Statistical Analysis Plan (SAP)

Protocol Title:	A RANDOMIZED, DOUBLE-BLINDED PHASE 3 STUDY TO DEMONSTRATE LOT-TO-LOT CONSISTENCY OF THREE LOTS OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN HEALTHY ADULTS AGED 18 TO 45 YEARS
Protocol Version No. / Date:	VLA1553-302 version 3.0 / 07-Apr-2021
CRF Version No. / Date:	Version 2.0 / 12-May-2021
SAP Version No. / Date	Version 5.0 / 31-Mar-2022

1.0 Approvals

Sponsor	
Sponsor Name:	Valneva Austria GmbH
Representative / Title:	[redacted] / Clinical Strategy Manager
Signature / Date:	
Representative / Title:	[redacted] / Clinical Operations Manager
Signature / Date:	
PRA	
Biostatistician / Title:	[redacted] / Principal Biostatistician
Signature / Date:	

2.0 Change History

Version/Date	Change Log
[redacted]	[redacted]

3.0 Table of Contents

4.0 Purpose

The Statistical Analysis Plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under Valneva Austria GmbH Protocol VLA1553-302 version 3.0.

5.0 Scope

The Statistical Analysis Plan outlines the following:

- Study Objectives
- Study Design
- Study Estimands (Endpoints to be Analyzed and the Analysis Sets)
- Applicable Study Definitions
- Statistical Methods

This SAP covers the analysis for Part A (Visit 3, Day 29) and Part B (Visit 5, Month 6) of the study.

6.0 Introduction

This SAP should be read in conjunction with the study protocol and case report form (CRF). Any further changes to the protocol or CRF may necessitate updates to the SAP.

Final approval of this document will occur prior to database lock and unblinding for the Part A analysis.

6.1 Changes from Protocol

The Per Protocol analysis set uses different visit windows to define out of window results than outlined in the protocol schedule of events. This is in order to align the visit windows allowable in this analysis in line with the phase 1 study of this vaccine. The visit windows in the protocol are shown in section 11.2, whilst the windows for the exclusion from the Per Protocol analysis set are outlined in Section 9.5.2. Further, if an Unscheduled Visit falls into a Scheduled Visit window that either did not occur or occurred outside of the defined Scheduled Visit window, the Unscheduled Visit will be used in analyses in the place of the Scheduled Visit.

The definition of adverse event of special interest in section 11.7.5 has been updated per FDA feedback for clarification of the anatomical location with cutaneous pruritus. This is not yet reflected in Protocol version 3.0, which is the approved version at the time of SAP finalization.

[redacted]

7.0 Study Objectives

7.1 Primary Objective

- To demonstrate Lot-to-Lot manufacturing consistency of a live-attenuated CHIKV vaccine candidate (VLA1553) 28 days following vaccination in a healthy population aged 18 to 45 years after a single immunization.

7.2 Secondary Objective

- To evaluate immunogenicity and safety of VLA1553 up to 180 days following vaccination in a healthy population aged 18 to 45 years after a single immunization.

8.0 Study Design

This is a prospective, randomized, double-blinded, multicenter Phase 3 clinical study investigating three Lots of VLA1553 at the final dose (target 1x 10E4 $TCID_{50}$ per 0.5 mL). Overall, approximately 402 subjects will be enrolled (i.e. ICF signed) into the study, approximately 134 subjects per VLA1553 Lot (see the subject distribution scheme presented in Table 8-1).

Subjects will be block-randomized in a 1:1:1 ratio into the three study arms to receive one of three lots of VLA1553 as a single i.m. vaccination on Day 1 (Visit 1).

All subjects will be asked to return to the study site at Day 8 (Visit 2), Day 29 (Visit 3), Day 85 (Visit 4) and Month 6 (Day 180, Visit 5) for immunogenicity sampling. Safety data collection will capture solicited AEs until Day 11 and unsolicited adverse events (AEs) up to Day 180 (Month 6, Visit 5) from all subjects. Adverse events of special interest (AESIs) will be captured 2 to 21 days post-vaccination. Subjects presenting with acute arthralgia within this time period will be followed-up until resolution and monitored for recurrences until the end of the study. Serious adverse events (SAEs) will also be assessed until the end of the study (Month 6, Visit 5).

Table 8-1 Subject distribution scheme

Study Arm	Vaccine	Cohort	Number of subjects (n)	Target dose ($TCID_{50}$/dose)	Injection volume (mL)
1	VLA1553	Lot 1	134	1x 10E4 $TCID_{50}$ per 0.5 mL	0.5
2	VLA1553	Lot 2	134		0.5
3	VLA1553	Lot 3	134		0.5
Total N:			**402**		

The overall study design is displayed in **Figure 8-1** below.

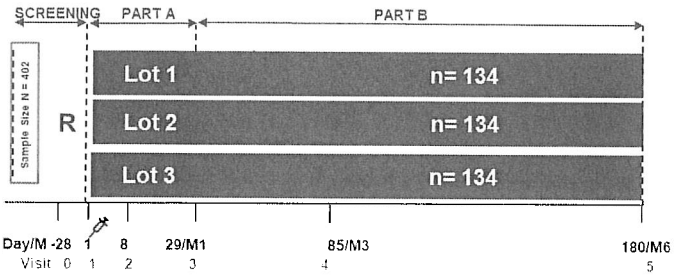


Figure 8-1 Overall Clinical Phase 3 Study Design

The overall duration of the study is estimated to be approximately eight months from study initiation (first subject enrolled) to study completion (last subject last visit). The individual subject participation is approximately seven months from enrollment to study completion unless prematurely discontinued.

The study is split into two parts which will be analyzed sequentially:

- Part A includes immunogenicity and safety data after all subjects have completed Visit 3 (Day 29);
- Part B includes immunogenicity and safety data after all subjects have completed Visit 5 (Month 6).

Part A will be unblinded (blinding will be maintained for study sites). The detailed study schedule for both parts is presented in Sections 15.4.1 (Part A) and 15.4.2 (Part B) of the Protocol.

8.1 Sample Size Considerations

The sample size is selected to allow for a demonstration of Lot-to-Lot consistency based on the primary endpoint, CHIKV-specific neutralizing antibody titers as determined by microneutralization (μPRNT) assay on Day 29 post-vaccination. The primary analysis is powered to demonstrate equivalence between the three treatment groups based on a comparison of GMTs.

