Statistical Analysis Plan

Document Type:	Template	Document ID:	03.007A.03
Issue Date:	02 MAY 2016	Effective Date:	30 MAY 2016

Sponsor Name:	GeneOne Life Science
Protocol Number and Title:	**ZIKA-001** **PHASE I, OPEN-LABEL, DOSE RANGING STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF GLS-5700 ADMINISTERED ID FOLLOWED BY ELECTROPORATION IN DENGUE VIRUS-NAÏVE ADULTS**
Protocol Version and Date:	**V1.2 (16JUL2016)**
INC Research Project Code:	**1008314**
Author(s):	**Yuming Xue, Senior Biostatistician**
SAP Version:	**1.0**
SAP Version Date:	**17OCT2016**

Statistical Analysis Plan

Protocol: ZIKA-001 & TLF shells

Version: 1.0 Version Date: 17-Oct-2016

I confirm that I have reviewed this document and agree with the content.

APPROVALS	
INC Research	
Yuming Xue, Senior Biostatistician	21-Oct-2016 Date (dd-Mmm-yyyy)
Laura Truskowski, Principal Biostatistician	21-Oct-2016 Date (dd-Mmm-yyyy)
GeneOne Life Science	
Joel Maslow, MD PhD MBA, Chief Medical Officer	21 Oct 2016 Date (dd-Mmm-yyyy)
Susan Spruill, MS, Biostatistician	23/Oct/2016 Date (dd-Mmm-yyyy)
Christine Roberts, PhD, Dir Clinical Lab Development	21 OCT 2016 Date (dd-Mmm-yyyy)
Celine Reimgio, RN DPT, Senior Program Manager	21 Oct 2016 Date (dd-Mmm-yyyy)

TABLE OF CONTENTS

Contents

1. GLOSSARY OF ABBREVIATIONS

Abbreviation	Description
AE	Adverse Reaction
AICD	Automatic Implantable Cardioverter Defibrillator
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CD4+	Cluster of Differentiation 4+
CD8+	Cluster of Differentiation 8+
CKD	Chronic Kidney Disease
CI	Confidence Interval
CPK	Creatine Phosphokinase
Cr	Creatinine
CRF	Case Report Forms
CSR	Clinical Study Report
DNA	Deoxyribonucleic Acid
E	Zika Virus Envelope Protein
ECG	Electrocardiogram
ELISA	Enzyme Linked Immunosorbent Assay
ELISpot	Enzyme Linked Immunosorbent Spot-forming Assay
EP	Electroporation

Abbreviation	Description
ERER	Event requiring expedited reporting
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus antibody
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICS	Intracellular Staining
ID	Intradermal
IFN-γ	Interferon gamma
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Drug Regulatory Affairs
mITT	Modified Intention-to-Treat
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PP	Per-Protocol
prME	pre-membrane and envelope
PT	Preferred Term
Q1	First Quartile
Q3	Third Quartile
REB	Research Ethics Board
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation

Abbreviation	Description
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
TLFs	Tables, listings and figures
TNF-α	Tumor Necrosis Factor alpha
WHO-DD	World Health Organization Drug Dictionary

2. PURPOSE

This Statistical Analysis Plan (SAP) is created based on Protocol Number ZIKA-001, Version 1.2, Version Date 1 July 2016 and ZIKA-001 Protocol Notification to sites dated 22 September 2016. The purpose of this SAP is to outline the planned analyses by INC to support the completion of the Clinical Study Report (CSR). This SAP describes in detail the statistical methodology and the statistical analyses to be conducted that includes an Interim Analysis following the Week 14 study visit at which time the primary endpoint of the protocol will be reached (as denoted in the Protocol Notification of 22 September) and the Final Analysis to occur after the completion of all study visits as presented in the ZIKA-001 ver 1.2. The planned analyses identified in this SAP will be included in regulatory submissions and/or future manuscripts.

2.1. RESPONSIBILITIES

INC Research will perform the statistical analyses and are responsible for the production and quality control of all tables, listings and figures (TLFs).

2.2. TIMINGS OF ANALYSES

This SAP details the analysis of the study. The primary analysis of safety and tolerability is planned after all subjects complete the 14 week study visit corresponding to the completion of the Primary Study endpoint with a second analysis to correspond to when all have completed all study visits (either the final study visit or early termination from the study) to assess long-term safety and immunogenicity.

3. STUDY OBJECTIVES

3.1. PRIMARY OBJECTIVE

The primary study objective is to evaluate the safety and tolerability of GLS-5700 when administered by intradermal (ID) injection followed by electroporation (EP) in healthy dengue virus-naïve adult participants within 14 days post final vaccination.

