Statistical Analysis Plan (SAP)

Protocol Title:	AN OPEN-LABEL, SINGLE ARM STUDY TO EVALUATE ANTIBODY PERSISTENCE AND LONG TERM SAFETY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADULTS AGED 18 YEARS AND ABOVE
Protocol Version No./Date:	4.0 / 12Jun-2023
CRF Version No./Date:	7.0 / 07-Jul-2022
SAP Version No./Date:	2.0 / 11-Jul-2023

1.0 Approvals

Sponsor	
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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
1.0 / 06-Oct-2022	Initial version of the SAP
2.0 / 11-Jul-2023	**General:** • Change from 'Seroprotection' to 'Seroresponse' throughout • A revised titer threshold was introduced for subjects who are discontinued from the study, to reflect the titer level distinguishing between seropositivity and seronegativity **Section 6.1 Change from Protocol** ○ Added clarification that the per-protocol and whole analysis sets were defined in the Statistical Analysis Plan (as not included in the protocol) ○ Added clarification that not permitted medications are considered an intercurrent event and does not lead to exclusion from the per-protocol population. ○ Clarify that the model used to derive the annual change rate is different from the model described in the protocol. ○ Clarified that Seroprotection' to 'Seroresponse' for consistency with other studies on the same vaccine. ○ Added clarification that some imputation of missing data will be implemented **Section 9.3 Estimand Attributes** • Clarified the definition of intercurrent events – outside of visit window, discontinuation due to μPRNT$_{50}$ ≤40 and event likely to interfere with immune response and associated imputation (where applicable). • Added a potential sensitivity analysis for Part E for the imputation of missing data. • Corrected the definition of the per-protocol (PP) set definition to be consistent with Section 10.2. • Added exploratory analysis table for Annual Rate of Change on WS population **Section 10.0 Population Sets** • Added the invited population for consistency with Table 14.1.1.1 (Subject analysis set). **Section 10.2 Per-Protocol Population** • Definition of the PP population has been redefined to allow the definition of intercurrent events. **Section 10.3 Sensitivity Per-Protocol Population** • Clarified that the sensitivity analyses for sPP1, sPP2 and sPP3 will only occur at Part A and not in future study parts. • Updated the definition of the sPP1, sPP2 and sPP3 to remain identical to previous version even though the definition of the PP population has been revised. **Section 10.4 Invited Population** • Section added to provide the definition of the invited subjects that is used in Table 14.1.1.1 (Subject Analysis Set) **Section 11.2 Study Year and Visit Windows** • Clarified that visit window is only applicable to the PP set analysis, unless it is used to map the end of study visit for the whole sample set. • Added visit windowing rules for WSP analysis; windows have been widened to allow out of window (according to the protocol) visits to be mapped to each study parts for analysis **Section 11.3 Immunogenicity Endpoints:**

	• Added imputation rules for µPRNT50 titers reported as <20. Also, added details regarding discontinued subjects with value µPRNT50 ≤40. • Clarified that the analysis is only relevant for Part A. **Section 11.3.2 Seroconversion** • The sensitivity analysis for the seroconversion rate has been removed for analysis after Part B. **Section 11.4.3 Ongoing Adverse Events of Special Interest** • Added a clarification as there were no ongoing adverse event of special interest (AESI) at the end of the VLA1553-301 study. **Section 11.5 Missing Data** • Added details on the imputation to be consistent with Section 9.3. **Section 11.7 Prior and Concomitant Medications** • Added clarification of the concomitant medications that are collected in the VLA1553-303 study. **Section 14.0 Statistical Methods** • Added reference to Section 11.5 for the missing data **Section 14.2 Protocol Deviations** • Revised to state that COVID-19 Protocol Deviations will only be summarized at Part A and B **Section 14.5.2 Primary Immunogenicity Analysis** • Details on the formal testing has been removed as it is not detailed in the protocol and not required. **Section 14.5.3.1 Geometric Mean Titer** • Revised the description of the analysis of annual rate of change. • Added additional steps to be implemented if the mixed model with repeated measures is not converging. • Added Kenward-Rogers degrees of freedom will be used. • Added details that Annual Rate of Change analysis will also be performed on the WS population. **Section 14.5.1 Other Immunogenicity Endpoints** • Section removed as no subject are baseline positive (so the analysis is not relevant). **Section 16.0 Glossary of Abbreviations** Added Mixed Model with Repeated Measures (MMRM) and revised seroprotection rate (SPR) to Seroresponse Rate (SRR)

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 VLA1553-303.

