Statistical Analysis Plan (SAP)

Protocol Title:	A MULTICENTER, RANDOMIZED, CONTROLLED, DOUBLE-BLINDED PIVOTAL STUDY TO EVALUATE SAFETY AND IMMUNOGENICITY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADOLESCENTS AGED 12 YEARS TO <18 YEARS
Protocol Version No./Date:	6.0/08Nov2022
CRF Version No./Date:	6.0/14Nov2023
SAP Version No./Date:	3.0/19Jul2024

1.0 Approvals

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(NOTE: Electronic Signatures should only be used if all parties have the ability to eSign.)

2.0 Change History

Version/Date	Change Log
0.1/20Jul2021	Created as new (First Draft).
0.2/30Jul2021	Minor updates for submission of draft to regulatory authorities.
0.3/12Oct2021	Text clarified throughout.
0.4/19Jan2022	Updated wording throughout to match Protocol Version 3: Definition on Seroconversion to >4-fold increase for all subjects; baseline positive serostatus updated from >20 to >40.
0.5/18Mar2022	Updated seroconversion definition and added to section 6.1 Changes from Protocol Aligned wording to Day 180 (Visit 5) from Month 6 (Visit 5). Added Day 29 to the 1st bullet point under safety endpoints. Clarification using $\mu PRNT_{50}$ for statistical analysis instead of CHIKV ELISA Updated NT result/value to $\mu PRNT_{50}$. Added study part (part A and part B) under safety endpoints for section 9.4. Added GMFI definition to section 11.3.4 Fold-Increase in Neutralizing Antibody Titer and 14.6.4.4 Secondary Immunogenicity Endpoints. Updated seroconversion definition. Added new section for GMFI under 9.4 Estimand Attributes. Added part A to Late Onset of AESI. Added viremia subset section under Analysis Population section.
0.6/15Jul2022	Delete contents of Section 6.1 Changes from Protocol. Updated descriptions of Section 8.0 Study Design and added the inclusion of sentinel subjects in IMM subgroup. Updated and added viremia subset to Section 10.0 Analysis Population section. Clarified analysis populations and subgroups. Added section 11.6 for handling of laboratory results. Align throughout the document to “µPRNT baseline seronegative/ seropositive”.
0.7/13Dec2022	Changes to section 11.6 Handling of Laboratory Records due to the change from central to local laboratory pages for safety lab collection. Also included the DSMB decision to remove ESR collection. Update to CHIKV case reporting from 14 days to entire study period. Also noted as change from protocol endpoint. Addition of Cox regression for time to CHIKV cases in section 14.7.1. Addition of overall AESI summary to the overall adverse event table. Study design section updated to cover the addition of 3 additional subjects to the Cohort II sentinel group, to a total of 33. Seroprotection updated to Seroresponse throughout SAP, and noted in change from Protocol section. Wording clarified throughout.
0.8/08Feb2023	Aligned with Protocol Version 6 – AESI definition clarified, confirmatory SARS-CoV-2-PCR requirement removed, laboratory testing procedures clarified. Diary-only solicited events added into a sensitivity reporting output.

0.9/15Mar2023	Added details on split delivery scenarios for Part A safety reporting, including potential interim analysis. Adverse event end date imputation rules updated to add a second imputation version for sensitivity. Updated version numbering, down-versioning prior versions as not yet approved. Version change impacts 13Dec2022 and 08Feb2023 versions.
0.10/24Mar2023	Wording clarified surrounding the Part A safety ELISA deliverable and interim analysis, including additional steps for minimizing bias. Addition of 1 adverse event output for non-serious AEs with incidence >=5% in a single study arm, and minor update to the summary AE table. Minor wording changes within the incidence of CHIKV infections section.
0.11/17Jul2023	Section for interim analysis for ELISA safety deliverable removed, as this is done on the same data cut as the full Part A analysis. Visit windowing rules updated in section 11.2 so all visits are assigned a windowed visit. Clarifications of PP analysis set and handling of missing samples at Part A. Output for Diary compliance added. Wording updated for clarity throughout. Immunogenicity Analyses section updated to provide further clarity to the modelling including the additional conditional analyses specified. An additional supplemental analysis has also been added for the primary endpoint looking at subjects in respect to their baseline ELISA result. Details added to PD section in respect to borderline or negative Ig subjects, temperature excursions and to clarify major classification is relevant for IMM subjects only. A modified PP population has been added to the SAP to produce a supplemental analysis excluding the borderline Ig subjects.
1.0/21Jul2023	Minor wording updates throughout for clarity, particularly focusing on the handling of ELISA Ig indeterminate cases. Up-versioned for signatures.
2.0/26Feb2024	Minor revisions throughout for clarity. **Section 11.4.5 Adverse Event of Special Interest**: Clarified that the analysis of adverse event of special interest (AESI) by early/late onset will be based on assigning the AESI cluster to either an early or late onset based on the earliest symptom(s) (i.e. assessment of the investigator will not be used) as it is more important to assess the timing of the cluster AESI instead of each of the individual symptoms separately. **Section 11.7 Missing Data:** a sentence added to clarify that the analysis was not performed as the number of missing data was < 5%. **Section 12.1 Planned Analysis Timepoints:** Clarified that some tables initially planned to be delivered for only Part A, will be delivered at Part B due to data changes post Part A analysis. **Section 13.6.3.1 Pooling of Sites:** added a note to clarify the analysis is an ad-hoc analysis and it was required at Part A. **Section 13.6.4.1 Comparison of Geometric Mean Titer**: • Added clarification that in case of non-convergence of the sensitivity analysis model the interaction between the study site and study arm may be dropped from the analysis of covariance (ANCOVA) model. • Added clarifications that the normality assumption is not expected to be met for the ANOVA and ANCOVA models at Day 8, but no alternative modeling will be applied unless the normality is also not met at Day 29.

	• Provided a description of the reverse cumulative distribution plots by visits, treatment groups and baseline µPRNT serostatus strata as was previously not included but produced. **Section 13.7.1 Incidence of CHIKV Infections:** • Added details on how the CHIKV case ascertainment will be performed. • Clarified the reporting for Part B and Part C requirements. **Section 13.8.1.1 Secondary Adverse Event Analyses:** Summaries for related late-onset AESI by System Organ Class (SOC) and Preferred Term (PT) has been added for completeness. **Section 13.8.1.2 Other Adverse Event Analyses**: • Summaries for related late-onset AESI by SOC and PT, and by SOC, PT and maximum severity have been added for completeness. • Clarified which summaries planned to be delivered for only Part A, will be delivered at Part B due to data changes post Part A analysis. **Section 13.8.2 Viremia:** • Added details that viremia is also collected at acute and convalescent visits and associated listing will include all the results and will be delivered for each study part reporting. • Added description of figures for solicited fever and arthralgias as was previously not included but produced. **Section 13.8.3 Laboratory Data:** added details on how the free text data will be summarized for urinalysis parameters. **Section 13.8.6 Subject Diary Entry Compliance**: new section added to describe the diary entry compliance tabulation. **Section 15.0 Important Protocol Deviation Identification Listings**: section removed as not performed. Subsequent sections were renumbered.
3.0/19Jul2024	Minor revisions throughout for clarity. **Section 11.4.5.2 AESI according to USPI FDA Broad Definition of Chikungunya-like Adverse Reactions**: New section to include: • Definition for AESI by the United States Prescribing Information (USPI) Food and Drug Administration (FDA) Broad Definition of Chikungunya-like Adverse Reactions and definitions for cases and symptoms of such AESIs. • Content from section 11.4.5 Adverse Events of Special Interest has been moved into (new) subsection 11.4.5.1 Protocol Definition. **Section 11.4.7 Duration of AEs:** Rules for handling partial stop dates have been added. **Section 13.3 Prior and Concomitant Medication:** Added clarification about the summary of Traveller Vaccination History and removed details from section 13.4 Demographics and Baseline Characteristics. **Section 13.6.4 Secondary Immunogenicity Endpoints:** For Part C reporting, additional analysis of seroresponse rate (SRR) and seroconversion rate (SCR) by age group have been included. **Section 13.6.4.1 Comparison of Geometric Mean titer:** added new analysis for Part C only for the comparison of the Geometric Mean of Titers (GMT) between Baseline µPRNT seronegative and Baseline µPRNT seropositive groups in the VLA1553 treatment arm. **Section 13.7.1 Incidence of CHIKV infections:** • Removal of timepoint analyses for CHIKV infections. • Removal of time-to-event analysis

	• Added summary and analyses of the categories “All probable CHIKV cases” and “All asymptomatic CHIKV cases”. • Added sentence to describe that data will be also listed. **Section 13.8.1.2 Adverse Events by Age Group Analyses:** New section added to describe the following Part C analysis and subsequent subsections renumbered accordingly. • Overview of AEs by age group • Additional inferential analysis for the solicited adverse events. **Section 13.8.1.3 USPI FDA-Broad Definition of Chikungunya-like Adverse Reactions Analyses:** • New section to describe summary of cases and symptoms of USPI FDA-Chikungunya-like Adverse Reactions. • Subsequent sections have been renumbered accordingly. **Section 13.8.1.4 Other Adverse Event Analyses:** • Following frequency count and percentage has been added to match the associated table shell: any AESI symptom as assessed by the investigator, any early onset AESI symptom as assessed by the investigator and any late onset AESI symptom as assessed by the investigator. • For Part C, AE overall summary for the VLA1553 arm, split by baseline serostatus has been added. • Arthralgia and solicited fever bar charts category changed from ‘>31 days’ to ’31-35 days’. **Section 13.8.2 Viremia:** • Section updated to clarify under which condition the Visit 3 results are summarized. Added sentence for “Not Detected” viremia results being taken as 0 GCE/ml for viremia plots. • Details added on the sequencing of the viremia samples and that data will be listed only

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 VLA1553-321.

5.0 Scope

The Statistical Analysis Plan outlines the following:

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

This SAP covers the analysis for Part A (Visit 3, Day 29), Part B (Visit 5, Day 180) and Part C (Visit 6, Month 12) 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.

The SAP v1.0 was approved prior to database lock and unblinding for the Part A analysis. Revisions to the SAP have been implemented to provide clarifications for the analysis in Part B and Part C, and to include descriptions of ad-hoc tables required to support the interpretation of the safety data.

6.1 Changes from Protocol

Wording was clarified to specify that all subjects in sentinel cohorts will be included in the immunogenicity subgroup, in order to collect full safety samples for these subjects. Updates were also made to indicate that 3 additional subjects were added to the sentinel cohort II due to missing data for some originally identified sentinel subjects. This was added to sections 8.0 and 8.2.

Wording for seroprotection has been updated throughout in the SAP to seroresponse. This is based on a change of terminology to be applied across all studies in the VLA1553 program, and implemented here for consistency across studies.

The protocol states that confidence intervals (CIs) for adverse event rates should be calculated according to Altman (ie. Wilson score intervals), however within this SAP exact Clopper-Pearson CIs are planned throughout for all analyses. This change is for the conservative control of low counts expected across the reporting, as well as for consistency with other VLA1553 study analyses.

Additional wording has been added for a split delivery for safety outputs based on Enzyme-Linked Immunosorbent Assay (ELISA) stratification, termed the preliminary safety summary which is not stated in the protocol. This is outlined in section 12.2.

In section 13.6.4.1 the wording for the analysis of covariance (ANCOVA) models has been updated slightly from the protocol for clarity. In the SAP, the baseline serostatus is classed as a fixed factor in the model, as it is a categorical variable. The protocol however uses the term covariate for this.

The protocol mentions that immunogenicity analysis will be displayed by treatment group and a model with factors treatment group and baseline Micro Plaque Reduction Neutralization Test (µPRNT) serostatus will be used. The analysis will instead be displayed by serostatus with treatment group within the serostatus and as such a model in these groups will not include baseline µPRNT serostatus and only the overall column of each output will utilize baseline µPRNT serostatus in its model.

To comply with International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH) guidelines to use the randomization strata in modelling, an additional supplemental analysis of primary endpoint using the baseline ELISA serostatus (the randomization serostatus) is added.

The sensitivity analysis mentioned in the protocol in regard to analysis of variance (ANOVAs) with factors study site, treatment group, study site*treatment group, baseline µPRNT serostatus will not include baseline µPRNT serostatus as multiple factors of site and serostatus will lead to non-convergence in the model due to small number of subjects in some sites.

The protocol mentions that analysis associated with the Chikungunya virus (CHIKV) cases will be revisited at the blinded data review meeting (BDRM). However, due to the requirement that certain unblinded information for CHIKV case classification is required (e.g. the confirmatory Quantitative reverse transcription polymerase chain reaction (RT-qPCR) for CHIKV case, µPRNT values/seroconversion status, as well as the clinical symptoms) this cannot be assessed at the time of the BDRM. Final Categorization of potential CHIKV infections and CHIKV analyses will be performed at Part C. Preliminary CHIKV case ascertainment and classification has been performed for Part B by the Sponsor and discussed with an independent Data Safety Monitoring Board (DSMB). Findings of this intermediate assessment have been presented in the Part B Clinical Study Report (CSR).