3.2. SECONDARY OBJECTIVES

Secondary objectives are to evaluate:

- the safety to 1 year post vaccination of GLS-5700 in dengue virus-naïve adults
- the cellular and humoral response of GLS-5700 when delivered ID followed by EP in dengue virus-naïve adults

3.3. EXPLORATORY OBJECTIVES

Exploratory objectives are to explore:

- whether end point antibody titers are dose related
- immunogenicity and longevity of immune responses, both humoral and cellular
- if increasing dose levels of GLS-5700 more rapidly induce cellular and humoral immunity
- the epitope specificity for T cell reactions of GLS-5700 to Zika virus

3.4. STUDY DESIGN

This Phase I clinical trial will evaluate whether GLS-5700 administered via ID injection and followed by EP is safe, well-tolerated, able to generate an immune response against Zika virus in dengue virus-naïve participants, and whether the immune reactivity is dose-dependent. Injections will be given in the deltoid region followed immediately by EP with the CELLECTRA®-3P device.

GLS-5700 contains plasmid pGX7201 that encodes for a consensus sequence of the pre-membrane and envelope (prME) proteins of Zika virus.

Evaluation of ID administration of GLS-5700:

There are two arms for ZIKA-001. Participants (n=20 per group) will be administered GLS-5700 at one of two dose levels: 1 mg or 2 mg DNA/dose. Vaccine will be administered as 0.1 ml ID injections followed by EP with the CELLECTRA®-3P device. Participants will receive one (1 mg/dose) or two (2 mg/dose) injections into the deltoid region at vaccination at 0, 4, and 12 weeks (3 vaccination series).

Safety assessment: Participants will be monitored for adverse events utilizing the "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" with labs assessed as per site normal values. Pain will be assessed immediately after EP and at 30 minutes post-EP. Laboratory safety assessments will be obtained at screening, 1 week following the 1st vaccination, and 2 weeks following the 2nd and 3rd vaccinations. Adverse events, including assessment of injection site reactions, will be monitored through 12 months after the final vaccination.

In the event that a stopping criterion is reached, the study will not continue until a full discussion has been conducted with the Medical Monitor, Principal Investigator, and Institutional Review Board (IRB)/Research Ethics Board (REB) (if applicable).

- One third (1/3) or more participants experience an ERER assessed as related to Study Treatment;
- Three or more participants in the same treatment arm discontinue due to an AE related to the Study Treatment;
- Any participant experiences a potentially life threatening AE, Grade 4 AE or death assessed as related to Study Treatment;
- Two or more participants within a treatment arm experience the same or similar grade 3 or 4 adverse event, assessed as related to Study Treatment;
- Seven or more participants across all treatment arms experience the same or similar grade 3 or 4 adverse event, assessed as related to Study Treatment;
- Any report of anaphylaxis of Grade 3 or greater assessed as related to Study Treatment.

Immunogenicity assessment: Blood will be obtained for Zika immune responses at baseline; at weeks 1, and 4; at 2 and 8 weeks following the 2nd and 3rd vaccinations; and at study weeks 36 and 60. Serum will be separated and sent for analysis for humoral responses (neutralizing and binding antibody titers). Whole blood will be processed to obtain peripheral blood mononuclear cells (PBMC) for Enzyme Linked Immunosorbent Spot-forming Assay (ELISpot) and/or Intracellular Staining (ICS) analysis.

3.5. SUBJECT SELECTION

The eligible population for this study is subjects who meet the following inclusion/ exclusion criteria.

3.5.1. Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for this study.

a. Age 18-65 years;

b. Able to provide consent to participate and having signed an Informed Consent Form (ICF);

c. Able and willing to comply with all study procedures;

d. Women of child-bearing potential agree to use medically effective contraception (oral contraception, barrier methods, spermicide, etc.), or have a partner who is sterile from enrollment to 3 months following the last injection, or have a partner who is medically unable to induce pregnancy.

e. Sexually active men who are considered fertile must agree to use either a barrier method of contraception during the study, and agree to continue the use for at least 3 months following the last injection, or have a partner who is permanently sterile or medically unable to become pregnant;

f. Normal screening ECG or screening ECG with no clinically significant findings;

g. Screening labs must be within normal limits or have only Grade 0-1 findings, except that creatinine may be grade 2 at baseline;

h. No history of clinically significant immunosuppressive or autoimmune disease.

i. No history of dengue virus vaccination or illness; no history of yellow fever vaccination

j. Dengue seronegative at baseline by screening laboratory evaluation

k. Not currently or within the previous 4 weeks taking immunosuppressive agents (excluding inhaled, topical skin and/or eye drop-containing corticosteroids, low-dose methotrexate, or prednisone at a dose less than 10 mg/day or steroid dose-equivalent).