Four hundred and two (402) randomized subjects (i.e. 134 per batch) will ensure that the three pair-wise comparisons have an overall power of approximately 90% based on a two-sided significance level of 5%, an assumed SD of 0.32 (on a log10 scale), and acceptance margins of [redacted] for the GMT ratios, while correcting for an assumed drop-out rate of 10% and 5% of subjects with major protocol deviations and 2% of subjects expected to be baseline positive for CHIKV (as determined by ELISA).

8.2 Randomization

The approximately 402 subjects will be block-randomized into the three study arms in a ratio of 1:1:1 at Visit 1 as described in Table 8-1.

In order to minimize bias, assignment into these study arms will be blinded for these subjects and the site staff, as well as the biostatistician (i.e. double-blind).

Each subject will have a unique subject identification code obtained from the interactive voice response system/interactive web response system (IXRS) assigned at the screening visit. The Investigator will keep a record (i.e. the subject screening log) of subjects who entered screening.

Randomization will be performed via the IXRS. At Day 1 (Visit 1, day of vaccination), eligible subjects will be assigned to the respective IMP lot.

8.3 Blinding/Unblinding

The study is conducted in a double-blind manner. Investigators and all other study staff involved in study conduct, safety assessments and IMP handling (i.e. perform preparation of the study vaccine, maintain the drug accountability logs detailing the dates and quantities of IMP administered to each subject); study participants, biostatistician (except the unblinded independent reporting statistician involved in the Data Safety Monitoring Board [DSMB]), CRAs responsible for monitoring study data, IMP handling and verifying drug accountability, and lab staff will all be blinded to treatment allocation.

The randomization assignment is not to be revealed except in emergency cases in which unblinding is necessary for the clinical management of an SAE. In such events, the Investigator must either inform the Sponsor before breaking the blind or immediately after unblinding has been performed.

In case of emergency, the vaccine administered to the subjects can be revealed through the web-response system (IXRS).

The study will be unblinded for analysis purposes at the end of Part A (after all subjects have completed Visit 3/ Day 29), but sites and subjects will remain blinded until the end of study.

9.0 Study Estimands

9.1 Target Population

The target population consists of healthy subjects aged 18 to 45 years receiving one of three lots of VLA1553, which is a candidate vaccine intended to prevent CHIKV infections in the general population living in endemic regions, as well as to serve as a prophylactic measure for travelers to epidemic areas or areas at risk for an upcoming outbreak or ongoing endemic transmission. Subjects are screened for their CHIKV serostatus by ELISA at study entry; subjects who test positive for CHIKV as measured by ELISA assay are excluded from the PP analysis set. For the primary and secondary endpoint analysis the µPRNT assay is used to determine neutralizing antibody titers.

9.2 Primary Endpoint

The primary immunogenicity endpoint is the geometric mean titer (GMT) of CHIKV-specific neutralizing antibodies as determined by µPRNT assay on Day 29 post-vaccination in subjects who tested negative for CHIKV antibodies (as determined by ELISA) at baseline.

9.3 Secondary Endpoints

The following secondary immunogenicity and safety endpoints will be evaluated:

Immunogenicity

- Immune response as measured by CHIKV-specific neutralizing antibody titers on Day 8, Day 85 and Month 6 post-vaccination as determined by µPRNT assay;
- Proportion of subjects with seroprotective levels (defined as [redacted] for baseline (µPRNT) negative subjects) at Days 8, 29, 85 and Month 6 post-vaccination by µPRNT assay;
- Proportion of subjects with seroconversion at Day 29 and Month 6 as determined by µPRNT assay (seroconversion defined as CHIKV-specific neutralizing antibody titer of [redacted] for baseline (µPRNT) negative subjects and [redacted] for baseline (µPRNT) positive subjects);
- Fold increase of CHIKV-specific neutralizing antibody titers determined by µPRNT assay at Day 8, 29, 85 and Month 6 post-vaccination as compared to baseline;
- Proportion of subjects reaching an at least 4-fold, 8-fold, 16-fold or 64-fold increase in CHIKV-specific neutralizing antibody titer compared to baseline as measured by µPRNT assay.

Safety

- Frequency and severity of solicited injection site and systemic AEs within ten days post-vaccination;
- Frequency and severity of unsolicited AEs within 28 days postvaccination;
- Frequency and severity of any AE during the entire study period;
- Frequency and severity of any SAE during the entire study period;
- Frequency and severity of any AESI within 2 to 21 days post-vaccination.

The below table links the endpoints to the study objectives and specify the estimands of the study. Analysis sets are defined in section 9.4.

Table 9-1 Overview of study estimands

Objectives	*Endpoints*	*Estimands*	*Study Part*	*Analysis Set*
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Objectives	*Endpoints*	*Estimands*	*Study Part*	*Analysis Set*
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Objectives	*Endpoints*	*Estimands*	*Study Part*	*Analysis Set*
	[redacted]	[redacted]	[redacted]	[redacted]

9.4 Analysis Sets

9.4.1 Safety Analysis Set

The safety analysis set (SAF) contains all subjects who entered into the study and received the vaccination. Subjects will be analyzed as treated.

9.4.2 Full Analysis Set

The full analysis set (FAS) contains all subjects who were randomized, received the vaccination and have evaluable immunogenicity data at the time point for the primary endpoint. Subjects will be analyzed as randomized.

9.4.3 Per Protocol Analysis Set

The per protocol (PP) analysis set contains all subjects who were baseline negative for CHIKV antibodies as determined by ELISA assay, have received the vaccination and have evaluable immunogenicity data at baseline and the time point for the primary endpoint without a major protocol deviation. This analysis set will be the primary analysis set for immunogenicity analyses. Subjects will be analyzed in the PP analysis set according to the study arm they have been randomized to.

9.4.4 [redacted]

9.5 Protocol Deviations

The study specific Protocol Deviation Guidance Document defines all important protocol deviations and all major protocol deviations.