Secondary endpoints mention Day 1 being displayed also for fold increases. As an increase is calculated from Day 1 result this is not appropriate. This has been clarified in the relevant section.

An additional supplemental analysis based on a new analysis population are defined within this SAP to exclude subjects who are Immunoglobulin M (IgM) indeterminate and Immunoglobulin G (IgG) negative by ELISA at screening. This case was not clearly described within the Protocol inclusion/exclusion criteria, and so the sensitivity is included to ensure the inclusion of these subjects does not impact the trial results.

7.0 Study Objectives

7.1 Primary Objectives

- To evaluate immunogenicity and safety of the adult dose of the live-attenuated CHIKV vaccine candidate (VLA1553) 28 days following vaccination in adolescents aged 12 years to <18 years after a single immunization.

7.2 Secondary Objectives

- To assess the immunogenicity and safety of the adult dose of VLA1553 following vaccination in adolescents aged 12 years to <18 years after a single immunization up to Month 12.
- To assess the immunogenicity and safety of VLA1553 in subjects previously exposed to chikungunya virus.

7.3 Exploratory Objectives

- To evaluate the efficacy of VLA1553 in adolescents aged 12 years to <18 years after a single immunization.
- To collect information on the CHIKV disease clinical spectrum and associated risk factors in an adolescent population.

8.0 Study Design

This is a multicenter, prospective, randomized, double-blinded, pivotal clinical study evaluating the adult dose (1x10E4 TCID50 per 0.5 mL) of VLA1553 in comparison to a placebo control. VLA1553 and control will be administered as single immunization on Day 1. Overall, 750 male and female subjects aged 12 years to <18 years will be enrolled (i.e. Informed Consent Form (ICF) signed) in the study, stratified by ELISA baseline serostatus: 20% seropositive and 80% seronegative for CHIKV.

Subjects will be randomized in a 2:1 ratio to VLA1553 (n=500) or control group (n= 250). The 750 subjects in this study will be stratified into two strata of subjects seropositive at baseline for CHIKV (Stratum A: overall 150 subjects) and subjects seronegative at baseline for CHIKV (Stratum B: overall 600 subjects). The serostatus at baseline will be determined at screening visit in a central diagnostic laboratory using a qualitative ELISA assay. The ELISA will be used in order to drive the enrollment of 20% seropositive subjects. In addition, the baseline CHIKV serostatus of neutralizing antibodies will be determined for all participants by a validated µPRNT assay. This µPRNT baseline serostatus will be used to stratify participants into seropositive and seronegative subjects for tabulation. Additionally, the validated µPRNT assay will be used for all immunogenicity analyses as baseline and serostatus definitions. Approximately 385 subjects will be randomized to the immunogenicity subset. Thereof, approximately 75 subjects will constitute the viremia subset.

As safety precaution, the study will be initiated with an age de-escalation of sentinel cohorts. Enrollment will start with 30 sentinel subjects from Cohort I (15 to <18 years) that will allow the generation and review of safety data before enrollment of sentinel subjects from Cohort II (12 to <15 years) is initiated. Age de-escalation will only be conducted if the safety profile is considered favorable, as outlined in the protocol in section 12.1. Enrollment will be performed in an age-descending, staggered manner for the two age cohorts. (See Figure 2). For full details on the sentinel subject recruitment see Protocol section 12.1.1. The sentinel subjects of Cohort I (30 subjects) and Cohort II (30 subjects, plus 3 additional subjects due to incomplete safety data) will be allocated to the immunogenicity only subset to allow for full safety evaluation.

Table 8-1 below illustrates the subject distribution scheme.

Table 8-1. Subject Distribution

Study Arm	Study Arm	Stratum (baseline status based on ELISA)	Number of subjects (n)	Immunogenicity (Viremia) Subset (n)
1	**VLA1553**[a]		**500**	**335 (50)**
		A (seropositive)	100	67 (10)
		B (seronegative)	400	268 (40)
2	**Control**		**250**	**50 (25)**
		A (seropositive)	50	10 (5)
		B (seronegative)	200	40 (20)
		Total N:	**750**	**385 (75)**

[a] dose used for Phase 3 trial in adults

All subjects will return to the study site at Day 8 (Visit 2), Day 29 (Visit 3), Month 3 (Day 85, Visit 4) and Month 6 (Day 180, Visit 5) for safety evaluations and immunogenicity sampling. At Day 1 (Visit 1), immunogenicity analysis will be performed for all subjects for stratification of baseline µPRNT serostatus. Thereafter, immunogenicity analysis and evaluations will only be done in the immunogenicity subset. Subjects in the viremia subset will have viremia samples collected at Visits 1, 2 and 3. Samples from Visit 1 and 2 will be analyzed. The collected viremia sample of Visit 3 will only be analyzed if sample of Visit 2 is positive. In addition, for clinically indicated retrospective analysis viremia samples will be collected throughout the study from all subjects. Safety data collection will capture all adverse events (AEs) up to Day 180 (Visit 5). After Day 180 (Visit 5), AE collection will be limited to SAEs.

Subjects from the immunogenicity subset will also return to the study site at Month 12 (Day 365, Visit 6) for collection and assessment of immunogenicity samples. In addition, a clinical sample for safety laboratory evaluations will be obtained at all study visits from the immunogenicity subset only.

The overall study design is displayed in Figure 1 below.

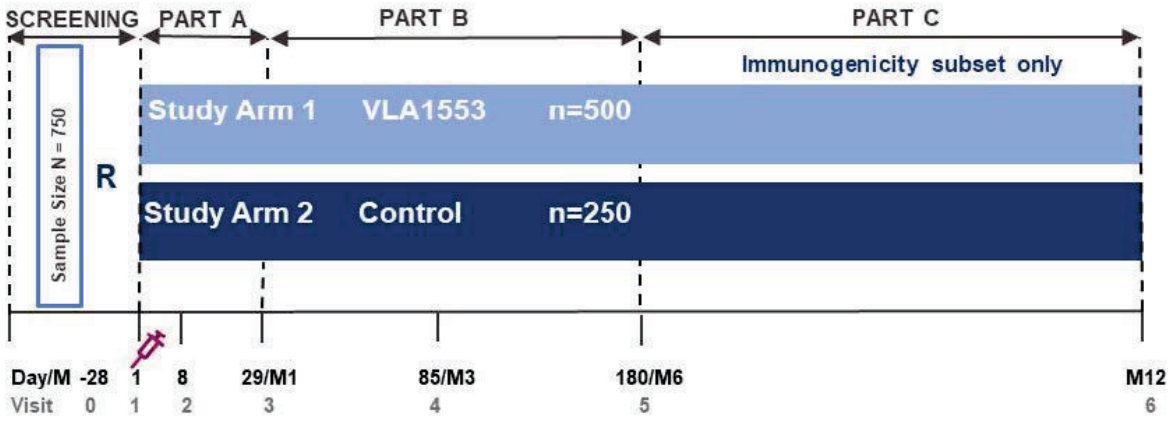

Figure 1 Adolescents Pivotal Clinical Study Design.
M = month

The Enrollment procedure for the two cohorts is shown here in Figure 2.

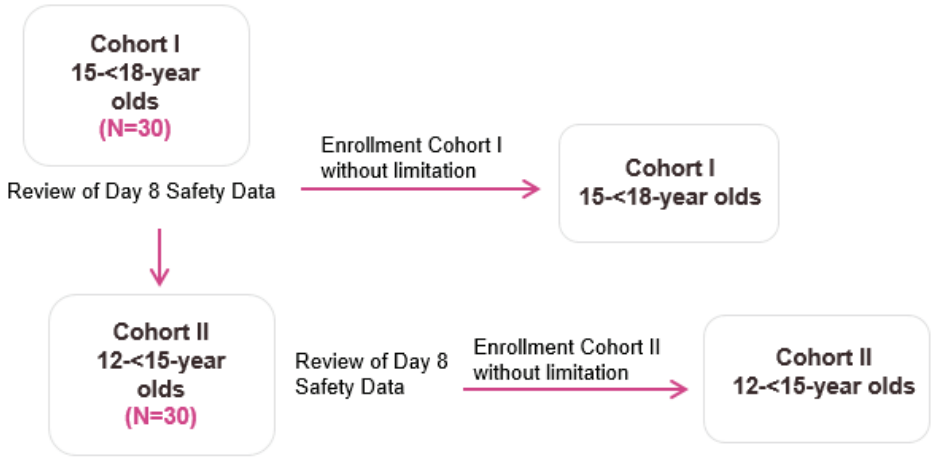

Figure 2 Subject Enrollment Process

The individual subject participation is approximately 7 months or 13 months (immunogenicity subset) from enrollment to study completion unless prematurely discontinued. The screening period will last up to 1 month (28 days) prior to randomization at Visit 1.

The study is split into 3 parts which will be reported separately:

- Part A: Screening to Visit 3 (up to Day 29)
- Part B: Visit 4 to Visit 5 (up to Day 180)
- Part C: Visit 6 (up to Month 12)

8.1 Sample Size Considerations

The total number of 500 subjects exposed to VLA1553 in this study has been selected to provide a sufficient number of subjects for proper safety evaluation in the adolescent's subgroup. With 500 subjects exposed, the study will provide 95% confidence that an AE does not occur at a frequency of 1:166 or 0.6% or higher, if not observed in the study.

The immunogenicity subset of 268 VLA1553-vaccinated ELISA baseline seronegative subjects will allow for sufficient statistical power when applying a one-sided exact binomial test with a significance level of 2.5% against a non-acceptance threshold of 70% on the proportion of subjects with a seroprotective level (defined as $\mu PRNT_{50}$ ≥150 for µPRNT baseline seronegative subjects) at Day 29. A seroresponse rate (SRR) of 80% is assumed, and 200 VLA1553-vaccinated subjects would thus be necessary for a statistical power of 90%. With an expected drop-out/major protocol deviations rate of approximately 10%, at least 223 ELISA baseline seronegative subjects vaccinated with VLA1553 need to be allocated to the immunogenicity subset.

8.2 Randomization

The 750 male and female adolescent subjects will be randomized in a 2:1 ratio to VLA1553 or control group, stratified by baseline serostatus based on ELISA: 20% seropositive and 80% seronegative for CHIKV as described in Protocol Section 12.1. In order to minimize/avoid bias, assignment into one of these study arms will be blinded for the subjects and the site staff performing the safety assessments as well as the biostatistician and study teams (i.e. double-blind). Each subject will have a unique subject screening number obtained via the interactive response technology (IRT) and 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 IRT. At Day 1 (Visit 1, Day of vaccination) eligible subjects will be assigned to VLA1553 or control. Each subject will receive a unique randomization number when he/she is assigned to the study arm. Subjects will be allocated to study arms according to the randomization code. The Investigational Medicinal Product (IMP) will be prepared by unblinded study staff in accordance with the information in the IRT.

30 sentinel subjects of Cohort I and 33 sentinel subjects of Cohort II (3 additional subjects were enrolled due incomplete safety data) are allocated to the Immunogenicity subset to allow safety evaluation, as outlined in the protocol (e.g. safety laboratory sampling, and additional safety follow-up). All other subjects are randomized as outlined above into the subgroup of either No subset, Immunogenicity subset, or Viremia subset, based on their serostatus and treatment arm, according to the subject distribution described in Table 8-1.

8.3 Blinding/Unblinding

The study is conducted in a double-blind manner. Investigators, study staff (apart from those designated to randomize subjects and handle the IMP), study participants, biostatistician (except the unblinded independent reporting statistician involved in the DSMB) and the ICON and Sponsor (Valneva and Butantan) Clinical Safety teams, Clinical Research Associates (CRAs) responsible for monitoring study data, lab staff and all other Sponsor and Clinical Research Organization (CRO) staff will all be blinded to treatment allocation.

At the time of Part A analysis, the study team (Butantan, Valneva and ICON) will be unblinded. For the 'Preliminary Safety Summary' prior to Part A full reporting, relevant ICON staff members will be fully unblinded, but Sponsor teams (Valneva and Butantan) will only be unblinded to overall summaries by treatment level, with no subject-level summaries or listings provided. Sites and subjects will remain blinded until the end of the study, except in emergency cases for the clinical management of an SAE (serious adverse event).

Full details of the handling of unblinding are detailed in the study Unblinding Plan, which was signed prior to the initial Part A data extract and unblinding.

9.0 Study Endpoints, Variables and Covariates

9.1 Primary Endpoint

- Proportion of subjects with a seroprotective CHIKV antibody level defined as $\mu PRNT_{50}$ ≥150 for $\mu PRNT_{50}$ baseline seronegative subjects 28 days post-vaccination.