3.5.2. Exclusion Criteria

Subjects who meet any of the following criteria will not be eligible for participation in this study.

a. Administration of an investigational compound either currently or within 30 days of first dose;

b. Previous receipt of an investigational product for the treatment or prevention of Zika virus infection except if participant is verified to have received placebo;

c. Administration of any vaccine within 4 weeks of first dose;

d. Administration of any monoclonal or polyclonal antibody product within 4 weeks of the first dose

e. Administration of any blood product within 3 months of first dose;

f. Pregnancy or breast feeding or have plans to become pregnant during the course of the study;

g. Positive serologic result for dengue virus (any serotype) or history of receipt of either dengue virus or yellow fever virus vaccination at any time in the past;

h. Positive serologic test for HIV, hepatitis B surface antigen (HBsAg); or any potentially communicable infectious disease as determined by the Principal Investigator or Medical Monitor;

i. Positive serologic test for hepatitis C (exception: successful treatment with confirmation of sustained virologic response);

j. Baseline evidence of kidney disease as measured by creatinine greater than 1.5 (CKD Stage II or greater);

k. Baseline screening lab(s) with Grade 2 or higher abnormality;

l. Chronic liver disease or cirrhosis;

m. Immunosuppressive illness including hematologic malignancy, history of solid organ or bone marrow transplantation;

n. Current or anticipated concomitant immunosuppressive therapy (excluding inhaled, topical skin and/or eye drop-containing corticosteroids, low-dose methotrexate, or prednisone at a dose greater than 10 mg/day or steroid dose-equivalent);

o. Current or anticipated treatment with TNF-α inhibitors such as infliximab, adalimumab, etanercept;

p. Prior major surgery or any radiation therapy within 4 weeks of group assignment;

q. Any pre-excitation syndromes, e.g., Wolff-Parkinson-White syndrome;

r. Presence of a cardiac pacemaker or automatic implantable cardioverter defibrillator (AICD)

s. Metal implants within 20 cm of the planned site(s) of injection;

t. Presence of keloid scar formation or hypertrophic scar as a clinically significant medical condition at the planned site(s) of injection.

u. Prisoner or participants who are compulsorily detained (involuntary incarceration) for treatment of either a physical or psychiatric illness;

v. Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements or assessment of immunologic endpoints; or

w. Not willing to allow storage and future use of samples for Zika virus related research

x. Any illness or condition that in the opinion of the investigator may affect the safety of the participant or the evaluation of any study endpoint.

3.6. DETERMINATION OF SAMPLE SIZE

As indicated in protocol section 8.3 and 8.4 for the probabilities of observing adverse events and immunological responses in the present trial, a sample finding of no grade 3 or grade 4 adverse events in a total evaluable sample of 40 participants would provide 95% confidence that the rate in the population from which the sample was drawn is no greater than 8.8%. With 40 participants receiving vaccine at any level, the study provides 80% probability of detecting an event if the incidence is greater than 4% in the vaccinated population (shown in table below).

True Event Rate in the Population Represented by the Study Sample	Probability of Observing Event(s) in the Study Sample (N = 40)	
	1 or more Event	2 or more Events
0.5%	18%	2%
1%	33%	6%
2%	55%	19%
3%	70%	33%
4%	80%	48%
5%	87%	60%

In immunogenicity analysis, a sample finding of 75% responders in the total sample of 40 participants would provide 95% confidence that the rate in the population from which the sample was drawn was no lower than 61% (Shown in table below).

N	Sample rate	Lower Limit	Upper Limit
40	25%	14%	39%
	50%	36%	64%
	75%	61%	86%
	90%	79%	97%
	95%	85%	99%

3.7. TREATMENT ASSIGNMENT & BLINDING

This study is an open-label study.

3.8. ADMINISTRATION OF STUDY MEDICATION

Group 1 Participants (n=20) and Group 2 Participants (n=20) will be administered 1 mg and 2 mg GLS-5700 DNA/dose given as an ID injection respectively followed by EP with the CELLECTRA®-3P device. Participants will receive a 3-dose series with immunizations at 0, 4, and 12 weeks.

3.9. STUDY PROCEDURES AND FLOWCHART

For an individual subject, study duration from screening through follow-up will be up to 1 year post final vaccination.