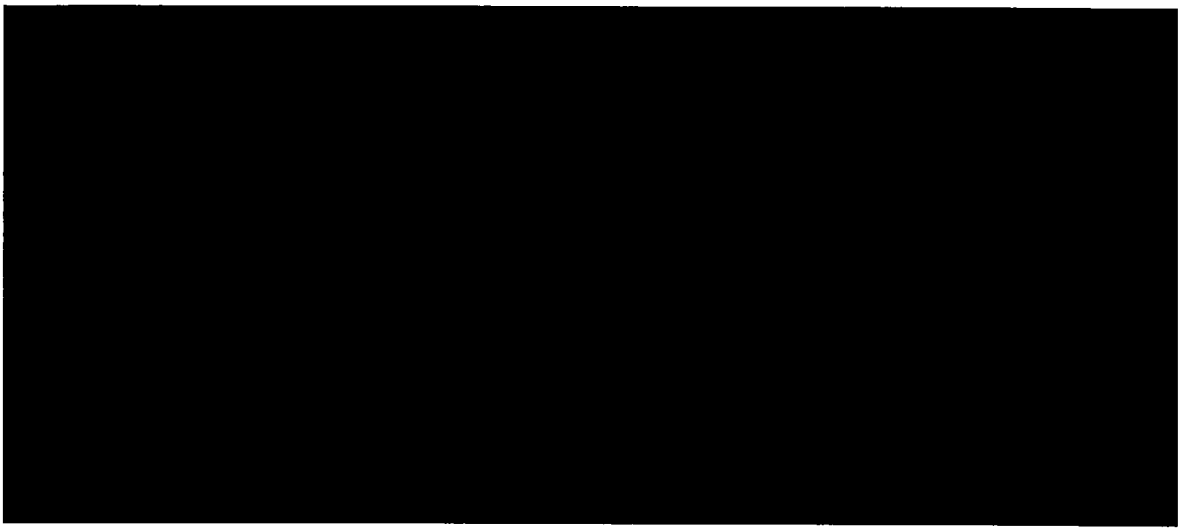
Per PRA processes, protocol deviations data will be entered into the system of record (PSO). The study team and the Sponsor will conduct on-going reviews of the deviation data from PSO and the resulting set of evaluable subjects throughout the study, adjusting the deviation criteria as seems appropriate. The evaluable subjects set must be finalized at the post-freeze data review meeting (or earlier), prior to the database lock for each part of the study.

In addition, a separate table and listing of COVID-19 specific protocol deviations will be produced.

9.5.1 Classification of Protocol Deviations

Protocol deviations will be classified as major or minor protocol deviations based on their possible impact on the study results in a blind data review meeting prior to the database snapshots at each study part.

All protocol deviations will be tabulated, separated by minor and major deviations. Major deviations are those which will lead to the exclusion of subject from the PP analysis set. A tabulation of the number and percentage of protocol deviations by deviation type will be provided for each study part. A by-subject listing of all protocol deviations will be presented.



Final decisions on whether any protocol deviation could impact immune response and thus lead to the exclusion from the PP analysis set will be made by the sponsor on a case by case basis in a blinded manner (prior to study unblinding). Sample testing issues may also lead to the exclusion from the PP analysis for particular time points.

In order to help identify protocol deviations which would lead to exclusion from the PP analysis set, listings of concomitant medications, protocol deviations, and subject visits outside of time window will be produced at the time of database freeze activities.

9.5.2 Exclusion of Time Points in Per Protocol Analysis

In the PP analysis of immunogenicity, samples with extensive time window deviations will be excluded from the analysis, even if the subject may remain in the PP analysis set. Any scheduled immunogenicity sample collected outside of the visit windows defined in the table below will be excluded from the PP analysis, but analyzed in the FAS.

Due to the fast onset of titer generation after immunization with VLA1553 and the long-term persistence of the titer without significant decrease over one year, the following protocol deviations related to time window deviations are classified as "minor protocol deviations" and do not exclude these subjects from the PP set (except for Visit 3 out of window as defined in section 9.4.3).

Table 9-2 Protocol deviation for immunogenicity samples collected outside of the visit window

Study Part	*Visit*	*Study Day (Visit Window)*
[redacted]	Visit 0 (Screening)	Day -28 to 0 (prior to Visit 1)
	Visit 1	Day 1
	Visit 2	Day 8 (Week 1)[redacted]
	Visit 3	Day 29 (Month 1)[redacted]
	Visit 4	Day 85 (Month 3)[redacted]
	Visit 5	Day 180 (Month 6)[redacted]

10.0 Analyses Part A and Part B

10.1 Planned Analysis Timepoints

There are 2 planned data analyses on this study:

- Part A includes safety and immunogenicity data after all subjects have completed Visit 3 (Day 29).
- Part B includes safety and immunogenicity data after all subjects have completed Visit 5 (Month 6).

Individual study parts will be analyzed sequentially.
The study will be unblinded for Part A analysis once the final subject has completed Visit 3 and database lock for Part A has occurred (blind will be maintained for study sites and subjects). Unblinding of the study for Part B analysis will occur once the final subject has completed Visit 5 and database for Part B database has been locked.
The analyses to be done at each study part are specified within each section of the statistical methods part of this SAP.
For the Part A analysis, data up to and including Visit 3 (Day 29) or ET if earlier will be kept for all subjects, for the Part B analysis all data will be kept for all subjects. All AEs and concomitant medications with a start date up to and including the date of the cutoff visit for each subject will be included in the analysis. Any record with a start date prior to the cutoff which has a stop date recorded after the cutoff visit for the analysis will be classed as ongoing for the relevant study part.

11.0 Conventions and Derivations

11.1 Baseline and Change from Baseline

Unless otherwise specified, baseline will be defined as the latest assessment taken prior to the administration of the study drug. All procedures/assessments (apart from AE assessment) taken at Visit 1 are assumed to occur prior to vaccination.

Change from baseline is defined as:

Observed result at nominal time point – observed result at baseline.

11.2 Study Days and Visit Windows

Study day is defined relative to the day of vaccination (Visit 1). Study Day 1 is the day of vaccination (Visit 1).

The scheduled study visits along with the predefined allowable visit window are included in the table below.