9.2 Secondary Endpoints

Immunogenicity

- Immune response as measured by CHIKV-specific neutralizing antibody titers on Day 8, Day 29, Day 85, Day 180, and Month 12 post-vaccination as determined by µPRNT assay;
- Proportion of subjects with seroprotective levels (defined as $\mu PRNT_{50}$ ≥150 for µPRNT baseline negative subjects) on Day 8, Day 85, Day 180 and Month 12 post-vaccination as determined by µPRNT assay;
- Proportion of subjects with seroconversion (defined as > 4-fold increase of $\mu PRNT_{50}$ compared to baseline) at Day 29, Day 180 and Month 12 as determined by µPRNT assay;
- Fold increase of CHIKV-specific neutralizing antibody titers determined by µPRNT assay at Days 8, 29, 85, 180 and at Month 12 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;
- Antibody titers, seroresponse and fold increases for CHIKV-specific neutralizing antibodies, determined by µPRNT assay at Days 1 (not fold increase), 8, 29, 85, 180, and Month 12 post-vaccination stratified by $\mu PRNT_{50}$ baseline serostatus.

Safety

- Frequency and severity of unsolicited AEs until Day 29 (Visit 3) and Day 180 (Visit 5) post-vaccination;
- Frequency and severity of solicited injection site and systemic reactions within ten days post-vaccination;
- Frequency and relatedness of any SAE during the entire study period;
- Frequency and severity of any early onset adverse event of special interest (AESI) starting within 2 to 21 days post-vaccination (i.e. Day 3 – Day 22);
- Frequency and severity of any late onset AESI during the entire study starting after 22 days post-vaccination (i.e. Day 23 – study end);
- Assessment of viremia on Days 1 and 8 (and Day 29, if applicable) after vaccination.

9.3 Exploratory Endpoints

- Incidence of CHIKV infections with onset 14 days post-vaccination as evidenced by viremia by virus specific RT-qPCR, clinical diagnosis and seroconversion by $\mu PRNT_{50}$ for the entire study period;
- Accumulate data of CHIKV disease signs and symptoms in adolescent population as assessed following vaccination on Day 1 for the entire study period.

9.4 Estimand Attributes

The below table links the endpoints to the study objectives, and describes the estimands to be used on the study.

Objectives	Estimand or Endpoint	Study Part	Population
Primary	Primary estimand		
• To evaluate immunogenicity and safety of the adult dose of the live-attenuated CHIKV vaccine candidate (VLA1553) 28 days following vaccination in adolescents aged 12 years to <18 years after a single immunization.	• Treatment of Interest: VLA1553 • Population of interest: Subjects in the per protocol (PP) analysis population who receive a vaccination, are μPRNT$_{50}$ baseline seronegative and have a non-missing Day 29 result. • Variable of interest: Seroresponse (μPRNT$_{50}$ ≥150) status at Day 29 • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis. • Population-level summary: Exact binomial test of seroresponse (μPRNT$_{50}$ ≥150) rate against a non-acceptance threshold of 70%.	Part A and Part B	PP
	Supplemental Analysis of Primary Estimand • As Primary, except: • Population of interest: immunogenicity (IMM) analysis population • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis. Multiple imputation (MI) of missing values based on Day 8 μPRNT$_{50}$ values (detailed in section 13.6.3.2). Only performed if >5% of missing Day 29 values for an immunogenicity comparison involving statistical test. Note that this condition was not met and the analysis was not performed.	Part A	IMM
	Supplemental Analysis of Primary Estimand • As Primary, except: • Using the immunogenicity (IMM) analysis population. All randomized and vaccinated subjects of the immunogenicity subset who are μPRNT$_{50}$ baseline seronegative and have evaluable results at baseline and at least one post-baseline titer measurement after vaccination. Subjects will be analyzed according to the study arm they had been allocated to, rather than by the actual treatment they received	Part A	IMM

	• Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are included in the analysis. Sensitivity Analysis of Primary Estimand • As Primary, except: • Population of interest will include subjects in the PP population with $\mu PRNT_{50}$ baseline seronegative subjects and $\mu PRNT_{50}$ baseline seropositive subjects Supplemental Analysis of Primary Estimand • As Primary, except: • Subjects in the PP analysis population who are IRT ELISA seronegative at screening and have a Day 29 result. Supplemental Analysis of Primary Estimand • As Primary, except: • Population of interest: Modified Per Protocol (mPP) analysis population - subjects with IgM indetermined/IgG- IRT ELISA results at screening to be also removed from the PP in addition to the pre-specified PP population. • Handling of Intercurrent Events: Same as Primary.	Part A Part A and Part B Part A and Part B	PP PP mPP
Secondary			
Secondary: Immunogenicity			
• To assess the immunogenicity of the adult dose of VLA1553 following vaccination in adolescents aged 12 years to <18 years after a single immunization up to Month 12. • To assess the immunogenicity of VLA1553 in subjects	Secondary Estimand #1 • Treatment of Interest: VLA1553 • Population of interest: PP population – ie subjects in the immunogenicity subset who receive a vaccination, with a non-missing post baseline immunogenicity result. • Variable of interest: Neutralizing titer ($\mu PRNT_{50}$) value at Day 8, Day 29, Day 85, Day 180 and Month 12. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis. • Population-level summaries: 95% CIs for geometric mean titer (GMT) in each treatment group, plus analysis of covariance (ANCOVA) to assess treatment differences.	Parts A, B and C	PP

previously exposed to chikungunya virus.	Supplemental Analysis of Secondary Estimand #1 • As Secondary #1, except: • Using the IMM population. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are included in the analysis.		IMM
	Secondary Estimand #2 • Treatment of Interest: VLA1553 • Population of interest: PP population - ie subjects in the immunogenicity subset who receive a vaccination and have a non-missing post baseline immunogenicity result. • Variable of interest: Seroresponse ($\mu PRNT_{50}$ ≥150) status at Day 8, Day 29, Day 85, Day 180 and Month 12. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis. • Population-level summaries: 95% CIs for seroresponse ($\mu PRNT_{50}$ ≥150) rate in each treatment group, plus Fisher's Exact test to compare treatment arms.	Parts A, B and C	PP
	Supplemental Analysis of Secondary Estimand • As Secondary #2, except: • Using the IMM population. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are included in the analysis.		IMM
	Secondary Estimand #3 • Treatment of Interest: VLA1553 • Population of interest: PP population – ie subjects in immunogenicity subset who receive a vaccination, with a non-missing post baseline immunogenicity result. • Variable of interest: Seroconversion (defined as > 4-fold increase of $\mu PRNT_{50}$ compared to baseline) status at Day 29, Day 180 and Month 12 • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis. • Population-level summaries: 95% CIs for seroconversion rate (SCR) in each treatment group, plus Fisher's Exact test to compare treatment arms.	Parts A, B and C	PP

	Supplemental Analysis of Secondary Estimand • As Secondary #3, except: • Using the IMM population. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are included in the analysis.		IMM
	Secondary Estimand #4 • Treatment of Interest: VLA1553 • Population of interest: PP population – ie subjects in immunogenicity subset who receive a vaccination, with a non-missing post baseline immunogenicity result. • Variable of interest: Fold-increase ($\mu PRNT_{50}$ result at nominal time point / $\mu PRNT_{50}$ at baseline) in neutralizing titer ($\mu PRNT_{50}$) value at Day 8, Day 29, Day 85, Day 180 and Month 12. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis. • Population-level summaries: Descriptive summary of continuous fold-increase ($\mu PRNT_{50}$ result at nominal time point / $\mu PRNT_{50}$ result at baseline) values.	Parts A, B and C	PP
	Supplemental Analysis of Secondary Estimand • As Secondary #4, except: • Using the IMM population. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are included in the analysis.		IMM
	Secondary Estimand #5 • Treatment of Interest: VLA1553 • Population of interest: PP population – ie subjects in immunogenicity subset who receive a vaccination, with a non-missing post baseline immunogenicity result. • Variable of interest: Fold-increase ($\mu PRNT_{50}$ result at nominal time point / $\mu PRNT_{50}$ result at baseline) in neutralizing titer ($\mu PRNT_{50}$) value at Day 8, Day 29, Day 85, Day 180 and Month 12. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis.	Parts A, B and C	PP

	• Population-level summaries: Categorical summary of the rate of subjects reaching prespecified thresholds for fold-increase ($\mu PRNT_{50}$ result at nominal time point / $\mu PRNT_{50}$ result at baseline) in neutralizing antibodies, including 95% CIs for percentages. Supplemental Analysis of Secondary Estimand • As Secondary #5, except: • Using the IMM population. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are included in the analysis.		IMM
	Secondary Estimand #6 • Treatment of Interest: VLA1553 • Population of interest: Subjects in the immunogenicity subset who receive a vaccination, with a non-missing post baseline immunogenicity result. • Variable of interest: Fold-increase in neutralizing titer ($\mu PRNT_{50}$) value at Day 8, Day 29, Day 85, Day 180 and Month 12. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are excluded from the analysis. • Population-level summaries: 95% CIs of Geometric Mean Fold Increase (GMFI) in each treatment group, plus ANCOVA to assess treatment differences. Supplemental Analysis of Secondary Estimand • As Secondary #6, except: • Using the IMM population. • Handling of Intercurrent Events: Subjects with events likely to interfere with immune response are included in the analysis.	Parts A, B and C	PP IMM
Secondary: Safety	Note that all summaries of AEs are detailed in section 13.8.1.		
• To assess the safety of the adult dose of VLA1553 following vaccination in	• Frequency and severity of unsolicited AEs until Day 29 (Visit 3) and Day 180 (Visit 5) post-vaccination.	Parts A and B. [Part C in case of	Safety Analysis Population

adolescents aged 12 years to <18 years after a single immunization up to Month 12. • To assess the safety of VLA1553 in subjects previously exposed to chikungunya virus.		ongoing events]	
	• Frequency and severity of solicited injection site and systemic reactions within ten days post-vaccination.	Part A. [Parts B and C in case of ongoing events]	Safety Analysis Population
	• Frequency and relatedness of any SAE during the entire study period.	Parts A, B and C	Safety Analysis Population
	• Frequency and severity of any early onset AESI starting within 2 to 21 days post-vaccination (i.e. Day 3 – Day 22).	Part A. [Parts B and C in case of ongoing events]	Safety Analysis Population
	• Frequency and severity of any late onset AESI during the entire study starting after 22 days post-vaccination (i.e. Day 23 – study end);	Parts A, B and C	Safety Analysis Population
	• Assessment of viremia on Days 1 and 8 (and Day 29, if applicable) after vaccination.	Part A (and repeated at Part B and Part C – if required*)	Safety Analysis Population (Viremia Subset)
Exploratory	Exploratory Endpoints		

• To evaluate the efficacy of VLA1553 in adolescents aged 12 years to <18 years after a single immunization.	• Incidence of CHIKV infections as evidenced by viremia by virus specific RT-qPCR, clinical diagnosis and seroconversion by $\mu PRNT_{50}$ for the entire study period. • Frequency of any definite, any definite or probable, and any definite, probable or asymptomatic CHIKV cases throughout the whole study period. Exact Clopper-Pearson 95% CIs will be presented for the incidence rates.	Part C	Safety Analysis Population
• To collect information on the CHIKV disease clinical spectrum and associated risk factors in an adolescent population.	• Accumulate data of CHIKV disease signs and symptoms in adolescent population as assessed following vaccination on Day 1 for the entire study period. No formal presentation is planned for this endpoint.		

* If not all samples have been tested for Part B reporting.

10.0 Population Sets

10.1 Study Subsets

10.1.1 Immunogenicity Subset

The immunogenicity subset is defined as all subjects who were initially randomized into the immunogenicity evaluation group or the viremia subset of the immunogenicity group, regardless of any other factors.

10.1.2 Viremia Subset

The viremia subset is defined as subjects from the immunogenicity subset with viremia samples analyzed from Visit 1 and Visit 2, (and Visit 3, if Visit 2 sample is positive) who were initially randomized into the viremia evaluation group.

10.2 Analysis Populations

10.2.1 Safety Analysis Population

The safety analysis population contains all subjects who entered into the study and received one vaccination. Subjects will be analyzed as treated.

10.2.2 Immunogenicity Analysis Population

The Immunogenicity (IMM) population is defined to include all randomized and vaccinated subjects of the immunogenicity subset who have evaluable µPRNT$_{50}$ antibody titer results at baseline and at least one post-baseline titer measurement after vaccination. Subjects will be analyzed according to the study arm they had been allocated to, rather than by the actual treatment they received.

10.2.3 Per Protocol Analysis Population

The Per Protocol (PP) analysis population contains all IMM subjects who have no major protocol deviations that could impact immune response. Examples that may lead to exclusion from the PP are provided here, further criteria may be defined later in the study in this SAP:

- Subject has a history of immune-mediated or clinically relevant arthritis/arthralgia;
- Subject has a known or suspected defect of the immune system that can be expected to influence the immune response to the vaccine, such as subjects with congenital or acquired immunodeficiency, including infection with human immunodeficiency virus (HIV), status post organ transplantation or immuno-suppressive therapy within 4 weeks prior to Visit 1. Immuno-suppressive therapy is defined as administration of chronic (longer than 14 days) prednisone or equivalent ≥2 mg/kg/day within 4 weeks prior to study entry, radiation therapy or immunosuppressive cytotoxic drugs/ monoclonal antibodies in the previous 3 years; topical and inhaled steroids are allowed;
- Subject is positive for HIV, hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV);
- Subject has received the wrong (not according to randomization) or no IMP;
- Subjects who received an excluded concomitant medication which could influence the immune response;
- Subjects missing Visit 3, missing Visit 3 immunogenicity sample or with Visit 3 out of window as defined in section 10.2.5.