The below flowchart details the study procedure in Protocol 1.1:

Tests and Observations	Screen (Day -7 to -30)	Day0	Day1	Wk1 (±2d)	Wk4 (±5d)	Wk6 (±5d)	Wk12 (±5d)	Wk14 (±5d)	Wk20 (±10d)	Wk36 (±10d)	Wk60 (±10d) / Early termination
Clinical Assessments											
Consent	X										
Med history	X	X									
Demographics	X										
Travel hx [a]	X										
Medications [b]	X	X		X	X	X	X	X	X	X	X
Phys Exam [c]	X	X		X	X	X	X	X	X	X	X
Vital signs [c]	X	X		X	X	X	X	X	X	X	X
Laboratory Assessments											
12-lead ECG	X										
CBC w/diff	X			X		X		X			
Chemistries [d]	X			X		X		X			
Serologies [e]	X										
Pregnancy [f]	X	X			X		X				
Serum [g]		X		X	X	X	X	X	X	X	X
PBMCs [g]		X		X	X	X	X	X	X	X	X
Blood Vol. per visit (mL)	15	70		80	70	80	70	80	70	70	70
Study Related Procedures											
Vaccine + EP		X			X		X				
EP Data [h]		X			X		X				
Memory aid [i]		X			X		X				
AEs		X	X [j]	X	X	X	X	X	X	X	X

a Travel history to Central America, South America, Caribbean Islands, and South Pacific Islands at any time in past
b Prior and concomitant, new medications will be recorded at all study visits (Day 0 through study discharge)
c Full physical examination performed at screening and last visit or early termination only; perform targeted examinations at other visits as determined by Investigator or per participant complaints; record history of weight lifting or other significant physical activity; Vital signs will be performed pre and post vaccination at week 0, 4, and 12 week visits; height and weight will be recorded at screening.
d Sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO3), glucose, BUN, Cr, ALT, AST, CPK
e HIV antibody or rapid test, HBsAg, HCV antibody, dengue, West Nile, and chikungunya virus antibodies
f Serum pregnancy test at screening and urine pregnancy test thereafter
g Collect 4 tubes whole blood in ACD (yellow top) tubes for PBMCs; and 3 tubes of whole blood (red top) to obtain ~10 mL serum at the indicated time points
h Download EP data within 48 hours of dose and transfer to GeneOne or its designee
i Provide memory aid to study participants and review on next visit
j Detail AEs assessed via telephone.

3.10. PRIMARY ENDPOINT

The primary safety endpoints are as following:

1. Incidence of adverse events classified by system organ class (SOC), preferred term (PT) severity, and relationship to Study Treatment and schedule to 14 days post-final vaccination

2. Administration (injection) site reactions (described by frequency and severity grade) and administration site pain to 14 days post-final vaccination

3. Changes in safety laboratory parameters described by frequency and severity grade (e.g., liver panel tests, vital signs)

3.11. SECONDARY ENDPOINTS

Secondary immunologic endpoints of this study are:

1. Binding antibody titers to the Zika envelope (PrME) protein as measured by ELISA

2. Neutralizing antibody titers against Zika virus as measured in viral neutralization assay

3. Antigen specific cellular immune responses to Zika virus as determined by Interferon-gamma (IFN-γ) ELISpot and/or Intracellular Staining (ICS) assays

3.12. EXPLORATORY ENDPOINTS

Exploratory endpoints of this study are:

1. Comparison of ELISA, neutralization titers, IFN-γ ELISpot and/or ICS across different vaccine regimens

2. Other analyses as indicated to assess protective mechanisms against Zika virus.

3. Epitope mapping of CD4+ and CD8+ T lymphocyte responses

4. ANALYSIS SETS

Four analysis sets, the Intent-to-treat (ITT) analysis set, the safety analysis set, the modified intent-to-treat (mITT) analysis set, and the per-protocol (PP) analysis set, will be used for the analyses.

Analyses	Safety	PP	mITT
Primary Safety endpoints	X		
Secondary Immunologic endpoints		X	X
Exploratory endpoints		X	X

4.1. INTENTION-TO-TREAT ANALYSIS SET

The Intention-to-treat Analysis Set includes all enrolled participants who passed the screening and are eligible for vaccination, and enrolled into the study regardless whether they receive treatment or not.

4.2. MODIFIED INTENT-TO-TREAT ANALYSIS SET

The modified intention to treat (mITT) analysis set includes all ITT participants who receive at least one dose of Study Treatment, are Zika naïve at baseline, have a baseline immune response assessment and have at least one available post-baseline immune response assessment.

4.3. SAFETY ANALYSIS SET

The safety analysis set includes all ITT participants who receive at least one dose of Study Treatment.

4.4. PER PROTOCOL ANALYSIS SET

Per-protocol (PP) analysis set to comprise mITT participants who receive all planned doses of Study Treatment and have no major protocol violations.

4.5. PROTOCOL DEVIATIONS

The participant failed to adhere to the protocol requirements (e.g., treatment noncompliance, failure to return for defined number of visits). The deviations are collected from the CRF.