Table 11-1 Predefined visit windows

Study Part	*Visit*	*Study Day (Visit Window)*
[redacted]	Visit 1	Day 1
	Visit 2	Day 8 (Week 1)[redacted]
	Visit 3	Day 29 (Month 1)[redacted]
	Visit 4	Day 85 (Month 3)[redacted]
	Visit 5	Day 180 (Month 6)[redacted]

Subjects who withdraw from the study prior to completion of the study will attend an Early Termination (ET) Visit where possible. For the purposes of analysis and reporting, each ET visit will be assigned to one of the two study parts. If the ET visit falls within the visit window for a scheduled visit then it will be summarized under that planned visit, unless a scheduled visit already exists within the time window. Otherwise, if a subject has an ET visit but no Visit 3 or later visits then the ET visit will be assigned to Part A, if the subject has an ET visit and a Visit 3 or later visit then the ET visit will be assigned to Part B.

Data will be analyzed according to the visit recorded on the CRF, except in the case of the immunogenicity analyses on the PP analysis set and ET visits. Unscheduled visits may be held at any time during the study as necessary, and may be included in the analyses of planned visits. Further, if an Unscheduled Visit falls into a Scheduled Visit window that either did not occur or occurred outside of the defined Scheduled Visit window, the Unscheduled Visit will be used in analyses in the place of the Scheduled Visit. Data at ET visits within the visit window for a scheduled visit will be summarized under the scheduled visit. Data at ET visits outside these visit windows will be listed only and may be included in summary tables. Similarly planned visits which fall into the window for a different planned visit will be analyzed according to the visit window they fall into rather than the recorded visit number. If there are multiple results falling into one visit after re-windowing, then a conservative approach will be taken, and the lower titer value will be used.

Analyses described as being presented at a study day will include all data up to the corresponding visit number. For example an analysis presented at Day 29 will include all data up to the subject's visit 3 timepoint, even if this is after study day 29. Similarly endpoints analyzed up to Day 180 will include all data collected on study, up to their Visit 5 or ET visit.

11.3 Re-Screened Subjects

In this study subjects can be rescreened if they initially fail at screening and randomized into the study.

All available prior/concomitant medications and medical history will be re-entered into the latest applicable ID for a subject that is re-screened.

All other data will be mapped from the original ID to the latest applicable ID for a subject that is re-screened.

11.4 Handling of Duplicate Records

If there is duplicate entry of records into the system for any data then both records will appear in tabulation and analysis datasets and listings but only the first record will be flagged for use in the tables and figures.

11.5 Handling of Laboratory Records

If there are irreconcilable discrepancies between eCRF and vendor files in regards to differing visit label, visit date or accession number within a subject's results then subjects will be mapped by visit date and accession number.

The eCRF will be used as the ultimate source of data for these three variables. For frozen samples (including baseline samples, immunogenicity, and viremia samples), results will not be used from records where both visit date and accession number do not match. All other laboratory samples where a mismatch may occur between the ultimate source EDC data and vendor data, the samples will be reconciled and cleaned accordingly.

If there are multiple, non-duplicate records at the same visit date and accession number, then the worst case shall be used in tables and figures but all reported in listings. The worst case will be individual for each laboratory parameter and will be the lowest, highest or furthest from midpoint of normal range dependent on clinical relevancy. For example if there are two records of Calcium, as both high and low calcium is clinically relevant, the record furthest from the midpoint of normal range will be used.

Primary samples of baseline samples (all subjects), immunogenicity samples and selected Viremia Samples underwent sample reconciliation and cleaning – the eCRF is the ultimate source (except for irreconcilable items) for the associated back-up samples even in case of mismatches between visit date and accession number. For all other remaining samples where lab vendor records are mapped to EDC records, samples will be matched using Accession ID and date to reduce mismatches between the two records due to limitations in visit naming for the vendor samples.

11.6 Immunogenicity Endpoints

Immunogenicity assessment measuring neutralizing antibodies will be performed on samples collected at Days 1, 8, 29 (Month 1), 85 (Month 3) and 180 (Month 6) after a single immunization.

[redacted]

11.6.1 Geometric Mean Titer

The geometric mean titer (GMT) will be calculated as the anti-logarithm of the mean of the log-transformed titer. The geometric standard deviation (GSD) will be calculated as the anti-logarithm transformation of the standard deviation of the log-transformed titer. The 95% confidence interval (CI) will be calculated as the

anti-logarithm transformation of the upper and lower limits for a two-sided CI for the mean of the log-transformed titers.

11.6.2 Geometric Mean Fold Increase

The fold increase is calculated as the ratio of the post-vaccination titer value to the pre-vaccination value to determine the multiplicative increase from baseline result. Then, geometric mean is calculated as the anti-logarithm of the mean of the log-transformed values of (titer divided by the baseline assay result).

11.6.3 Seroconversion

Seroconversion is defined as a CHIKV-specific [redacted] for baseline µPRNT negative subjects (PP, sPP1 for definition see section 9.4.3 and 9.4.4) . Seroconversion for baseline µPRNT positive subjects (included in PP) is defined as a [redacted] increase over baseline.

[redacted]

The seroconversion rate (SCR) is defined as the proportion of subjects meeting the criteria for seroconversion at the relevant study timepoint.

11.6.4 Seroprotection

Seroprotective levels of CHIKV-specific NT are defined as [redacted] at any post-baseline timepoint for baseline µPRNT negative subjects.

The seroprotection rate (SPR) is defined as the proportion of baseline µPRNT negative subjects meeting the criteria for seroprotection at the relevant study timepoint.

11.6.5 Fold-Increase in Neutralizing Antibody Titer

The fold-increase from baseline in the CHIKV-specific NT is defined as:

µPRNT result at nominal time point / µPRNT result at baseline.

The fold-increase will be summarized as a continuous endpoint. In addition, the number of subjects reaching pre-specified fold-increase categories of at least 4, 8, 16 and 64-fold increases compared to baseline will be summarized as the number and percentage of subjects in each category.

11.7 Adverse Events

An AE is defined as any untoward medical occurrence in a subject administered an investigational product that does not necessarily have a causal relationship with the treatment. All new abnormalities or any exacerbation in intensity or frequency (worsening) of a pre-existing condition during or after vaccination have to be documented as AEs.

Any untoward medical occurrence experienced before vaccine exposure (for example, from the time of signed informed consent up to but not including vaccine exposure) will not be considered an AE and will be described in the medical history.