- Subjects missing Visit 1, missing Visit 1 immunogenicity sample.
- Subjects with serostatus IgM+/IgG- at screening by ELISA.
- Subjects given an IMP kit identified as being in a temperature excursion that was not approved for use.

Subjects will be analyzed in the PP according to their actual treatment.

As described above major protocol deviations (PDs) will only be defined on subjects in the immunogenicity subset. All subjects not in the immunogenicity subset will have only minor PDs reported even if their PD falls into the above categories.

The PP population will be the primary analysis set for all immunogenicity analyses. Part A, Part B and Part C are defined in section 12.1. The same PP population will be defined for Part A, Part B and Part C analysis, and if excluded from the PP population, the subject as a whole will not be included in the PP analysis for Part A, Part B or Part C. In addition, subjects may be excluded from the PP analysis at specified visits only. As an example, some major protocol deviations may occur post Day 29, such as certain prohibited concomitant medication. In this instance the deviation is deemed as an intercurrent event at the time of the deviation, and immunogenicity results after the occurrence of the protocol deviation will be removed from the PP analysis; the relevant subject remains in the PP population.

Note that at the time of the Part A data extract, there may be some outstanding immunogenicity samples not yet analyzed. Any missing samples will be identified at the initial Part A freeze and BDRM based on the CRF data, with final populations revised once the analytic data is available. If samples are not analyzed in time for the Part A reporting, these will be deemed as missing samples for the purposes of the PP population definition. In the case that these sample results become available at a later point, the samples will be included in analyses. However, if a subject was excluded from the PP population at Part A due to a missing immunogenicity sample at Visit 1 or Visit 3 which is later available, the subject will not be added back into the population for future reporting. The PP population is fixed at Part A.

As the PP population encompasses the definition of the IMM Population, missing Visit 1 immunogenicity samples will be deemed a major protocol deviation at the time of Part A analysis due to the need of an evaluable $\mu PRNT_{50}$ antibody titer result at baseline and to compare $\mu PRNT_{50}$ titers to baseline.

The list of evaluable PP subjects and exclusionary protocol deviations were finalized at the blind data review meeting prior to unblinding at Part A.

10.2.4 Modified Per Protocol Analysis Population

Generally, subjects with any indetermined ELISA result at screening will be included in the study, but the indetermined element is mapped to positive for presentation. Hence, IgM indetermined/IgG- results would map to IgM+/IgG- and will be mapped to the seropositive group for baseline tabulation as described in section 13.4.

However, neither epidemiological nor clinical evidence indicated a Chikungunya circulation in the region at the time of enrolment and investigators regarded indetermined ELISA results as truly CHIKV negative for the case of IgM indetermined. As such cases of IgM indetermined/IgG negative ELISA screening results were allowed into the study on the assumption of being a truly negative subject.

A modified per protocol population will be used for a supplemental analysis to the primary endpoint. This population will include the same defined population as for the per protocol population but will in addition exclude those subjects identified with indetermined IgM ELISA results at screening who would have mapped to the exclusionary IgM+/IgG- serotype if the indetermined result was positive (e.g. subjects with IgM indetermined/IgG- results), if they have not been excluded already through other exclusion criteria.

10.2.5 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 (i.e. samples collected outside of the visit windows defined in the table below), even if the subject may remain in the PP population. Any scheduled immunogenicity Visit 3 Day 29 sample collected outside of the visit window defined in the table below will be excluded from the PP analysis.

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 protocol deviations related to time window deviations are classified as “minor protocol deviations” and do not exclude these subjects from the PP population (except for Visit 3 out of window as defined below).

Study Part(s)	VISIT	Study Day (Time Window)
A, B, C	Visit 0 (Screening)	Day -28 to -0 (prior to Visit 1)
A, B, C	Visit 1	Day 1
A, B, C	Visit 2	Day 8 (Week 1) (+/- 4 days)
A, B, C	Visit 3	Day 29 (Month 1) (+/- 8 days)
B, C	Visit 4	Day 85 (Month 3) (+/- 16 days)
B, C	Visit 5	Day 180 (Month 6) (+/- 42 days)
C	Visit 6	Day 365 (Month 12) (+/- 42 days)

Sample testing issues may also lead to exclusion from the PP for particular time points. Such issues are identified by review of samples by medical and clinical teams and discussed during the data review meetings at each study part, and samples to be excluded from the statistical analysis are flagged for exclusion in the datasets.

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.

Note that for log-transformed endpoints, the above derivation is applied after the log-transform, and back-transformed differences are displayed. This means that a change from baseline for a log-transformed endpoint is presented as a ratio.

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 visit window per clinical study protocol are included in the table below.

Study Part(s)	VISIT	Study Day (Visit Window)
A, B, C	Visit 0 (Screening)	Day -28 to -0 (prior to Visit 1)
A, B, C	Visit 1	Day 1
A, B, C	Visit 2	Day 8 (Week 1) (+/- 1d)
A, B, C	Visit 3	Day 29 (Month 1) (+/- 4d)
B, C	Visit 4	Day 85 (Month 3) (+/- 7d)
B, C	Visit 5	Day 180 (Month 6) (+/- 14d)
C	Visit 6	Day 365 (Month 12) (+/- 14d)

Data will be analyzed according to the visit windowing rules below (Section 11.2.1), except in the case of the immunogenicity analyses on the PP population (see Sections 10.2.3 and 10.2.5). Unscheduled visits may be held at any time during the study as necessary and are mapped per the visit windowing rules below (Section 11.2.1).

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 Visit 5 (Day 180) will include all data collected on study, up to their Visit 5 or Early Termination (ET) visit.

11.2.1 Visit Windowing Rules

Visit windowing will be included in the analysis of planned timepoints on this study. To this end, all visits (including planned, unscheduled or ET) of immunogenicity, hand stiffness, physical examination and safety samples will be assigned to the visit number of the closest planned study visit. For Viremia tables the windows will be applied for planned visits only. In order to do this, the mid-point between two planned study visits is used as the cut-off for visit windowing, according to the mapping per the below. All visits will be windowed for analysis per the below.

Study Part(s)	Windowed Visit	Study Day Range for Window Mappings
A, B, C	Visit 0 (Screening)	Day -28 to -0 (prior to Visit 1)
A, B, C	Visit 1 – Day 1	Day 1
A, B, C	Visit 2 – Day 8	Day 2 – Day 18
A, B, C	Visit 3 – Day 29	Day 19 – Day 57
B, C	Visit 4 – Day 85	Day 58 – Day 132
B, C	Visit 5 – Day 180	Day 133 – Day 272
C	Visit 6 – Month 12	Day 273 onwards.

All visits will be analyzed according to the windowed visit per the above, rather than the recorded visit number. If there are multiple results for a specific endpoint falling into one visit after windowing is applied, then the visit closest to the planned visit day will be used in the first instance. If these are tied, then a

conservative approach will be taken, i.e. the worse value will be taken, and the lower titer value will be used for post-baseline immunogenicity values. Detailed approach is seen in section 11.2.1.1.

In listings, both the visit name as reported in the CRF, and the windowed visit will be displayed, with an indication of which results are used in the analysis for each windowed visit.

Subjects who withdraw from the study prior to completion of the study will attend an ET visit where possible. For the purposes of analysis and reporting, each ET visit will be assigned to one of the three study parts. If a subject has an ET visit mapped to Visit 3 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 but no Visit 5 or later visits then the ET visit will be assigned to Part B and if the subject has an ET visit after Visit 5 and no Visit 6 visit then the ET visit will be assigned to Part C.

Acute and convalescent visits may occur at any time throughout the study, and are handled with visit windowing as described above for immunogenicity, physical examination hand stiffness and safety samples. Viremia results from acute and convalescent visits are explained in sections 11.2.1.1 and 13.8.2. These visits will map to study parts based on their relation to the cut-off for each study part. If the acute or convalescent visit is on or before the subjects Part A cutoff date (described in section 12.1 as the Visit 3 actual date, ET date if prior Visit 3, or end of window for Visit 3 if neither are available), then it is mapped to Part A. If the visit falls between the Part A cutoff and Part B cutoff, it will be assigned to Part B, and if it falls after Part B then it will be assigned to Part C.

11.2.1.1 Handling of Multiple Visits on the Same Study Day

In some cases, subjects may have two (or more) planned study visits on the same day. In particular, an Acute or Convalescent visit may be done on the same day as a planned study visit, or in some cases Acute and Convalescent visits may be on the same day. In these cases, sites were instructed to collect data only once, but enter the results into both visit folders (eg Acute visit and Visit X folders). In these cases, the data will be windowed as per the rules in section 11.2.1, so that data will be presented at the planned visit name (Visit X) in outputs if it falls into a visit window, according to the following additional rules:

- For tables/figures, if the results data entered into multiple pages is exactly the same, the planned visit result will be presented. In cases where two visits are on the same day but no planned visit, eg an Acute and Convalescent visit are on the same day but no other visit, then the Acute visit result will be used where results are exactly the same.
- Else, if there are differences in data reported for the same variable/analyte on the same day, the worst result will be used in each case for presentation, with the rules for the worst result in applicable forms as follows:
    - Safety labs: rules are as defined in section 11.6 with furthest out of range per analyte used;
    - Pregnancy test and SARS-CoV-2 tests: worst is a positive result;
    - Viremia: worst result is higher copy number value;
    - µPRNT: worst result is a lower $\mu PRNT_{50}$ titer value (eg a more conservative value);
    - ELISA and RT-PCR: worst result would be Positive, Indetermined, Negative in that order;
    - Physical exams: where a body system has differences between abnormal and normal the abnormal record will be used. If both are abnormal and one is clinically significant, then the clinically significant will be used. If both abnormality and clinical significance are the same but details differ (eg in free-text abnormal findings fields), then the planned study visit (or acute visit if no planned visit) will be used.
    - Hand stiffness – worst is a higher result.
- In listings, all data entered on the CRF will be presented (eg some duplicate data will be shown), with the CRF and windowed visit, and flags showing the records used for analysis where Visit is included.

Note that data checks were in place to minimize the chances of mis-matched data on these duplicated visit forms, however the above rules are in place for cases where such differences are irreconcilable. If data are entered only at one visit (eg a form is only applicable for one of the overlapping visits), then this is presented, but data are windowed to the visit windows as applicable.

11.3 Immunogenicity Endpoints

Immunogenicity of VLA1553 throughout the study will be evaluated using µPRNT. The µPRNT leads to a single CHIKV-specific neutralization value from each sample analyzed, referred to as the $\mu PRNT_{50}$.

Any $\mu PRNT_{50}$ value less than 20 (lower limit of quantification (LLOQ)) will be imputed as 10 (LLOQ/2). Any subject with a baseline $\mu PRNT_{50} \leq 40$ will be classified as baseline negative; subjects with baseline $\mu PRNT_{50} > 40$ are classed as baseline positive. Subjects with baseline titer values from 20 – 40 will not be imputed and the reported value will be used. The upper limit of quantification was determined as 13498. However, values above 13498 will be reported in the data, and the reported values will be used in the analysis as they are.

In general, all statistical analysis will be stratified based on the results of the $\mu PRNT_{50}$ at baseline (on or before Day 1), apart from sensitivity utilizing the ELISA result.

11.3.1 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 (SD) of the log-transformed titer. The 95% 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.

11.3.2 Seroconversion

Seroconversion is defined as > 4-fold increase of $\mu PRNT_{50}$ compared to baseline.

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

11.3.3 Seroresponse

Seroresponse levels of CHIKV-specific neutralizing antibodies are defined as $\mu PRNT_{50} \geq 150$ at any post-baseline timepoint. This is defined for both $\mu PRNT_{50}$ baseline positive and negative subjects.

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

11.3.4 Fold-Increase in Neutralizing Antibody Titer

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

$\mu PRNT_{50}$ result at nominal time point / $\mu PRNT_{50}$ 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.

The geometric mean of the fold increase is called Geometric Mean Fold Increase (GMFI) where in simple sense the fold increase is calculated as the ratio of the post-vaccination titer value to the pre-vaccination value. So, GMFI is calculated as the anti-logarithm of the mean of the log-transformed titer divided by the baseline assay result.

11.4 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. Furthermore, routine health checks required due to pre-existing diseases will not be recorded. 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.

After data entry, AEs and medical history will be coded according to MedDRA (Medical Dictionary for Regulatory Activities) version available at time of Part A reporting. The strategy for up-versioning of coded terms to a later MedDRA version for Part B and C reporting is described in the coding conventions of the study. 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.