A list of protocol deviations will be provided with the date the deviation occurs, the category, and the description of the deviation.

5. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

5.1. GENERAL METHODS

Descriptive statistics will be presented from the data collected in this Phase I study.

For categorical variables, the number and percentage of subjects in each category will be summarized. Percentages will be displayed to one decimal place, except 100%, which will not show any decimals. Counts of zero will not have percentage displayed in order to draw attention to non-zero counts.

Continuous variables will be summarized with the number of observations (n), mean, standard deviation (SD), median, first and third quartiles (Q1 and Q3) minimum, and maximum values. The minimum and maximum will be displayed to the precision with which the data were collected. The mean, median and quartiles will be displayed to one additional decimal place and the SD will be displayed to two additional decimal places.

The data analyses will be conducted using the SAS System (SAS Institute Inc., Cary, NC) Version 9.3 or a later version. All SAS programs used to generate analytical results will be developed and validated according to INC programming standards and SAS validation procedures.

5.2. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographic and baseline data, vital signs, medical history, concomitant illnesses, and current medications/treatments will be summarized by means of descriptive statistics: continuous variables as mean, median, standard deviation, and interquartile ranges and categorical variables as frequencies and percentages, stratified by treatment arm based on the safety population.

5.3. SAFETY ANALYSIS

The frequencies of TEAEs will be presented separately by dose and overall by system organ class and by preferred term, the number and percentage of participants affected.

Additional frequencies will be presented with respect to maximum severity and to strongest relationship to Study Treatment. Multiple occurrences of the same AE will be counted only once following a worst-case approach with respect to severity and relationship to Study Treatment.

All serious AEs and administration site events will also be summarized as above.

The proportion of participants experiencing grade 3+ (grade 3 or grade 4) TEAEs, as well as investigational drug-related grade 3+ TEAEs following the vaccine will be presented

by treatment groups. The adverse event rates in each treatment group will be reported, with of 95% binomial exact CIs. Proportion difference between treatment groups will be tested by Fisher's Exact test and 95% binomial exact CIs will be reported.

5.4. IMMUNOGENICITY ANALYSIS

Data classified as positive/negative or responder/non-responder will be analyzed as the frequency of response for each assay within treatment arm at each time point at which an assessment is performed. For secondary immunogenicity outcomes, estimates of proportions and exact 95% binomial CIs for the differences between the groups will be calculated. We will use the chi-square test to evaluate the significance of the differences between groups.

The proportion of participants meeting the criteria for immunologic response following vaccination will be presented for each study arm. The precision with which this sample result estimates the rate of immunologic response rates in the population represented by the study sample will be reported in terms of 95% CIs around the sample proportions. This will provide 95% confidence that the rate of immune response is not less that the lower limit of the proportion of study participants meeting immune response criteria. The primary immunogenicity analysis will include all subjects having evaluable data.

Comparison of immune response between treatment arms will be provided.

The median of immunogenicity titers of binding antibody titers, neutralizing antibody titers, and antigen specific cellular responses will be tested for significant differences between treatment groups, using a Mann Whitney U test. The median (95% Distribution-free CIs) and geometric mean (95% Geometric CIs) will also be tabulated.

The corresponding immune responses (positive/negative), i.e. binding antibody titers, neutralizing antibody titers, and antigen specific cellular responses, will be tested for the proportion difference between treatment groups using Fisher's exact test. The primary immunogenicity analysis will include all participants having evaluable data.

A secondary qualitative analysis will assess immune responses relative to presence of pre-existing antibodies to other flaviviruses (West Nile or dengue) or chikungunya virus.

5.5. KEY DEFINITIONS

Baseline is defined as last non-missing assessment prior to first dose. This definition will be used for all baseline and change from baseline analyses, unless specified otherwise.

5.6. MISSING DATA

Missing data will not be replaced. For quantitative assessments summarized by visit, summary statistics at each study visit will include data from subjects with non-missing values. Partial dates will be listed as recorded on CRF. No imputation of missing or

incomplete date/data will occur unless otherwise specified.

5.7. VISIT WINDOWS

There will be no derivation for visit windows in terms of summary of assessments. Nominal visits will be used for listings and tables.

6. DEMOGRAPHIC, OTHER BASELINE CHARACTERISTICS AND MEDICAL HISTORY

6.1. SUBJECT DISPOSITION

The following frequencies (number and percent) will be displayed by part and by treatment:

- Subjects enrolled (number only)
- Subjects who completed the study
- Subjects who discontinued early overall and by reason

The denominators for the percent calculations will be the number of subjects in safety set per treatment and combined, whether or not they are included in any of the analyses. No formal statistical comparison between the groups will be performed.