A subject's death per se is not an event, but an outcome. The event which resulted in the subject's death must be fully documented and reported, regardless of being considered related to treatment or not.

Preexisting diseases that are described in the medical history, and that manifest with the same severity, frequency, or duration after vaccine exposure, will not be recorded as AEs. However, when there is an increase in the severity of a preexisting disease, the event will be recorded as an AE.

There is no definition of treatment-emergent in this study. Any AEs with an onset date prior to the date of vaccination will be raised with data management and should be moved to medical history.

All AEs entered on the CRF will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1 or higher. The following information will be documented on the CRF for each AE: severity, causality, outcome, seriousness, medically-attended, action taken to treat AE, start and stop dates.

11.7.1 Adverse Events Severity

All AEs will be assessed for severity by the Investigator using his/her clinical expertise. Severity will be categorized as Mild (Grade 1), Moderate (Grade 2) or Severe (Grade 3).

If the severity rating for an ongoing AE changes before the event resolves, the AE will not be reported a second time. Instead the original AE report will be revised. For purposes of data capture the highest severity rating during the course of a single AE will be the severity rating entered on the AE CRF. Any AE with missing severity will be classed as severe.

11.7.2 Causality

For AEs, the Investigator will assess the causal relationship between the IMP and the AE using his/her clinical expertise and judgement. Causality will be recorded as probable, possible, unlikely or not related.

AEs with a causality reported as probable or possible will be considered related to the IMP. AEs with missing causality assessment will be regarded as related unless further specified. All other AEs will be considered as not related to IMP.

11.7.3 Medically Attended Adverse Events

All AEs where subjects are seeking medical care (i.e. doctor's office, emergency service, hospital, but not including use of self-medication). This will be identified by the Investigator and recorded on the CRF.

11.7.4 Serious Adverse Events

An SAE is defined as any untoward medical occurrence that at any dose meets one or more of the following criteria:

- Outcome is fatal/results in death (including fetal death);
- Is life-threatening – defined as an event in which the subject was, in the judgment of the Investigator, at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe;
- Requires inpatient hospitalization or results in prolongation of an existing hospitalization;
- Results in persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions);
- Results in congenital anomaly/birth defect;
- Is a medically important condition – a medical event that may not be immediately life-threatening or result in death or require hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the definitions above. This definition also applies to progression of disease leading to a serious outcome.

All criteria leading to an AE being classified as an SAE will be recorded on the eCRF. AEs graded as potentially life-threatening (Grade 4) as per FDA Guidance on Toxicity Grading Scales will be reported as SAEs and reported as Severe (Grade 3).

11.7.5 Adverse Events of Special Interest

An AESI is an event of scientific and medical concern specific to the Sponsor's product. In addition to nonspecific transient muscle pain and joint pain which may occur after any vaccination, the AESI for VLA1553 include signs and symptoms suggesting an acute stage CHIKV-associated event.

The following cluster of symptoms suggestive of CHIKV infection with or without remissions or exacerbations will receive particular consideration:

1. Fever (≥ 38.0 °C / 100.4 °F measured orally);

AND

2. Acute (poly)arthralgia/arthritis most frequently in the extremities (wrists, ankles and phalanges, often symmetric), back pain and/or neurological symptoms (e.g. confusion, optic neuritis, meningoencephalitis or polyneuropathy) and/or cardiac symptoms (e.g. myocarditis);

   OR

   One or more of the following signs and symptoms: macular to maculopapular rash (sometimes with cutaneous pruritus (foot plant[1]) and edema of the face and extremities), polyadenopathies;

AND

3. Onset of symptoms 2 to 21 days after vaccination;

AND

4. Duration of event ≥3 days.

Any suspected clinical case of CHIKV-associated event shall be referred to a clinical expert, be evaluated according to standard diagnostic procedures and treated according to current medical standard until resolved or stabilized.

All AESI will be identified by Investigator assessment, using the symptoms listed above as a guideline, and will recorded on the CRF. AESI are only captured from 2 to 21 days post-vaccination. Only those AEs identified as AESIs on the CRF will be included in the analysis of AESIs.

All AESIs will be adjudicated by the DSMB to see if the board agree with the investigator decision. Adjudication will be a Y/N flag from the DSMB for each AESI case. This will be added into the database and used for additional reporting.

Additionally, subjects presenting with acute arthralgia within 2 to 21 days post-vaccination will be followed-up until resolution and monitored for recurrences until the end of the study.

11.7.6 Solicited Adverse Events

Solicited AEs are defined only in the first 10 days post-vaccination (until study Day 11). All solicited AEs will be reported by the subject in the Subject eDiary and will be recorded on the AE page of the CRF. The same information on severity and causality will be collected for these events as for the unsolicited AEs and they will be coded in the same way. Only those solicited AEs recorded as such on the AE page of the CRF will be included in the main analysis of solicited AEs.

Any solicited adverse events which are reported on the AE page of the CRF but not reported on the eDiary are flagged as recall events. This flag is used in a sensitivity analysis of the solicited AEs.

11.7.6.1 Injection Site Adverse Events

Solicited injection site AEs include injection site pain, tenderness, erythema/redness and induration/swelling. The severity for these AEs will be rated based on the FDA Guidance on Toxicity Grading Scales as described in Table 17.2-1 of the Protocol. Any grade 4 injection site AE should be reported as an SAE and will be reported as Severe (Grade 3) (see Section 11.7.4).

[1] The update regarding the anatomical location of cutaneous pruritus is included to provide further clarification. This information is not included in the current Protocol Version 3.0. However, this definition will be included in all subsequent CSPs.

11.7.6.2 Systemic Adverse Events

Systemic AEs include fever, nausea/vomiting, headache, fatigue, myalgia (muscle pain), arthralgia (joint pain) and rash will be reported in a standardized manner over a period of 10 consecutive days after vaccination.

Severity for systemic AEs will be rated based on the FDA Guidance on Toxicity Grading Scales as described in Table 17.2-2 of the Protocol. Any grade 4 systemic AE should be reported as an SAE and will be reported as Severe (Grade 3) (see Section 11.7.4).

11.7.7 Duration of Adverse Events

Duration of AEs is calculated as (End date – Onset Date) + 1.

If end date for AE is missing, then for the calculation of the duration the date of study completion/early termination/LTFU will be used.