11.4.1 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) according to the (Food and Drug Administration) FDA toxicity grading scale.

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.

In a sensitivity analysis, diary-only solicited symptoms (i.e. those symptoms reported on the diary but not included in the AE page) will be tabulated, and classed as severe in this case.

11.4.2 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.

In a sensitivity analysis, diary-only solicited symptoms (those symptoms reported on the diary but not included in the AE page) will additionally be tabulated, and classed as related to IMP in this case.

11.4.3 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.

11.4.4 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.

Hospitalization or prolonged hospitalization for diagnostic or elective medical procedures (including plastic surgeries) that were planned prior to vaccination are not reported as an SAE. The treatment of a pre-existing condition that did not change in severity, the condition leading to hospitalization or prolonged hospitalization and also the medical procedure itself are not reported as an SAE. In this case, the underlying diagnosis or condition should be reported in the medical history section of the CRF and the corresponding medical procedure should be documented as a comment to the underlying diagnosis or condition in the CRF's medical history section.

All criteria leading to an AE being classified as an SAE will be recorded on the CRF. 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).

11.4.5 Adverse Events of Special Interest

11.4.5.1 Protocol Definition

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 and are defined as early onset AESI:

1. Fever (≥ 37.8°C measured axillary);

AND

2. Acute (poly)arthralgia/arthritis, myalgia, neurological symptoms (e.g. meningoencephalitis, acute encephalitis, headache, seizures), retinitis/uveitis;

   OR

   One or more of the following signs and symptoms: macular to maculopapular rash (sometimes with cutaneous pruritus (foot plant)), pigmentary changes, bullous rash/ skin blistering, purpura and ecchymosis;

AND

3. Onset of symptoms 2 to 21 days after vaccination (i.e. Day 3 – Day 22);

AND

4. Duration of event ≥ 3 days.

The cluster of symptoms defined above but starting 22 days after vaccination (i.e. Day 23) until study end is defined as late onset AESI.

Within the CRF investigators can mark each symptom as early onset or late onset AESI, based on the start day of the symptom. For analysis (irrespective of the investigator assessment), the cluster of symptoms will be considered when determining the timing of the AESI, i.e. the date of the symptom(s) that occurred first will be used to determine if an AESI will be classed as to have an early or late onset. As such, a flag for early and a flag for late onset AESI will be derived in the datasets as follows (the flag are mutually exclusive). An AESI event (eg the whole cluster of symptoms) will be classed as early onset if the start date of the earliest symptom is between Day 3 and Day 22. All symptoms within the AESI cluster are marked as early onset, even if a symptom within the event starts on or after Day 23. An AESI cluster is deemed as late onset if the study day of the onset of any symptom within the cluster is Day 23 or later. As such all symptoms within an AESI event will have the same classification, even if symptoms started before/after the Day 23.

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 be recorded on the CRF. 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 agrees with the investigators' decisions. Adjudication will be a Y/N flag from the DSMB for each AESI case. This will be provided as an excel file and merged into the datasets and used for additional reporting.

Additionally, subjects presenting with acute arthralgia will be followed-up until resolution and monitored for recurrences until the end of the study.

11.4.6 AESI according to USPI FDA Broad Definition of Chikungunya-like Adverse Reactions

Additionally to the definition of AESI included in the protocol, AESI defined according to the United States Prescribing Information (USPI) FDA broad definition of chikungunya-like Adverse Reactions (AR) will be derived as described in Appendix 16.1.1.

1. Fever (≥ 37.8°C measured axillary);

AND

2. Any symptoms of arthralgia or arthritis, myalgia, headache, back pain, rash, lymphadenopathy, or certain neurological, cardiac or ocular symptoms

AND

3. Onset of symptoms 0 to 30 days post vaccination, regardless of the order of onset and duration. (no need of overlapping symptoms)

A case of an AESI according to the broad definition of chikungunya-like ARs is a collection of symptoms that collectively met the conditions as shown above. A severe case is defined as a collection of symptoms with at least one severe symptom that met the conditions as shown above.

11.4.7 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 Diary 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 AEs which are reported on the AE page of the CRF but not reported on the Diary are flagged as recall events. This flag will be used in a sensitivity analysis of the solicited AEs.

For a sensitivity analysis of solicited AEs, any symptom entered in the Diary but not the AE page will be included. For these tabulations, the diary-only AEs will be classed as severe and related.

11.4.7.1 Injection Site Reactions

Subjects will be provided with a measuring device to measure the size of any measurable injection site reaction that may develop after vaccination. The subject/legal representative(s) will be instructed on how to measure any such reactions over a period of ten consecutive days after vaccination along the longest diameter of the reaction area and record this measurement in the subject diary.

Solicited injection site reactions include injection site pain, tenderness, erythema/redness and induration/swelling. The severity for these reactions 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 reaction should be reported as an SAE and will be reported as Severe (Grade 3) (see Section 11.4.4). All injection site reactions will be considered as related to IMP.

11.4.7.2 Systemic Reactions

Systemic reactions 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 reactions 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 reaction should be reported as an SAE and will be reported as Severe (Grade 3) (see Section 11.4.4).

11.4.8 Duration of AEs

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

If end date is missing, then it will be assumed to be the date of study completion/ET/lost to follow-up.

If the end date is partial, the end date will be imputed as follows:

- If the day is missing, the end date will be imputed as the last day of the month,
- If the day and month are missing, the end date will be imputed as the earliest between the last day of the year and the date of study completion/discontinuation.

In an additional sensitivity analysis, the missing end dates will be calculated as follows:

- Subjects lost to follow-up will have end date imputed as the last attended visit or contact date in the study prior to being lost to follow-up;
- Completed subjects will have end date imputed as their end of study date;

Note at interim reporting points, subjects ongoing at time of reporting with AEs ongoing at reporting will have end date imputed as the latest day on the study for that part (eg the analysis cutoff date per subject as described in section 12.0).

11.5 Classification of CHIKV Cases

Assessment of the incidence of naturally occurring CHIKV infections for the entire course of the study from Day 1 will be compared between the study arms 14 days after vaccination for the exploratory assessment of efficacy. Suspected cases of CHIKV infection/disease will be classified into four different categories according to the criteria given below.

Following the end of the study, an attempt will be made to classify CHIKV infections into acute, post-acute and chronic stage of disease. The classification of these stages will be done based on the duration of symptoms or from the immunogenicity response over time. The acute stage is defined by symptoms until

the first three weeks after onset of illness. The post-acute stage is defined as having symptoms until the end of the third month. The chronic stage is defined by symptoms that persist for more than three months. All CHIKV cases will be discussed and assessed by the Sponsor and subsequently presented to the DSMB.

Details of this process are included in section 13.7.2.

11.5.1 Definite CHIKV Case

Any of the cluster of clinical manifestations of CHIKV events observed:

1. Fever (≥37.8°C measured axillary);

   AND

2. Acute (poly)arthralgia/arthritis, myalgia, neurological symptoms (e.g. meningoencephalitis, acute encephalitis, headache, seizures), retinitis/uveitis;

   OR

   One or more of the following signs and symptoms: macular to maculopapular rash (sometimes with cutaneous pruritus (foot plant)), pigmentary changes, bullous rash/ skin blistering, purpura and ecchymosis;

   AND confirmatory laboratory tests:

- A retrospective confirmatory CHIKV-specific quantitative RNA (ribonucleic acid) detection (RT-qPCR) will be performed for all samples collected at acute and if applicable at convalescent visits.

11.5.2 Probable CHIKV case

Any of the cluster of clinical manifestations of CHIKV events (as described above) observed, that cannot be confirmed by RT-qPCR.

A confirmatory laboratory test (with acute and convalescent samples) will be conducted retrospectively:

Seroconversion, defined as a >4-fold increase of $\mu PRNT_{50}$ compared to baseline (Day 1), for µPRNT baseline negative and positive subjects in the control group. In the VLA1553 treatment arm, it will not be possible to discriminate seroconversion induced by natural infection from vaccine induced within approximately 2 months after vaccination. Thereafter a more than 4-fold increase in $\mu PRNT_{50}$ titer could indicate natural exposure to circulating virus.

11.5.3 Asymptomatic CHIKV Case

Sera from subjects without apparent clinical symptoms of wild type CHIKV disease will be retrospectively tested for antibodies in some instances. In these subjects a serological confirmation is required as applicable:

- In the control group, seroconversion is defined as a >4-fold increase of $\mu PRNT_{50}$ compared to baseline (Day 1) at Day 180 (Visit 5) and Month 12 for $\mu PRNT_{50}$ baseline negative and baseline positive subjects.
- In the VLA1553 treatment arm, it will not be possible to discriminate seroconversion of CHIKV-specific neutralizing antibodies induced by natural infection or VLA1553 vaccination up to Day 85[1]. Thereafter a more than 4-fold increase of $\mu PRNT_{50}$ titer could indicate natural exposure to circulating virus.

11.5.4 Unconfirmed CHIKV Case

Unconfirmed cases are all suspected CHIKV cases that do not meet above criteria for CHIKV. Unconfirmed cases will not be included in the assessment of efficacy.

[1] Depending on actual titer kinetics in the study, this cut-off time point may be revisited.

11.6 Handling of Laboratory Records

In this study, data will be captured using a combination of central and local laboratory pages for safety labs, requiring manual results entry in the CRF by the sites. Results from vendor for specialist labs will be transferred to ICON by data transfer. Due to logistic reasons at central lab, who provides the kits for safety and speciality labs, the samples will be tracked in the CRF only by the subject code, lab panel/lab parameter, visit label (visit number) and collection date, and these variables will only be used for reconciliation. No additional barcode or accession number will be tracked in the CRF.

For the vendor data, if there are irreconcilable discrepancies between CRF and vendor files in regard to differing visit label or visit date within a subject's results then subjects will be mapped by visit date.

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

If there are multiple, non-duplicate records at the same visit date, 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. In case of ties, the measurement presenting the highest change from baseline in absolute values will be used. In case of a tie in this instance, the named visit is used as default.

Primary samples for safety, immunogenicity and viremia will undergo reconciliation and cleaning. The CRF is the ultimate source (except for irreconcilable items) for the associated back-up samples even in case of mismatches between visit date.

Safety lab data which are conducted by the central lab but entered locally, will be taken from the CRF entered values. These will be entered using the Rave local labs tool to capture associated normal ranges and units.

Some lab tests – namely urinalysis – are to be done locally at sites due to instability of samples, and these will also be taken from the local pages in the CRF. Where sites have differing normal ranges, these will be captured and included in listings.

According to the DSMB meeting of June 24th 2022, the erythrocyte sedimentation rate (ESR) may not represent an accurate parameter for detecting inflammatory conditions. Hence, it was agreed to not collect ESR (erythrocyte sedimentation rate) and use CRP (C-reactive protein) instead. Furthermore, the absence of ESR in the safety laboratory reports will not be classified as major or minor protocol deviation.

All data analyzed by the labs will be used for reporting, subject to the conditions for multiple or duplicate records described in section 11.2.1. Hemolysis of samples is assessed but only samples with acceptable levels are included in the reported results from each laboratory, and hence all results received are deemed to be valid.

11.7 Missing Data

All statistical analysis will generally be based on observed values, missing values will not be imputed. Missing severity and causality will be handled as described in Section 11.4.1 and Section 11.4.2 respectively.

In case of > 5% of missing values for the primary immunogenicity analysis, a separate sensitivity analysis will be performed where multiple imputation (MI) methods will be applied in order to evaluate the possible impact of missing values on these results. Further details are specified in Section 13.6.3.2. Note that this analysis was not required as the 5% missing threshold was not reached.

11.8 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/Placebo dosing, or AE end dates as described in section 11.4.8 for AE duration.

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/Placebo, 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, Part B and Part C

12.1 Planned Analysis Timepoints

There are 3 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 (Day 180).
- Part C includes safety and immunogenicity data after all subjects have completed Visit 6 (Month 12).

Individual study parts will be analyzed sequentially.

The study will be unblinded for Part A analysis once the last subject has completed Visit 3 and database freeze for Part A has occurred (blind will be maintained for study sites and subjects). See Unblinding Plan and Blind Maintenance Plan for full details.

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

For analysis, data are restricted at the study part cutoff date for each individual subject as follows. For the Part A analysis, data is from Visit 1 up to and including Visit 3 (Day 29) or ET if earlier will be kept for all subjects, for the Part B analysis, data is from Visit 1 up to and including Visit 5 (Month 6, Day 180) or ET if earlier will be kept for all subjects. As per the study design, the last visit for the non-IMM subjects is Visit 5 (Month 6, Day 180), so all their data is included in Part B analysis. For the Part C analysis (Month 12) all data will be kept for all subjects. If the relevant visit is missing and there is no earlier ET visit, then the end of the relevant visit window is used as the cutoff date.

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.