Subjects' completion/discontinuation status will be listed, including subject identifier, last visit/timepoint, and the specific reason(s) for discontinuation.

All inclusion/exclusion criteria will be listed. Inclusion/exclusion criteria violations will be listed.

6.2. DEMOGRAPHICS, OTHER BASELINE CHARACTERISTICS

Demographic and baseline characteristics, will be summarized by means of descriptive statistics: continuous variables as mean, median, standard deviation, and interquartile ranges and categorical variables as frequencies and percentages, stratified by treatment arm based on the safety analysis set. Calculation of age is as follows:

- Age = (Informed Consent Date - Date of Birth + 1) / 365.25 and truncated to complete years.

6.3. MEDICAL HISTORY

A by-treatment summary table of the number and percentage of subjects by medical history system organ class (SOC) will be produced for subjects in the safety analysis set. Medical history will be sorted alphabetically by SOC using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary, version 19.0. For the summary tables, a subject may appear more than once if he/she has more than one medical history coded under different SOC categories. However, the subject will be counted only once in the overall category. A by-subject listing with coded SOC and PT along with verbatim CRF term will also be provided.

6.4. PRIOR AND CONCOMITANT MEDICATION

Medications will be classified as prior if they were ongoing medications but stopped prior to the 1st dose of Study Treatment or concomitant (continued past or started on or after the first dose date of Study Treatment). Episodic medications (such as analgesics) will not be captured. The following rules will be applied to concomitant medications that were ongoing at the start of the trial, but discontinued during the clinical trial:

- If the stop date is completely missing, then it will be set to the last dose date;
- If the day is missing, then the last day of the month will be used. If this date is after the last dose date and the months are the same, then the last dose date will be used;
- If the day and month are missing, then the last day of the year will be used. If this date is after the last dose date and the years are the same, then the last dose date will be used.

All prior and concomitant medications will be classified using the Anatomical Therapeutic Chemical (ATC) classification codes and preferred drug names from the World Health Organization Drug Dictionary (WHO-DD), June 2016.

Summaries of prior and concomitant medications will be presented separately in tabular form using the second-level ATC term as an upper classification level and the preferred drug name as a lower classification level. All medications will be summarized by treatment group and sorted alphabetically by ATC level 2 and preferred drug name within a given ATC level 2 term. The summary will consist of the frequency and percent of safety subjects who used the medication at least once.

For each subject, the medication will be counted only once within a level-2 ATC and only once within a given preferred drug name level. A subject may appear more than once if he/she has more than one concomitant medication coded under different ATC categories; however, the subject will be counted only once in the overall category.

A by-subject listing with coded terms will also be provided along with calculated study day.

6.5. 12-LEAD ECG

An ECG will be performed at screening for all participants to determine eligibility. The ECG should include measurements of ventricular heart rate, PR, QRS, QT, QTc with assessment as to whether the ECG is normal or abnormal. Abnormal ECGs will be interpreted as clinically significant or not clinically significant.

A by-subject listing will be generated incorporating information and assessment results obtained from the designated central reading facility with provided investigator abnormality interpretations.

7. SAFETY ANALYSES

7.1. ADVERSE EVENTS

All AEs will be classified using MedDRA version 19.0.

Treatment-emergent AEs (TEAEs) will be defined as AEs onset or pre-existing conditions that worsen on/after the first study dose. Only TEAEs are included in the summaries and analyses, but all AEs, including non-treatment-emergent AEs will be presented in subject data listings. The following TEAEs will be summarized for each treatment group as follows:

- Number of TEAEs and number (%) of subjects with TEAEs by SOC and preferred term
- Number of TEAEs and number (%) of subjects with TEAEs by SOC, preferred term and severity (in hierarchical order: Potentially Life-threatening > severe > moderate > mild, missing severity will be considered as severe)
- Number of TEAEs and number (%) of subjects with TEAEs by SOC, preferred term and causality relationship to study drug (related vs unrelated)
- Number of TEAEs and number (%) of subjects with serious TEAEs by SOC and preferred term
- Number of TEAEs and number (%) of subjects with TEAEs leading to discontinuation of study drug by SOC and preferred term
- Number of Injection site reactions and number (%) of subjects as well as number (%) of injection sites with injection site reactions
- Deaths, if any, will be summarized, with reasons given

The summaries and analyses for TEAEs will be based on safety analysis set. TEAE will be summarized by proportions and exact 95% binomial CIs. The differences between the groups will be calculated in proportions. Fisher's Exact test will be used to evaluate the significance of the differences in frequency between groups.

Injection site reaction will be collected from CRF at the category of Tenderness, Pruritus, Erythema, Induration/Swelling and Bruising.