If end date for SAE is missing, then for calculation of duration the date of overall study completion (date last subject had their end of study visit) will be used.

11.7.8 Adverse Events at end of study

Only SAE information should be collected after the end of study for a subject. If a subject has an updated record for an AE that is not an SAE after end of study their AE details will be set to the status that would have been present at end of study.

11.8 Missing Data

All statistical analyses will generally be based on observed values, missing values will not be imputed. Missing severity and causality will be handled as described in Section 11.7.1 and Section 11.7.2 respectively.

In case of > 5% of missing values for the primary immunogenicity analysis, a separate sensitivity analysis will be performed where multiple imputation methods will be applied in order to evaluate the possible impact of missing values on these results.

11.9 Handling of Missing or Incomplete Dates

Missing or partial dates will not be imputed, except to determine the timing of AEs or concomitant medications in relation to VLA1553 dosing.

Untoward medical occurrences with missing or partial start dates will be considered AEs unless the partial start date indicates that the event began prior to vaccination with VLA1553, e.g. if the month and/or year are before the month of Visit 1.

Medications with incomplete end dates will only be considered prior if the partial end date indicates that the medication was stopped prior to dosing, e.g. if the month and/or year are before the month of Visit 1. All other medications with missing or incomplete end dates will be considered concomitant.

12.0 Analyses Part A and Part B

No formal interim analysis is planned before completing the part A of the study after day 29.

Nevertheless, data analyses will be provided sequentially by study parts (specified in Table 9-1) and a safety monitoring will be conducted by DSMB as described below.

12.1 Planned Analysis Timepoints

The data analyses will be performed by study parts:

- **Part A analysis** will be performed when all subjects have completed Visit 3 (Day 29) and will comprise immunogenicity and safety data collected to this timepoint.
- **Part A&B analysis** will be performed when all subjects have completed Visit 5 (Month 6) and will comprise all immunogenicity and safety data collected.

The study parts will be analyzed sequentially. Part A will be unblinded once the final subject has completed Visit 3 and database freeze for Part A has occurred (blind will be maintained for study sites). The final Part A&B report will be submitted for licensure.

The analyses to be done at each study part are specified within each section of the statistical methods part of this SAP.

For the Part A analysis, data up to and including Visit 3 (Day 29) or ET if earlier will be kept for all subjects, for the Part B analysis all data will be kept for all subjects. All AEs and concomitant medications with a start date up to and including the date of the cutoff visit for each subject will be included in the analysis. Any record with a start date prior to the cutoff which has a stop date recorded after the cutoff visit for the analysis will be classed as ongoing for the relevant study part.

12.2 Data Safety Monitoring Board

As specified in Section 17.11.1 of the Protocol, an independent Data Safety Monitoring Board (DSMB) will be utilized for this study to address any safety concerns arising during the conduct of the study. The DSMB will review cases of SAEs, AESIs and severe (Grade 3) solicited AEs and provide independent monitoring of safety issues in the subject's interest. In this function the DSMB may mandate changes to the protocol, suggest a protocol amendment and gives recommendations to the sponsor as to whether the study should progress unchanged, or the study requires changes, or the study should be terminated prematurely as the specified in the DSMB charter.

A DSMB charter including a detailed description has been prepared. A separate DSMB table and listing shell document has also been created which will specify the outputs to be produced for review at these meetings.

13.0 Statistical Methods

Unless otherwise noted, categorical variables will be summarized using counts and percentages. Percentages will be rounded to one decimal place, except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts.

Continuous variables will generally be summarized using the number of observations (n), mean, Standard Deviation (SD), median, 25th quartile (Q1), 75th quartile (Q3), minimum and maximum. Summaries of CHIKV-specific NT values will present the number of observations (n), GMT, GSD, median, minimum and maximum. The minimum and maximum values will be displayed to the same level of precision as the raw data, the mean, GSD, median, Q1, and Q3 to a further decimal place, and the SD and GSD to two additional decimal places.

Where relevant, estimates will be presented with 95% two-sided CIs.

All statistical analysis will generally be based on observed values, missing values will not be imputed. In case of >5% of missing values for the primary immunogenicity analysis, a separate sensitivity analysis will be performed where multiple imputation methods will be applied in order to evaluate the possible impact of missing values on these results. Further details are given in Section 13.4.3.3

For the analyses of each study part, all available data will be included, regardless of the subject status during that part of the study. For example, if a subject discontinued the study in part A, they would still be included in any summaries of Part A timepoints in Part B.

Subjects will be always analyzed according to the study arm they have been randomized to. The last column in corresponding summary tables will contain the column TOTAL of all three treatment arms.

Unless otherwise specified, all data collected during the trial will be presented in the subject data listings.

All analyses will use SAS version 9.4 or higher.

13.1 Subject Disposition

The number and percentage of subjects screened, randomized and vaccinated in the study will be presented, together with the number and percentage of subjects who withdrew from the study prematurely during each part and a breakdown of the corresponding reasons for early termination and discontinuation.

Tabulations of the number and percentage of subjects included in each analysis set will be provided. Reasons for exclusion from each analysis set will not be tabulated, but will be listed.

The number and percentage of subjects in the study at each timepoint will also be presented.

A tabulation of the number and percentage of subjects randomized at each center will be presented.

13.2 Demographic and Baseline Characteristics

Demographic characteristics to be summarized will include gender, ethnicity, race, age at screening (years), height (cm), weight (kg) and body mass index (BMI) (kg/m^2).

Medical history will be summarized by system organ class (SOC) and PT using the current version of MedDRA.

A separate summary of vaccination history at baseline will be provided.

All demographic and baseline summaries will be provided on the Safety analysis set. Additionally the summary of demographic characteristics will be presented for the PP analysis set. All demographic and baseline data will be listed.

13.3 Prior and Concomitant Medications

Prior and concomitant medications, categorized by ATC level 2 and preferred term (PT) according to WHO Drug (Version B3 Sep2020 or later), will be summarized separately. The number and percentage of subjects using each medication will be displayed together with the number and percentage of subjects using at least one medication within each medication group and subgroup. Each concomitant medication will be coded to a single ATC code, taking into account the indication for the use of the medication as documented on the CRF.