For the purpose of reporting any durations for AEs that stop after the cutoff for the analysis in Part A and Part B the end date will be imputed to date of Visit 3 and Visit 5 respectively. In cases where Visit 3 or Visit 5 are missing and an ET visit is earlier, this is used for the imputation date for end dates. In a case where there is no ET visit or Visit 3 or Visit 5, the end of study date will be used, which could be after the cutoff for Part A or B respectively.

Note that at the time the Part A data were extracted there were some outstanding data issues, which were resolved after the Part A data was reported. In particular there were some changes to immunogenicity data, with additional samples analyzed after the Part A reporting. In addition, there were changes to some AE data from Part A, including solicited AEs. As such, safety data which were planned to be reported only at Part A are repeated at Part B to account for data changes. For the efficacy data, most of the results from the specific Part A tables (i.e. the tables that only include Day 29) are already included in the tables planned

for Part B and Part C with the exclusion of the primary analysis hypothesis testing, and the sensitivity analysis per ELISA stratification and the sensitivity analysis on the mPP population. As the additional immunogenicity data is very limited (< 5 subjects) the changes will not impact the conclusion and interpretation of the primary endpoint. Changes to outputs which impact the conclusion and interpretation of the Part A will be noted in the Part B CSR.

12.2 Preliminary Safety Summary

A split delivery of safety data prior to Part A final analysis is planned to support study activities. To accommodate an expedited reporting of safety data prior to Part A final analysis, an initial delivery of safety and demographic outputs is planned, and will not include lab vendor data. This deliverable is based on the Part A CRF safety data, it will take place after the full CRF data cleaning activities for Part A have been completed, and hence run only on the final clean Part A snapshot. Final lab vendor data files may not be available at the time of the snapshot, but sample reconciliation will be as complete as possible. A full cleaning status at the Part A snapshot will be available in the Data Management Plan. The outputs reported in this analysis will be stratified by ELISA serostatus, instead of $\mu PRNT_{50}$ serostatus. As such the ELISA serostatus will be taken from the serostatus entered into the IRT system, rather than derived based off screening ELISA lab results, but both will be presented in the baseline table, see section 13.4.

The outputs to be included in the 'Preliminary Safety Summary' will be all those from the disposition, demographic and safety sections, excluding any outputs requiring lab vendor data, such as the immunogenicity, viremia and alphaviruses data. Outputs using the immunogenicity or per-protocol populations will not be presented as these populations will not have been fully defined prior to the full receipt of the immunogenicity data. Note that all relevant major PDs will be assessed prior to the unblinding at time of the 'Preliminary Safety Summary', and populations will be defined based on information available at this point and documented in the BDRM minutes (which will be approved prior to Part A freeze and unblinding). Any additional exclusions based on final immunogenicity data being available will be noted once these immunogenicity results are available and will be documented in an amendment to the BDRM minutes, which will also be approved.

By-subject listings and datasets will not be delivered to sponsor teams for the 'Preliminary Safety Summary' to minimize potential bias due to unblinding in the event that full immunogenicity data is not available at the time of the snapshot. This will ensure that the sponsor teams are not unblinded to treatment on a subject-level prior to the finalization of all immunogenicity data cleaning and verification. The relevant ICON team will be unblinded at a subject-level, but will not have direct communication with the immunogenicity data vendor, limiting potential bias in this process.

A sponsor Unblinding Plan will be created and followed to minimize risk of unblinding of specific team members, and this plan will be approved prior to the Part A freeze and unblinding being performed.

12.3 Data Safety Monitoring Board

An independent unblinded DSMB will be utilized for this study. The DSMB will meet to review accumulating safety data on a regular (bi-weekly to bi-monthly) basis until all subjects have completed Visit 3 (Day 29), at timings per member discretion. In addition, the DSMB will periodically review accruing safety information throughout the study, as applicable. Furthermore, the DSMB will meet ad-hoc to provide a recommendation on further age de-escalation and vaccination if at any time during the sentinel recruitment DSMB review criteria as outlined in Section 12.1.2. of the Protocol are met. Additional meetings of the DSMB may be called at the discretion of the Sponsor, e.g. to address any safety concerns arising during the conduct of the study.

The DSMB will perform ad hoc review of SAEs, AESIs and severe (Grade 3) solicited AEs. The DSMB will periodically review listings and summary tabulations of SAEs, Deaths, Solicited AEs, and Unsolicited AEs.

A DSMB charter including a detailed description will be prepared. A separate DSMB table and listing shell document will also be 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, SD, median, 25th percentile (Q1), 75th percentile (Q3), minimum and maximum. Summaries of CHIKV-specific $\mu PRNT_{50}$ results will present the number of observations (n), GMT, GMFI, 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, GMT, GMFI, 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 MI methods will be applied in order to evaluate the possible impact of missing values on these results. Further details are given in Section 13.6.3.2.

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 Parts B and C.

In general, all tables will be presented by baseline serostatus determined by $\mu PRNT_{50}$ serostatus and study arm.

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 ET 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 Protocol Deviations

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

Per ICON processes, protocol deviations data will be entered into the ICON system of record (Predictivv Study Operations [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 freeze for each part of the study.

Protocol deviations are classified into important or not important based on the protocol deviation guidance based on the impact to the study results, subject safety, or data integrity. In addition, protocol deviations will be classified into major or minor protocol deviations (Major PDs are only defined for Part A), based on their possible impact on subject efficacy results, in a Blind Data Review Meeting prior to the database snapshots at each study part (for Part A, DRM was blinded). Major deviations are those which will lead to exclusion from the Per Protocol analysis set, i.e. those which would have a major impact on the validity of the immunogenicity result, as described in section 10.2.3. It is possible for subjects to be deemed important

but not exclusionary from the Per Protocol analysis set, and hence not major. Within the system of record PSO, all major deviations are captured with grade major, and any records with missing grade are classed as minor, as minor grade is not necessarily captured.

A tabulation of the number and percentage of protocol deviations by deviation types and deviation category will be provided for each study part on the Safety population and repeated on the IMM population. A by-subject listing of all protocol deviations will be presented for the Safety population.

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

From Part B onwards data may be excluded on a timepoint basis from the PP analysis.

13.3 Prior and Concomitant Medications

Prior and concomitant medications, categorized by Anatomic Therapeutic Chemical (ATC) classification level 2 and preferred term (PT) according to WHODrug Dictionary (World Health Organization Drug Dictionary) (Version B3 Sep2021 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 and route of administration 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.

A summary of prior vaccinations for relevant traveller diseases will be produced including records from the CRF form “Prior Vaccination Against Relevant Traveller Diseases in the last 3 years prior to Screening”. The number and percentage of subjects for each traveller vaccine administered will be displayed together with the number and percentage of subjects using at least one traveller vaccination within each treatment group and subgroup. Traveller vaccines will be listed separately along with the ATC Level 2 term, preferred term, start data, and study day. The Safety Population will be used for both the listing and summary.

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.

Some medications are prohibited during the study (unless such treatment has to be administered in an emergency situation), these drugs are specified in the protocol section 17.6.2 and these will be documented as protocol deviations, even if in an emergency situation. Additionally, some medications/procedures may delay the vaccination also specified in the protocol section 17.6.2. Medications that are not permitted prior to study enrollment, resulting in exclusion from the study, are reflected in the exclusion criteria in the protocol section 13.2.

13.4 Demographic and Baseline Characteristics

Demographic and baseline characteristics to be summarized will include gender, ethnicity, race, age at screening (years), age group, height (cm), weight (kg), body mass index (BMI) (kg/m^2), baseline serostatus according to µPRNT (seronegative (≤40) vs seropositive (>40)), serostatus strata according to ELISA based on the IRT randomization groupings, and the serostatus based on the baseline ELISA lab results including the serotype results for IgG and IgM. Subjects with any indetermined ELISA result at screening will be mapped to positive for this presentation, ie the following mappings apply:

- IgG indetermined / IgM negative maps to IgG+/IgM-;
- IgG indetermined / IgM indetermined maps to IgG+/IgM+;
- IgG negative / IgM indetermined maps to IgG-/IgM+.

BMI = weight/height^2, where weight is in kg and height in meters. Note that baseline BMI is to be derived in the ADaM (Analysis Data Model) datasets for all subjects using the baseline height and weight records, rather than using the derived BMI from the CRF.

Medical history will be summarized by system organ class (SOC) and PT according to the latest MedDRA version available at time of Part A reporting. The strategy for up-versioning of coded terms to a later MedDRA version for Part B and C reporting is described in the coding conventions of the study.

All demographic and baseline summaries will be provided on the Safety population by treatment arm and by baseline µPRNT serostatus strata. Additionally, the summary of demographic and baseline characteristics will be presented for the IMM and PP populations. All demographic and baseline data will be listed.

13.5 Study Drug Exposure

No summary tables will be produced for the study drug exposure, however all drug accountability data (including kit numbers) will be listed showing subjects administration of the vaccine on day 1.

13.6 Immunogenicity Analyses

13.6.1 Hypothesis Testing Strategy and Multiplicity

A formal hypothesis test is defined for the primary immunogenicity analysis, using a one-sided significance level of 2.5%. There will be no adjustment for multiplicity for any immunogenicity endpoints.

13.6.2 Sub-group Analyses

All immunogenicity analyses will be presented by baseline µPRNT$_{50}$ serostatus, apart from seroresponse primary analysis outputs as this analysis is only specified for µPRNT$_{50}$ baseline seronegative subjects. The µPRNT test result will be used for the determination of seropositivity, per section 11.3.

13.6.3 Primary Immunogenicity Endpoint

The primary immunogenicity endpoint is the proportion of subjects with a seroprotective CHIKV antibody level (defined as µPRNT$_{50}$ ≥150 for baseline negative subjects) 28 days post-vaccination (study day 29) in µPRNT$_{50}$ baseline seronegative subjects (µPRNT$_{50}$ ≤ 40).

The primary immunogenicity analysis will compare the SRR against a non-acceptance threshold of 70%. An exact binomial test for the null-hypothesis H0: SRR ≤70% against the alternative H1: SRR >70% with a one-sided significance level of 2.5% will be applied and exact (Clopper-Pearson) two-sided 95% CIs will be calculated. This will be presented for the PP population.

13.6.3.1 Pooling of Sites

Potential ad-hoc immunogenicity analyses may be performed for the sites with higher CHIKV circulation/ high seropositivity rate. If required, a logistic regression using SAS® LOGISTIC procedure will be used with seroresponse as the dependent variable and treatment group, study site, treatment group*study site as the independent variables.

At Part A this has not been noted and thus not performed.

Other separate summaries by site are not planned.

13.6.3.2 Sensitivity and Supplemental Analyses

The primary analysis will be repeated for the IMM population, and also repeated including baseline positive and negative subjects based on µPRNT$_{50}$ for the PP population.

A supplemental analysis of the primary endpoint will be performed where subjects are assessed based on their IRT ELISA serostatus at screening rather than the µPRNT$_{50}$.

Another supplemental analysis of the primary endpoint will be performed using a modified per protocol population where in addition to the derived per protocol population those subjects with indetermined ELISA results at screening (e.g. IgM indetermined/IgG-) will also be removed from the population going into the analysis.

Additionally, if there are >5% of missing values out of the number of subjects in the IMM population on Day 29 for the immunogenicity analysis of seroresponse on Day 29, a separate sensitivity analysis will be performed applying MI strategy under missing at random (MAR) assumption.

Treatment group, baseline serostatus and Day 8 $\mu PRNT_{50}$ values will be used to impute the missing Day 29 $\mu PRNT_{50}$, that will then be used to calculate estimated seroresponse. A seed number of 15532021 will be used.

STEP 1: In general, the missing pattern will not be monotone therefore the first step will be to use the Markov Chain Monte Carlo (MCMC) method in conjunction with the IMPUTE=MONOTONE statement of the SAS® MI procedure to partially impute quantitative parameters of the $\mu PRNT_{50}$, separately for each treatment regimen. The MCMC model will include in this order an indicator variable for treatment arm, an indicator variable for baseline serostatus (negative and positive), and $\mu PRNT_{50}$ values of each immunogenicity visit ordered chronologically [Day 8 to Day 29].

STEP 2: Thereafter the 500 datasets generated at step 1 will be used to create full imputation of the missing titer values under a MAR assumption using a monotone regression model including in this order an indicator variable for treatment arm, an indicator variable for baseline serostatus and D29 $\mu PRNT_{50}$ value using SAS MI procedure to output 500 imputed datasets.

STEP 3: In the 500 imputed datasets, Day 29 seroresponse will be derived from the Day 29 $\mu PRNT_{50}$ value using the cutoff (≤40/ >40).

An exact binomial test for the null-hypothesis H0: SRR ≤70% against the alternative H1: SRR >70% with a one-sided significance level of 2.5% will be applied and exact (Clopper-Pearson) two-sided 95% CIs will be calculated.

