjects.

Summaries of prior and concomitant medications and vaccines will be provided for the safety population. No p-values or inferences regarding comparisons of the usage of concomitant medications and vaccines in the two treatment groups will be generated.

A listing of prior and concomitant medications and vaccines will be provided, with a column indicating if a medication is prior, Y or N.

7.4. Vital Signs

Vital signs will be listed by subject and timepoint for the randomized population.

7.5. Physical Examination

A data listing will be provided for physical examination results based on the randomized population.

7.6. Other Safety Measures

A data listing of all pregnancy test results will be provided for subjects.

8. ADDITIONAL INFORMATION

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

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

9. References

[1] Agresti, A., & Coull, B. A. (1998). Approximate is better than "exact" for interval estimation of binomial proportions. The American Statistician, 52(2), 119-126.

[2] PXVX-CV-317-001. Protocol Version 1.0: 'A Phase 2 Parallel-Group, Randomized, Double-Blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], unadjuvanted or alum-adjuvanted)'. February 16, 2017.

[3] PXVX-CV-317-001. Protocol Amendment_Fianl_v2.0: 'A Phase 2 Parallel-Group, Randomized, Double-Blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], unadjuvanted or alum-adjuvanted)'. January 31, 2019.

[4] PXVX-CV-317-001. Protocol V3.0 Amendment 2_v3.0: 'A Phase 2 Parallel-Group, Randomized, Double-Blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], unadjuvanted or alum-adjuvanted)'. July 24, 2019.

[5] PXVX-CV-317-001. Protocol V4.0 Amendment 3_4.0: 'A Phase 2 Parallel-Group, Randomized, Double-Blind Study to Assess the Safety and Immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], unadjuvanted or alum-adjuvanted)'. May 22, 2020.

10. Software

[redacted]

11. SAP Revision

Revision Date (dd month, yyyy)	Section	Summary of Revision	Reason for Revision
08 August, 2019	Whole SAP	Add all information of additional Group 9 Groups 2, 3, 5, 6, and 7 will complete study after Day 365. Groups 1, 4, and 8 will complete study after Day 760, but only Group 4 will have the booster injection on Day 547. Group 9 will complete study after Day 182.	Protocol Amendment 1 Protocol Amendment 2
14 July, 2020	Whole SAP	Add all information of additional Group 10. Group 10 will complete study after Day 29.	Protocol Amendment 3