The summary of prior medications will include any medication with a stop date prior to vaccination (Day 1). Prior medications with a stop date more than 14 days prior to vaccination will not be included in the summary table, but will be listed. Concomitant medications are those with a start or end date on or after date of vaccination.

The concomitant medication summaries for each study part will be cumulative, so the summary for each part of the study will contain any concomitant medications taken during previous study parts.

13.4 Immunogenicity Analyses

13.4.1 Hypothesis Testing Strategy and Multiplicity

A formal hypothesis test is defined for the primary immunogenicity analysis, using a two-sided significance level of 5%. There will be adjustment for multiplicity for primary immunogenicity endpoints if statistical significance (p-value ≤ 0.05 for any of the comparisons between the 3 Lots) is reached. To adjust for multiplicity in statistical comparison between the 3 Lots, the PDIFF=ANOM and ADJUST=NELSON option in PROC GLM will be used which analyzes all differences with the average least squares mean difference among the Lots. ANOM tests whether the treatment means differ from the overall mean (also called the grand mean); and if the least squares means are correlated, the ADJUST=NELSON option in PROC GLM

uses the factor-analytic covariance approximation described in Hsu (1992) for the underlying quantile calculation.

13.4.2 Primary Immunogenicity Analysis

The primary immunogenicity analysis will be a comparison of the GMTs in the PP analysis set between the VLA1553 Lots 1, 2 and 3 at Day 29 (i.e. 28 days postvaccination) by ANOVA, using the factor Lot as a fixed effect and study center as a covariate. The ANOVA model will be applied to the log-transformed (natural log base e) µPRNT values of the three Lots and the 95% CIs for the pair-wise ratios of the GMTs will be obtained by taking the anti-log of the resulting 95% CIs for the least square means differences. If these three pairwise 95% CIs for GMT ratios are all between [redacted] equivalence between the three commercial batches will be postulated.

The analysis will be performed on the PP analysis set and will be repeated on the Full analysis set.

For the PP analysis set there will be also produced the following graphical outputs:

- a line plot of the GMT at each study timepoint and treatment arm;
- a reverse cumulative distribution plot of the proportion of subjects by treatment arm.

13.4.3 Secondary Immunogenicity Analyses

The secondary immunogenicity analyses will be performed on the PP analysis set and repeated on the Full analysis set.

13.4.3.1 Comparisons of Geometric Means for Titer and Fold Increase

Secondary immunogenicity analyses will include the comparison of the GMTs between the three Lots at Day 1, Day 8, Day 85 and Month 6 post-vaccination by ANOVA using the factor Lot as a fixed effect and study center as a covariate, two-sided 95% confidence intervals will be calculated for the GMT. Day 1 values will be summarized to display values at baseline.

Values of geometric mean fold increase (GMFI) will be analyzed in the same way as for GMTs.

13.4.3.2 Seroprotection Rate

The number and percentage of subjects meeting the criteria for seroprotection will be presented for each study timepoint and treatment group. The denominator for the percentage will be the number of baseline negative µPRNT subjects with non-missing NT values at each timepoint. Two-sided exact (Clopper-Pearson) 95% CIs for the seroprotection rate (SPR) will be presented.

The treatment groups will be compared using Fisher's Exact test. In addition, the pairwise difference in risk between the three treatment groups, and exact two-sided 95% CIs for the pairwise differences in risk between treatment groups will be presented.

This summary will be repeated by titer threshold, evaluating the number of baseline negative subjects meeting the criteria $\mu PRNT_{50} \geq x$, where x takes the values [redacted]

Bar charts of the percentage of subjects meeting the seroprotective criteria will be produced by study visit and treatment group.

13.4.3.3 Seroconversion Rate

The seroconversion rate (SCR) will be summarized similarly to the SPR, but will only be presented on Days 29 and 180.

13.4.3.4 Fold-Increase in Neutralizing Antibody Titer

The fold-increase will be summarized as a continuous endpoint in descriptive statistics. In addition, the number of subjects reaching pre-specified fold-increase categories of at least 4, 8, 16 and 64-fold increases compared to baseline will be summarized as the number and percentage of subjects in each category

13.4.4 [redacted]

13.4.4.1 [redacted]

13.4.4.2 [redacted]

13.4.4.3 [redacted]

13.4.4.4 [redacted]

13.5 Safety Analyses

Descriptive statistics will be used in all safety analyses, unless otherwise specified.

13.5.1 Adverse Events

Safety tabulations will generally be provided separately for solicited AEs and unsolicited AEs, and for both types of AEs combined. Number and proportion of subjects, plus number of events in each category will generally be presented. 95% confidence intervals will be provided for all AEs, SOC and PT. Differences between the three Lots will be assessed for significance using Fisher's exact test.

Summaries of AEs categorized by SOC and PT coded according to the MedDRA dictionary will be produced. Within these summaries counting will be by subject not event and subjects are only counted once within each SOC or PT.

Where AEs are presented by severity (Mild, Moderate, Severe), SOC and PT, subjects with multiple events within a particular body system or preferred term will be counted once under the category of their most severe event within that SOC or PT.

In summaries of AEs which are categorized by relationship to IMP, SOC and PT, AEs with a causality reported as probable or possible will be considered related to the IMP. Subjects with multiple events within a particular SOC or PT will be counted under the category of their most drug-related event within that SOC or PT.

All AE tabulations will be presented for the Safety analysis set.

All AEs recorded on the CRF will be listed.