The VLA1553 and placebo groups will be compared using a two-sided Fisher's Exact test. The difference in proportion between the two treatment groups, and exact score (Chan-Zhang method using the EXACT RISKDIFF (METHOD=SCORE) option in SAS PROC FREQ) two-sided 95% CIs for the difference in treatment groups will be presented. The final estimates for the proportion of responders, difference in proportion of responders and p-value will correspond to the median of these estimates across all imputed data sets.

13.6.4 Secondary Immunogenicity Endpoints

13.6.4.1 Comparison of Geometric Mean Titer

A summary of the $\mu PRNT_{50}$ in each study arm will be presented at all timepoints (Day 8, Day 29, Day 85, Day 180 and Month 12) for the PP and IMM populations. Two-sided 95% CIs will be presented for the GMT in each study arm at each timepoint.

The GMT will be compared in all strata groups between VLA1553 and placebo groups and also overall. Where presented by baseline serostatus then treatment group, an analysis of variance (ANOVA) model including treatment group as a fixed effect will be used. An analysis of covariance (ANCOVA) model including treatment group and baseline serostatus as fixed effects will be used for the overall. Estimates of treatment differences in GMT and associated 95% CIs will be presented. In addition, sensitivity analyses (ANCOVAs with factors study site, treatment group, study site*treatment group) will be presented. Note in the case of non-convergence of this second model, the study site*treatment group interaction term may be dropped in order to allow for model convergence.

For all ANOVA and ANCOVA models, model checking for the assumptions will be included, including a residual check for normality of the data and outliers search will be included. The assumptions of normality is not expected to be met at Day 8 due to the low number of subjects with immunogenicity responses,

therefore even if the data is not normal at Day 8 the model will not be changed unless the log-transformed data is not normal at Day 29, in which case an appropriate alternative method such as a generalized linear model (GLM) will be applied

A line plot of the GMT at each study timepoint will be produced by treatment group in the PP population. This will be repeated by baseline µPRNT serostatus, and each plot will be repeated using a logarithmic scale.

Reverse cumulative distribution plots of the proportion of subjects by titer value will be produced and will include Day 29, Day 85, Day 180 and Month 12 for each treatment group. The reverse cumulative distribution will be repeated by visits, treatment groups and baseline µPRNT serostatus strata.

In addition, for Part C only, the GMT at each study timepoint within the VLA1533 treatment arm will be compared between baseline µPRNT seronegative group and the baseline µPRNT seropositive group. An ANOVA model including baseline µPRNT serostatus as a fixed effect will be used. Estimates of baseline µPRNT serostatus differences in GMT, associated 95% CIs and p-values will be presented. This analysis will be carried out of the IMM and PP populations.

13.6.4.2 Seroresponse Rate

The number and percentage of subjects meeting the criteria for seroresponse will be presented for each study timepoint. The denominator for the percentage will be the number of subjects with non-missing $\mu PRNT_{50}$ values at each timepoint. Two-sided exact (Clopper-Pearson) 95% CIs for the SRR will be presented.

The VLA1553 and placebo groups will be compared using a two-sided Fisher's Exact test. In addition, for the difference in proportion between the two treatment groups an exact score (Chan-Zhang method) two-sided 95% CIs for the difference in treatment groups will be presented.

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

All analyses of seroresponse rate will be presented by stratification group of baseline serostatus as determined by µPRNT, as defined in section 11.3.

For Part C, the following will be tabulated for the PP population. The number and percentage of subjects within each age group (12 to < 15 years, 15 to <18 years old and overall) meeting the criteria for SRR will be presented for Day 8, Day 29, Day 85, Day 180 and Month 12 by treatment group for the subset of baseline µPRNT seronegative subjects. The denominator for the percentages will be the number of subjects with non-missing $\mu PRNT_{50}$ values on Visit 3 Day 29 within each treatment group and age group. In addition, the two-sided exact (Clopper-Pearson) 95% CI for the SRR will be presented.

For each age group within each treatment group at Visit 3 Day 29, the p-value from an exact binomial test for the null-hypothesis H_0: SRR ≤ 70% against the alternative H_1: SRR > 70% with a one-sided significance level of 2.5% will be provided.

Finally, the difference in proportion between the two age groups (12 to < 15 years group minus 15 to <18 years group) will be tabulated alongside the two-sided exact 95% CIs (Chan-Zhang) and the p-value Fisher's Exact test for Day 8, Day 29, Day 85, Day 180 and Month 12.

13.6.4.3 Seroconversion Rate

The SCR will be summarized similarly to the SRR (including additional Part C analysis), but will only be presented on Days 29, 180 and Month 12.

For Part C, the following will also be tabulated for each treatment group separately on the PP population. The number and percentage of subjects within each age group (12 to < 15 years and 15 to <18 years old) meeting the criteria for SCR will be presented for Day 29, Day 180 and Month 12 by baseline µPRNT serostatus (seronegative and seropositive). The denominator for the percentages will be the number of subjects with non-missing $\mu PRNT_{50}$ values on Visit 3 Day 29 within each baseline µPRNT serostatus and age group. In addition, the two-sided exact (Clopper-Pearson) 95% CIs for the SCR will be presented.

For each age group and baseline µPRNT serostatus at Visit 3 Day 29, the p-value from an exact binomial test for the null-hypothesis H_0: SCR ≤ 70% against the alternative H_1: SCR > 70% with a one-sided significance level of 2.5% will be provided.

Finally, the difference in proportion between the two age groups (12 to < 15 years group minus 15 to <18 years group) within baseline µPRNT serostatus will be provided alongside the two-sided exact 95% CIs (Chan-Zhang) and the p-value Fisher's Exact test for Day 29, Day 180 and Month 12.

13.6.4.4 Fold-increase in Neutralizing Antibody Titer

A descriptive summary of the continuous fold-increase in CHIKV-specific $\mu PRNT_{50}$ result from baseline will be produced for all post-baseline timepoints. This summary will include n, mean, SD, median, Q1, Q3 and range.

In addition, a categorical summary of subjects reaching at least 4-fold, 8-fold, 16-fold, and 64-fold increase in $\mu PRNT_{50}$ compared to baseline will be produced for all post-baseline timepoints. Two-sided Clopper-Pearson 95% CIs for the percentages will be presented.

Values of GMFI will be analyzed in the same way as for GMTs.

13.7 Exploratory Endpoints

13.7.1 Incidence of CHIKV infections

All occurrences of possible CHIKV infections and clinical manifestations after 14 days post-vaccination will be analyzed based on classification of CHIKV cases.
A summary of the number of CHIKV cases meeting the following criteria will be tabulated:

- All definite, probable and asymptomatic CHIKV cases
- All definite and probable CHIKV cases
- All definite CHIKV cases
- All probable CHIKV cases
- All asymptomatic CHIKV cases

Two Excel listings will be produced to support the classification of the CHIKV cases:

- Listing of specific lab results (CHIKV PCR from DASA, CHIKV IgG ELISA, CHIKV IgM ELISA, Dengue PCR, Dengue IgG ELISA, Dengue IgM ELISA, Dengue IgG from Cerba, Zika PCR, Zika IgG ELISA, Zika IgM ELISA, Zika IgG from Cerba, CHIKV RT-qPCR from Nexelis, Mayaro virus IgG from Cerba, µPRNT titer from Nexelis, Rheumatoid factor, ACPA, Ferritin and CRP) by visit and date,
- Listings of specific AEs (see Appendix 16.1.2) alongside with immunogenicity results (>4-fold increase from baseline at Day 8, Day 29, Day 85, Day 180 and Month 12. More than 4-fold increase from Day 29 at Day 85, from Day 85 at Day 180, from Day 85 at Month 12 and from Day 180 at Month 12).

The listings will be provided to Valneva for their initial case ascertainment, and then the DMSB will adjudicate the classification of the infections.

Final classification of the CHIKV infections will be done once the database has been frozen (before the final lock). However, intermediate assessments of the CHIKV infection will be performed for Part B reporting and a description of the CHIKV infections will be included in the CSR from the adjudicated review performed by the DSMB.

The proportion of subjects with CHIKV cases as lined out above will be compared between the treatment arms by Fisher's exact test and exact score 95% confidence intervals (Chan-Zhang method) will be calculated. The denominator for the percentage will be the number of subjects in the analysis set. The Safety population will be used for these analyses. This table will be produced for data from the whole study

period (i.e. at Part C). As the CHIKV case classification requires the RT-qPCR and/or immunogenicity results, assessment will not be done at Part A. At Part B, a preliminary CHIKV case ascertainment and classification will be performed by the Sponsor and discussed with an independent DSMB, with findings of the intermediate assessment being shown in the Part B CSR. Final categorization of potential CHIKV infections and CHIKV analyses will be performed at Part C.

These summaries of CHIKV infections will be an exploratory analysis, and no inferences will be made from any p-values, apart from in an exploratory manner. These will be descriptive only, and will not form the basis of a formal test of significance. CHIKV infection classifications will be listed for Part C.

13.7.2 CHIKV disease signs and symptoms

Information on the CHIKV disease clinical spectrum and associated risk factors in an adolescent population will be collected and listed. No separate presentation is planned for this endpoint, but laboratory results and CHIKV assessment from Acute and Convalescent Visits following suspected CHIKV infection will be presented within the safety listings.

13.8 Safety Analyses

13.8.1 Adverse Events

Safety tabulations will include both solicited AEs and unsolicited AEs, unless otherwise specified. Number and proportion of subjects, plus number of events in each category will generally be presented. Two-sided exact (Clopper-Pearson) 95% CIs will be provided for overall AE rates in the summary AE table, and by SOC and PT. Differences between the study arms will be assessed for significance using a two-sided Fisher's exact test for overall rates.

Summaries of AEs categorized by SOC and PT will be provided. 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 PT 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 tables will be produced by baseline $\mu PRNT_{50}$ serostatus in addition to both baseline serostatus strata combined unless stated otherwise.

All AE tabulations will be presented for the Safety population.

All AEs recorded on the CRF will be listed.

13.8.1.1 Secondary Adverse Event Analyses

The following tabulations will be produced to analyze the secondary safety endpoints:

- All unsolicited AEs up to study Day 29 (Visit 3) and Day 180 (Visit 5) 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 and Part B. Part C in the case of ongoing events)
- Solicited injection site and systemic reactions within 10 days post-vaccination by PT (Part A. Part B in the case of ongoing events. For Part C, Fisher's exact test will be presented for each PT)
- Solicited injection site and systemic reactions within 10 days post-vaccination by PT and severity (Part A. Part B in the case of ongoing events)

- All AEs during the entire study period by SOC and PT (Parts A, B and C)
- All AEs during the entire study period by SOC, PT and severity (Parts A, B and C)
- All Related AEs during the entire study period by SOC and PT (Parts A, B and C)
- SAEs by SOC and PT during entire reporting period (Parts A, B and C)
- Related SAEs by SOC and PT during entire reporting period (Parts A, B and C)
- Early Onset AESI within 2 to 21 days post-vaccination by SOC and PT (Part A)
- Early Onset AESI ongoing after Part A by SOC and PT (Parts B, C)
- Late Onset of AESI by SOC and PT (Parts A, B and C)
- Late Onset of AESI by SOC, PT and severity (Parts A, B and C)
- Related Early Onset AESI within 2 to 21 days post-vaccination by SOC and PT (Part A. Part B in case of ongoing events)
- Related Late Onset AESI by SOC and PT (Parts B and C in case of ongoing events)

All AESIs recorded on the CRF will be listed.

13.8.1.2 Overall Summary of Adverse Events by Age Group Analyses

The summary of overall AEs (detailed in Section 13.8.1.4 will be repeated for Part C but will be tabulated for the following groups:

- By treatment group and age groups (12 to < 15 years, 15 to <18 years old and overall). Differences between the 12 to < 15 years and 15 to <18 years age groups within each treatment group will be assessed for each categories using Fisher's Exact test of independence; the p-value from the Fisher's Exact test will be presented
- Within each treatment group, by baseline µPRNT serostatus (seronegative and seropositive) and by age groups (12 to < 15 years, 15 to <18 years old and overall). Differences between the 12 to < 15 years and 15 to <18 years age groups within each baseline µPRNT serostatus and each treatment group will be assessed for each categories using Fisher's Exact test of independence; the p-value from the Fisher's Exact test will be presented

13.8.1.3 USPI FDA Broad-Definition of Chikungunya-like Adverse Reactions Analyses

For Part C only, the number and percentage of cases and severe cases as well as the number of associated symptoms of chikungunya-like ARs will be tabulated overall and for each age groups (12 to < 15 years and 15 to <18 years) by treatment group and by baseline µPRNT serostatus.

In addition, the number and percentage of cases and subjects with any symptom(s) of chikungunya-like ARs will be summarized along with the number and percentage of subjects for each symptom categorized by SOC and PT by treatment group and by baseline µPRNT serostatus. The summary will be repeated for severe cases and associated symptoms.

Cases and symptoms of chikungunya-like ARs that occurred up to 30 days after vaccination will be listed. The listings will be repeated for severe case and associated symptoms.