All AE summary tables including injection site reactions will only contain AEs within 14 days from the final vaccine administration.

Vital signs will be assessed at each visit starting from the screening visit. The by-visit summary table will include diastolic and systolic blood pressures, pulse, respiratory rate

and temperature. A by-subject listing will also be provided.

7.2. IMMUNOGENICITY ANALYSIS

The immunogenicity analyses will be based on per-protocol and mITT analysis set.

The baseline and post-baseline immunologic assessment results will be summarized for each treatment arm as follows:

- Frequency of subjects with positive immune responses (including binding antibody titers to the Zika virus, Neutralizing antibody titers against Zika virus, Antigen specific cellular immune responses) in each treatment arm at each time-point;
- Estimates of proportions and exact 95% binomial CIs with positive immune responses for each treatment arm at each time-point;
- p values of the differences between the treatment arms with positive immune responses from Fisher's Exact test;
- Median (95% Distribution-free CIs) and Geometric mean (95% Geometric CIs) of the immune responses in each treatment arm at each time-point;
- p values from Mann Whitney U test for the differences between the treatment arms at each time-point;

By-subject listings will be generated showing original units collected for all immunologic assessment results.

7.3. LABORATORY EVALUATIONS

Safety laboratory test values (hematology, blood chemistry, serology) and changes from baseline will be summarized by treatment group for the safety analysis set. In addition, the following clinical summaries will be presented by treatment group:

- The frequencies and percentages of subjects with clinically significant abnormalities at each study visit
- Tables summarizing the frequencies and percentages of subjects below, within, and above the normal ranges by each visit
- Tables displaying shifts from baseline to worst grade of post-baseline assessment in laboratory test values
- Tables displaying shifts from baseline to each assessment timepoints in laboratory test values by grade

By-subject safety lab listings will be generated incorporating information and

assessment results obtained from the designated laboratory which provided normal range and reported out of range flags. If multiple samples are taken at the same visit, repeats will be listed by the order of sampling times. However, the worst-case scenario will be applied in summarizing the test results.

7.4. HEIGHT, WEIGHT AND VITAL SIGNS

Vital signs (blood pressure, respiratory rate, and oral temperature), will be assessed at specified study visits; height and weight will be assessed at baseline as per Section 3.9.

Vital sign assessments will be captured as part of the safety analysis data set for Adverse Events.

Abnormality table will be provided for investigator assessment that show the number and percentage of subjects who have normal or abnormal non-Clinical Significant (NCS)/ Clinical Significant (CS) values at each visit by treatment group and overall, for the safety analysis set.

By-subject listings will be generated showing original units collected on the CRF.

7.5. PHYSICAL EXAMINATION

Physical examination results will be presented in a by-subject listing.

7.6. PREGNANCY TEST

Serum and urine pregnancy test results will be presented in a by-subject listing.

8. INTERIM ANALYSES

An interim analysis will be performed at the time of the primary endpoint is reached, 2 weeks post-final vaccination, corresponding to the Week 14 study visit. TLFs will include those delineating demographics, exposures, safety analyses, and antibody (binding and neutralizing antibody).

9. INDEX OF TABLES

No.	Title	Population
14.1.1.1	Subject Disposition - Number of Subjects Enrolled and Study Termination by Treatment Group	ITT
14.1.2	Protocol Deviations by Treatment Group	Safety
14.1.3.1	Demographic Characteristics and Other Baseline Characteristics	Safety
14.1.3.2	Baseline ECG Parameters	Safety
14.1.3.3	Medical History	Safety
14.1.4.1	Prior Medications	Safety
14.1.4.2	Concomitant Medications	Safety
14.1.5	Study Drug Administration	Safety
14.3.1.1	Treatment-Emergent Adverse Events–Overall Summary	Safety
14.3.1.2	Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety
14.3.1.3	Treatment-Emergent grade 3+ Adverse Events by System Organ Class and Preferred Term	Safety
14.3.1.4	Treatment-Emergent Adverse Events by System Organ Class and Preferred Term, and Relationship	Safety
14.3.1.5	Treatment Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety
14.3.1.6	Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety
14.3.1.7	Treatment-Emergent Adverse Events Leading to Permanent Discontinuation of Study Drug by System Organ Class and Preferred Term	Safety
14.3.1.8	Injection Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety
14.3.1.9	Injection Site Reaction by Visit	Safety
14.3.2.1.1	Hematology: Summary by Visit and Change from Baseline	Safety
14.3.2.1.2	Hematology: Proportion of Abnormal Results by Visit	Safety
14.3.2.1.3	Hematology: Shift from Baseline Grade to Maximum Post-Baseline Grade	Safety
14.3.2.2.1	Blood Chemistry: Summary by Visit and Change from Baseline	Safety
14.3.2.2.2	Blood Chemistry: Proportion of Abnormal Results by Visit	Safety
14.3.2.2.3	Blood Chemistry: Shift from Baseline Grade to Maximum Post-Baseline Grade	Safety
14.4.1.1	Frequency Summary of Binding Antibody Titers by Visit	mITT
14.4.1.2	Frequency Summary of Binding Antibody Titers by Visit	PP
14.4.2.1	Frequency Summary of Neutralizing Antibody Titers by Visit	mITT
14.4.2.2	Frequency Summary of Neutralizing Antibody Titers by Visit	PP