Furthermore, the following tabulations of AEs will be produced:

- All unsolicited AEs up to study Day 29 will be presented by SOC and PT, as well as summaries by SOC, PT and severity. Treatment groups will be compared using Fisher's exact test (Part A);
- Solicited injection site and systemic AEs within 10 days post-vaccination by PT (Part A);
- Solicited injection site and systemic AEs within 10 days post-vaccination by PT and severity (Part A);
- All AEs during the entire study period by SOC and PT (Parts A, B);
- All AEs during the entire study period by SOC, PT and severity (Parts A, B);
- All Related AEs during the entire study period by SOC and PT (Parts A, B);
- SAEs by SOC and PT during entire reporting period (Parts A, B);
- Related SAEs by SOC and PT during entire reporting period (Parts A, B);
- AESI within 2 to 21 days post-vaccination by SOC and PT (Part A, B);
- AESI for ongoing events from Part A (Part B);
- Related AESI within 2 to 21 days post-vaccination by SOC and PT (Part A, B);

In addition, the following tables will also be presented for the specified study part:

- AE summary table showing the overall number and percentage of subjects with any AE, any related AE, any severe AE, any related severe AE, any solicited AE, any severe solicited AE, any related solicited AE, any related severe solicited AE, any solicited injection site AE, any severe solicited injection site AE, any solicited systemic AE, any severe solicited systemic AE, any unsolicited AE, any related unsolicited AE, any severe unsolicited AE, any related severe unsolicited AE, any SAEs, any related SAEs, any related solicited SAE, any related unsolicited SAE, any AESI as assessed by the investigator, any AESI as assessed by the DSMB, any related AESI as assessed by the investigator, any medically attended AE, any related medically attended AE, any related medically attended solicited AE, any related medically attended unsolicited AE, any AE leading to withdrawal from the study, any unsolicited AE leading to withdrawal from the study and any solicited AE leading to withdrawal from the study. For each study arm 95% CIs will be presented for percentages. Differences between the three Lots will be assessed for significance using Fisher's exact test (Parts A, B);
- AE summary table as above, split by whether the subject was ELISA seropositive or seronegative at baseline (Parts A, B);

- AE summary table as above, split by whether the subject was μPRNT baseline seropositive (defined as [redacted] compared to baseline ELISA seropositive subjects
- Unsolicited related AEs up to Day 29 by SOC and PT (Part A);
- Unsolicited related AEs up to Day 29 by SOC, PT and maximum severity (Part A);
- Solicited injection site and systemic AEs within 10 days post-vaccination by PT excluding recall data (Part A);
- Solicited related injection site and systemic AEs (Part A);
- Solicited related injection site and systemic AEs by maximum severity (Part A);
- AESI by SOC, PT and maximum severity (Part A, B);
- Related AESI by SOC, PT and maximum severity (Part A, B);
- Solicited local and systemic AEs tabulated by subject diary day up to Day 11. This tabulation will be based on onset day of the AE. If not possible to assign a day due to partial onset date then it will be assumed to be Day 1 (Part A);
- Summary of duration of solicited local and systemic AEs. Summary statistics for duration of solicited AE (days) will be presented by symptom in each study arm (Part A);
- Maximum fever temperature post-vaccination up to Day 11, for subjects who experienced fever (Part A);
- Unsolicited AEs by SOC and PT (Parts A, B);
- Unsolicited related AEs by SOC, PT (Parts A, B);
- Any unsolicited related severe AE by SOC and PT (Parts A, B);
- Any AE occurring at a frequency of at least 10% in at least one study arm by PT (Parts A, B);
- Any AE occurring at a frequency of at least 1% in at least one study arm by PT (Parts A, B);
- Any related AE occurring at a frequency of at least 10% in at least one study arm by PT (Parts A, B);
- Any related AE occurring at a frequency of at least 1% in at least one study arm by PT (Parts A, B);
- Any medically attended AE by SOC, PT (Parts A, B);
- Any medically attended AE by SOC, PT and Maximum Severity (Parts A, B);
- Any AE leading to withdrawal from study by SOC and PT (Parts A, B);

AE rates will be plotted in forest plots by treatment group for all AEs, severe (related) AEs, related AEs, SAEs and AESIs. Radar plots will be produced for subjects with solicited AEs, displaying the maximum severity of any AEs within each of the categories of solicited AE.

Subject diary data will be listed.

13.5.2 Laboratory Data

Safety laboratory values will be assessed for all subjects at baseline and all subsequent visits from all subjects for standard clinical chemistry, hematology, coagulation panel and urinalysis. HIV / HBsAg / HCV testing will be provided only at screening.

A CHIKV baseline screening sample (5.0 mL) will be obtained from all subjects at Visit 0 (Screening Visit) for CHIKV-specific ELISA testing for baseline serostatus. Visit 1 samples will be included in reporting if no Visit 0 sample was collected.

[redacted]

13.5.3 Vital Signs

Vital signs will be summarized descriptively at each study timepoint they are collected, including screening and at the vaccination visit. Change from baseline values will be summarized for the post-vaccination timepoint. Vital signs parameters to be summarized include systolic blood pressure (mmHg), diastolic blood pressure (mmHg), pulse rate (bpm) and body temperature (°C).

All vital sign data will be included in a by-subject listing.

13.5.4 Physical Examination

Baseline physical examination will be performed on the following body systems being described as normal or abnormal: general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological.

A symptom-driven physical examination will be performed at all study visits except the screening, i.e. only in case a symptom is reported by the subject, a system-based assessment will be performed for a detailed check of the affected body system(s). A symptom-driven examination should also be performed in case the subject has complaints within the observation time after vaccination. A Hand Stiffness examination will be performed at all study visits irrespective of any clinical signs or symptoms.

Summary tables of symptom-driven examination and hand stiffness results by timepoint and change from baseline by timepoint will be presented.

All physical examination and hand stiffness data will be listed. Physical examination findings will be flagged for clinical significance in the listing.

14.0 References

[1] Food and Drug Administration, C. f. B. E. a. R. "Guidance for Industry: Toxicity Grading Scales for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials".

[2] Hsu, J. C. (1992). "The Factor Analytic Approach to Simultaneous Inference in the General Linear Model." Journal of Computational and Graphical Statistics 1:151–168.

15.0 Glossary of Abbreviations

Abbreviations	*Description*
AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomic Therapeutic Classification
BMI	Body Mass Index
CI	Confidence Interval
CHIKV	Chikungunya Virus
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
ET	Early Termination
FAS	Full Analysis Set
GMT	Geometric Mean Titer
GMFI	Geometric Mean Fold Increase
GSD	Geometric Standard Deviation
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
i.m.	Intra - muscular
IMM	Immunogenicity
IMP	Investigational Medicinal Product
IXRS	Interactive Voice/Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
NT	Neutralizing Titer
PP	Per Protocol
PT	Preferred Term
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SCR	Seroconversion Rate
SD	Standard Deviation
SOC	System Organ Class

Abbreviations	*Description*
SPR	Seroprotection Rate
WHODrug	World Health Organization Drug Dictionary
µPRNT	Micro Plaque Reduction Neutralization Test