13.8.1.4 Other Adverse Event Analyses

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 related solicited AE, any solicited

AE ongoing after day 11, any severe solicited AE, any related solicited AE, any related severe solicited AE, any solicited injection site reaction, any severe solicited injection site reaction, any related severe solicited injection site reaction, any solicited systemic AE, any severe solicited systemic AE, any related 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 AESI symptom as assessed by the investigator, any related AESI as assessed by the investigator, any early onset AESI as assessed by the investigator, any early onset AESI as assessed by the DSMB, any early onset AESI symptom as assessed by the investigator, any related early onset AESI as assessed by the investigator any late onset AESI as assessed by the investigator, any late onset AESI as assessed by the DSMB, any late onset AESI symptom as assessed by the investigator, any related late onset 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 study, any unsolicited AE leading to withdrawal from study and any solicited AE leading to withdrawal from study. For each study arm 95% CIs will be presented for percentages. Differences between the study arms will be assessed for significance using Fisher's exact test. (Parts A, B and C)

- AE summary table as above, split by whether the subject was µPRNT seropositive or seronegative at baseline (Parts A, B and C) AE summary table as above, split by µPRNT serostatus at baseline but only for the VLA1553 treatment group. Differences assessed for significance using Fisher's exact test will be between baseline serostatus as opposed to study arm. (Part C)
- Related unsolicited AEs up to Day 180 (Visit 5) by SOC and PT (Parts A and B. Part C in case of ongoing events)
- Related unsolicited AEs up to Day 180 (Visit 5) by SOC, PT and maximum severity (Parts A and B. Part C in case of ongoing events)
- Solicited injection site and systemic reactions by PT, sensitivity excluding recall data (Part A and repeated at Part B)
- Solicited injection site and systemic reactions by PT, sensitivity including Diary-only events (Part A and repeated at Part B)
- Solicited injection site and systemic reactions by PT and maximum severity, sensitivity including Diary-only events (Part A and repeated at Part B)
- Related solicited injection site and systemic reactions (Part A and repeated at Part B)
- Related solicited injection site and systemic reactions by maximum severity (Part A and repeated at Part B)
- Related solicited injection site and systemic reactions by maximum severity, sensitivity including Diary-only events (Part A and repeated at Part 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 and repeated at Part B)
- Summary of duration of solicited local and systemic reactions. Summary statistics for duration of solicited reaction (days) will be presented by symptom in each study arm (Part A and repeated at Part B)
- Unsolicited AEs by SOC and PT (Part C)
- Related unsolicited AEs by SOC and PT (Part C)
- Any related unsolicited severe AE by SOC and PT (Part C)

- AESI by SOC, PT and maximum severity (Parts A, B and C)
- Related early onset AESI by SOC, PT and maximum severity (Parts A, B and C in case of ongoing events)
- Related late onset AESI by SOC and PT (Parts B and C)
- Related late onset AESI by SOC, PT and maximum severity (Parts B and C)
- Maximum fever temperature post-vaccination up to Day 11, for subjects who experienced fever (Part A and repeated at Part B)
- Any AE occurring at a frequency of at least 10% in at least one study arm by PT (Parts A, B and C)
- Any AE occurring at a frequency of at least 1% in at least one study arm by PT (Parts A, B and C)
- Any related AE occurring at a frequency of at least 10% in at least one study arm by PT (Parts A, B and C)
- Any related AE occurring at a frequency of at least 1% in at least one study arm by PT (Parts A, B and C)
- Any medically attended AE by SOC and PT (Parts A, B and C)
- Any medically attended AE by SOC, PT and Maximum Severity (Parts A, B and C)
- Any AE leading to withdrawal from study by SOC and PT (Parts A, B and C)
- Any non-serious AE occurring at a frequency of at least 5% in at least one study arm by SOC and PT (Parts A, B and C)

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 reaction, by study arm and repeated by study arm and µPRNT serostatus. For each treatment group, the frequency of solicited fever and arthralgia events will be presented by way of bar charts stacked by severity and grouped by duration (1-5 days, 6-10 days, 11-15 days, 21-25 days, 26-30 days and 31-35 days). The bar chart will be repeat with the event duration imputed as per the rules in section 11.4.8. The same bar chars will be repeated for each Baseline µPRNT serostatus.

Adverse events of arthralgia or arthritis will be listed in a separate output by treatment arm only (not duration).

In addition, a table of subject diary compliance will be produced at Part A. This tabulation will show a categorical summary of the number of subjects who completed at least one entry on each day of the diary by diary day, as well as a continuous summary of the number of days entered (where a day is classed as entered if it has at least one symptom or temperature record present). All subject diary data will be listed.

13.8.2 Viremia

As per the protocol, subjects in the viremia subset of the immunogenicity subset will have viremia samples collected at Visits 1, 2 and 3. Samples from Visit 1 and 2 will be analyzed. The collected viremia sample of Visit 3 will only be analyzed if the sample at Visit 2 is a positive result, a below LLOQ result or an inconclusive result. In addition, for clinically indicated retrospective analysis viremia samples will be collected throughout the study from all subjects.

As acute and convalescent visits can be performed on the same day as a planned visit, such samples collected on the planned visits (Visit 2 Day 8 and Visit 3 Day 29) but associated with acute or convalescent visit will be considered for the data tabulation only of subjects included in the viremia subset. In addition to

the previous rules, Visit 3 Day 29 results will be analyzed if Visit 2 Day 8 samples are delayed (and falls within Visit 3 Day 29 visit window), i.e. the Visit 2 Day 8 results are deemed inconclusive in that case. Viremia visits mapping is described in section 11.2.1.1.

Descriptive summaries of viremia data by planned visit (Visit 1 Day 1, Visit 2 Day 8 and Visit 3 Day 29) and serostatus will be provided for active and placebo subjects. In the summary of viremia results, the count of subjects with a viremic result (split into Quantifiable and Below LLOQ), non-viremic result (including "<LLOD" [lower limit of detection] or "Not Detected") or inconclusive result will be displayed by timepoint. For all quantifiable results, the mean, min and max will be presented. Only the samples of the viremia subset planned to be analyzed by the specialty lab will be included in the summary, i.e. at Visit 3 Day 29 only results associated with Visit 2 Day 8 viremic results or Visit 2 Day 8 inconclusive results will be summarized.

In addition, a line plot of viral load, measured in genome copy equivalents (GCE) per ml, over time will be presented by $\mu PRNT_{50}$ serostatus and treatment group. A boxplot of viral load by $\mu PRNT_{50}$ serostatus stratification will also be produced for active arm subjects only. For Viremia plots, results of "Not Detected" are to be imputed as 0 GCE/mL.

All available viremia sample results of regular Visits 1, 2 (and Visit 3 if applicable) will be listed at each study Part.

Viremia samples are also collected and analyzed for Acute and Convalescent visits throughout the study for all subjects with suspected CHIKV cases for analysis of the exploratory endpoints. Sequencing of viremia samples were performed to identify the type of virus (wild-type or vaccine). Results will be listed for Part C.

13.8.3 Laboratory Data

Safety laboratory values will be assessed for all subjects at baseline, and for the immunogenicity subset post-baseline. Laboratory values will be evaluated according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA). Clinically significant laboratory values will be documented as AEs.

Changes in laboratory values from study entry will be analyzed descriptively for clinical chemistry, hematology, coagulation and urinalysis. System International units will be used for all laboratory parameters.

Note that for the summary of urinalysis parameters, due to a free-text field for Urobilinogen results, and results being reported in a mixture of Portuguese and English, some results are grouped for the reporting of categorical results. For presentation, any results starting with 'POS' (ie POSITIVE and POSITIVO) are displayed as POSITIVE. Similarly results starting with 'NEG' (NEGATIVE and NEGATIVO) are displayed as NEGATIVE.

The rate of subjects with urinalysis results according to the test manufacturer's results categories will be calculated overall and by visit.

Baseline values will be summarized separately for subjects in the safety population and subjects in the immunogenicity subset.

The rates of subjects with laboratory assessments with maximum post-baseline grade falling into the grade 0 vs. 1 through 4 will be calculated. Shift tables of laboratory results by grade will be presented for the maximum postbaseline grade. For urinalysis parameters, shift tables of laboratory results by whether normal or abnormal will be produced by timepoint.

COVID-19 testing results will be tabulated by timepoint and test type (Antigen Rapid Test vs RT-PCR confirmatory test if done). Covid-19 results will also be listed.

In addition, retrospective testing of antibodies to other alphaviruses at baseline, and retrospective Viremia results will be listed for those subjects where testing was done.

All laboratory data will be listed.

Laboratory results also collected throughout the study for all subjects with suspected CHIKV cases will be listed.

13.8.4 Vital Signs

Vital signs will be summarized descriptively at each study timepoint they are collected, including screening, Day 1 pre-vaccination and Day 1 post-vaccination. 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 signs data will be listed.

13.8.5 Physical Examinations and Other Observations Related to Safety

A summary table of 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.

Travel data will be listed in a by-subject listing, categorized into travel within or outside of Brazil.

13.8.6 Subject Diary Entry Compliance

A summary of subject diary entry compliance based on the Safety population will be produced at Part A to support the reporting of solicited AEs. This summary will include the number and percentage of subjects with at least one non-missing diary entry on each study day (1-11) and a descriptive summary of number of diary days with entered data, tabulated by baseline $\mu PRNT_{50}$ serostatus, baseline serostatus strata combined and by treatment groups (and overall). A diary day is classed as having data entered if there is at least one symptom or temperature recording present on that day.

14.0 References

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".

15.0 Glossary of Abbreviation

Glossary of Abbreviations:	
ADaM	Analysis Data Model
AE	Adverse event
AESI	Adverse Event of Special Interest
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AR	Adverse reaction
ATC	Anatomic Therapeutic Classification
BDRM	Blind Data Review Meeting
BMI	Body Mass Index
CI	Confidence Interval
CHIKV	Chikungunya Virus
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Clinical Research Organization
CRP	C-Reactive Protein
CSR	Clinical Study Report
DSMB	Data Safety Monitoring Board
ELISA	Enzyme-Linked Immunosorbent Assay
ESR	Erythrocyte sedimentation rate
ET	Early Termination
FDA	Food and Drug Administration
GCE	Genome Copy Equivalents
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
GSD	Geometric Standard Deviation
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form

ICH	International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use
Ig	Immunoglobulin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMM	Immunogenicity
IMP	Investigational Medicinal Product
IRT	Interactive Response Technology
LLOQ	Lower Limit of Quantification
MAR	Missing At Random
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple Imputation
mPP	Modified Per Protocol
NT	Neutralizing Titer
PCR	Polymerase Chain Reaction
PD	Protocol Deviation
PP	Per Protocol
PRNT50	Antibody titer at 50% virus neutralization determined by µPRNT assay
PSO	Predictivv Study Operations
PT	Preferred Term
Q1	First Quartile
Q3	Third Quartile
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SCR	Seroconversion Rate
SD	Standard Deviation
SOC	System Organ Class
SRR	Seroresponse Rate
USPI	United States Prescribing Information
WHODrug	World Health Organization Drug Dictionary
µPRNT	Micro Plaque Reduction Neutralization Test

16.0 Appendix

16.1.1 AESI according to USPI FDA Broad Definition of Chikungunya-like AR

As per USPI-FDA broad definition of chikungunya-like AR are those AR that meet the following conditions.

1. Fever (PT of Pyrexia)

AND

2. Any symptom of

   SOC Musculoskeletal and connective tissue disorders and PT: arthralgia, arthritis, polyarthritis, polyarthralgia, myalgia, joint pain, back pain
   SOC Nervous system disorders: all PTs
   SOC Cardiac disorders: all PTs
   SOC Skin and subcutaneous tissue disorders: all PTs
   SOC Blood and lymphatic system disorders: all PTs
   SOC Eye disorders and PT: optic neuritis, retinitis/uveitis

AND

3. Onset of symptoms 0 to 30 days post vaccination, regardless of the order of onset and duration. (no need of overlapping symptoms)

16.1.2 Chikungunya-like AR Preferred Terms

The following are the PTs considered for chikungunya-like ARs:

- SOC General disorders and administration site conditions – all PTs with the exception of Asthenia, Chest pain, Inflammation, Injection site erythema, Injection site induration, Injection site pain, Injection site pruritus, Injection site swelling, Nodule, Tenderness, Vaccination site pain.
- SOC Musculoskeletal and connective tissue disorders – PTs: arthralgia, arthritis, polyarthritis, polyarthralgia, myalgia, joint pain, back pain
- SOC Nervous system disorders – all PTs with the exception of Dizziness, Dizziness postural, Sciatica, Somnolence, Syncope
- SOC Skin and subcutaneous tissue disorders – all PTs with the exception of Dermatitis contact, Eczema, Sensitive skin
- SOC Blood and lymphatic system disorders – all PTs with the exception of Eosinophilia, Leukopenia, Lymphadenopathy, Lymphocytosis, Lymphopenia, Neutropenia
- SOC Eye disorders – PTs: optic neuritis, retinitis/uveitis