No.	Title	Population
14.4.3.1	Frequency Summary of Antigen Specific Cellular Immune Responses by Visit	mITT
14.4.3.2	Frequency Summary of Antigen Specific Cellular Immune Responses by Visit	PP
14.4.4.1	Summary of Immune Responses by Visit and Change from Baseline	mITT
14.4.4.2	Summary of Immune Responses by Visit and Change from Baseline	PP

* All tables (as data are available) are required for both the Interim (Week 14) and Final (Week 60) Analyses.

10. INDEX OF LISTINGS

No.	Title	Population
16.2.1	Subject Disposition	ITT
16.2.2	Protocol Deviations	mITT
16.2.3.1	Demographics	Safety
16.2.3.2	Medical History	Safety
16.2.3.3	Prior/Concomitant Medications and Therapies	Safety
16.2.3.4	Travel History	Safety
16.2.5.1	Study Drug Administration	Safety
16.2.5.2	Investigational Device Information	Safety
16.2.7.1	Adverse Events	Safety
16.2.7.2	Serious Adverse Events	Safety
16.2.7.3	Adverse Events Leading to Study Discontinuation	Safety
16.2.7.4	Injection Related Treatment Emergent Adverse Events	Safety
16.2.7.5	Death	Safety
16.2.7.6	Injection Site Reactions	Safety
16.2.8.1	Laboratory Assessments –Hematology	Safety
16.2.8.2	Laboratory Assessments –Blood Chemistry	Safety
16.2.8.3	Laboratory Assessments –Serology	Safety
16.2.8.4	Pregnancy Test-Serum and Urine	Safety
16.2.9	Vital Signs	Safety
16.2.10	12-Lead ECG Results and Interpretations	Safety
16.2.11	Physical Examination	Safety
16.2.12.1.1	Zika Binding Antibody Titers by Subject	mITT
16.2.12.1.2	Zika Binding Antibody Titers by Subject	PP
16.2.12.1.3	Zika Binding Antibody Titers by Visit	mITT
16.2.12.1.4	Zika Binding Antibody Titers by Visit	PP
16.2.12.2.1	Neutralizing Antibody Titers against ZIKA Virus by Subject	mITT
16.2.12.2.2	Neutralizing Antibody Titers against ZIKA Virus by Subject	PP
16.2.12.2.3	Neutralizing Antibody Titers against ZIKA Virus by Visit	mITT
16.2.12.2.4	Neutralizing Antibody Titers against ZIKA Virus by Visit	PP
16.2.12.3.1	Antigen Specific Cellular Immune Responses by Subject	mITT
16.2.12.3.2	Antigen Specific Cellular Immune Responses by Subject	PP
16.2.12.3.3	Antigen Specific Cellular Immune Responses by Visit	mITT
16.2.12.3.4	Antigen Specific Cellular Immune Responses by Visit	PP

11. REFERENCES

1. CDISC: Study Data Tabulation Model (SDTM), Final Version 1.2, dated November 12, 2008.
2. CDISC: Study Data Tabulation Model (SDTM) Implementation Guide: Human Clinical Trials (V3.1.2), dated November 12, 2008.
3. CDISC: Analysis Data Model (ADaM), Final Version 2.1, dated December 17, 2009.
4. CDISC: Analysis Data Model (ADaM) Implementation Guide, Final Version 1.0, dated December 17, 2009.
5. Kieser M, Hauschke D. Approximate sample sizes for testing hypotheses about the ratio and difference of two means. *Journal of Biopharmaceutical Statistics*. 1999;9:641–650.
6. Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Statistics in Medicine*. 1998;17:873-890.
7. Corder, G. W.; Foreman, D. I. (2014). Nonparametric Statistics: A Step-by-Step Approach. Wiley.
8. Kaplan, E. L.; Meier, P. (1958). "Nonparametric estimation from incomplete observations". J. Amer. Statist. Assn.

12. ATTACHMENTS

Attachment 1: Planned Table Shells.

Attachment 2: Planned Listing Shells