5.0 Scope

The Statistical Analysis Plan outlines the following:

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

This SAP covers the analysis for each study part; Part A (Visit 1, Year 1), Part B (Visit 2, Year 2), Part C (Visit 3, Year 3), Part D (Visit 4, Year 4) and Part E (Visit 5, Year 5).

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.

An earlier version of this document was approved prior to data base lock at Part A. An updated version 2.0 will be approved prior to database lock for the Part B.

6.1 Changes from Protocol

The definition of the invited population, per-protocol population and whole sample population has been included in the SAP (the populations were not defined in the protocol).

The definition of serious adverse events in section 11.4.1 is altered slightly from the protocol to match the collection of data on this study.

A set of sensitivity analyses for immunogenicity endpoints has been added, based on the updated cutoff value of 20. The sensitivity analyses will be carried out for Part A (Visit 1, Year 1) of the study. The justification for this is that based on the results obtained during study VLA1553-301 Part A, where it was observed that some baseline samples as well as sera from subjects obtained after placebo treatment tested with $\mu PRNT_{50}$ values close to the $\mu PRNT_{50}$ lower limit of quantitation of 20. In order to increase stringency for seropositivity the definition was changed from $\mu PRNT_{50} \geq 20$ to >40 in the main analysis, and a sensitivity analyses (detailed in section 10.3) is added using the original cutoff value. In addition, seroconversion is defined as a >4- fold increase of $\mu PRNT_{50}$ compared to baseline (Day 1). Therefore, the original VLA1553-301 definition of seroconversion as µPRNT50 of ≥20 for baseline negative subjects is no longer applicable in the main analysis and only used in the sensitivity. A new sensitivity definition of seroconversion has been added in section 11.3.2 and an additional analysis set to be used for the sensitivity analysis based on the updated baseline definition is defined in section 10.3. The additional outputs to be repeated using these definitions are outlined in section 14.5.2.2.1.

The protocol specifies that the decline in rate of antibody titers will be estimated using log-linear regression. In this SAP, the model has been updated to a mixed model with repeated measures on the log-titer values. This is to account for the inter-subject variability by the random intercept, and to account for the stratifying factors of baseline titer value and age stratum as fixed effect covariates. The yearly change is modelled by a fixed linear effect of time (years) within the model. Full model details are specified in section 14.5.3.1.

In the protocol, the intake of not permitted medications may lead to the exclusion from the per-protocol population, however to be within the estimand framework the intake of not permitted medication will be

defined as an intercurrent event instead, and the titers associated with that event may be excluded from the pre-defined analysis.

7.0 Study Objectives

7.1 Primary Objective

- To evaluate persistence of antibodies annually from 1 to 5 years after the single immunization with VLA1553.

7.2 Secondary Objective

- To evaluate long-term safety (i.e. serious adverse events [SAEs]) 6 months to 2 years after the single immunization with VLA1553.

8.0 Study Design

This is a prospective, multicenter, open-label Phase 3b, single arm clinical study evaluating the persistence of antibodies and long-term safety i.e. SAEs in subjects recruited from the VLA1553-301 study. In this precursor study 4,060 male and female subjects aged 18 years and above were randomized in a ratio 3:1 to either the final dose of VLA1553 or placebo. The first enrolled and randomized approximately 500 subjects constituted the initial group of subjects, i.e. the immunogenicity subset of study VLA1553-301, that will be approached to join VLA1553-303. Thereafter, additional subjects will be approached in a predefined manner (see Section 7.4.1 of the study protocol) to achieve a more robust number of participants in study VLA1553-303. Overall, up to 375 subjects vaccinated with VLA1553 as part of study VLA1553-301 will be recruited into the study and stratified into two age strata of subjects aged 18 to 64 years (Stratum A) and subjects of 65 years of age or above (Stratum B). This subject population will be enrolled at approximately 13 pre-selected study sites across the United States (U.S). representative of the whole study population.

All subjects will be asked to return to the study site for Visit 0 of VLA1553-303, followed by Year 1 (Visit 1), Year 2 (Visit 2), Year 3 (Visit 3), Year 4 (Visit 4) and Year 5 (Visit 5) for immunogenicity sampling. Visit 0 may be performed as a visit combined with Visit 5 of study VLA1553-301, or will be performed as a separate study visit, if participants already had their VLA1553-301 Visit 5 before joining study VLA1553-303. If subjects titers fall below µPRNT50 ≤ 40 during immunogenicity sampling they will have an end of study visit at the next scheduled visit. Previously to the protocol version 4.0, the seroresponse threshold which would trigger the end of study of a subject was $\mu PRNT_{50}$ <150. In the protocol version 4.0, this threshold has been revised to a titer $\mu PRNT_{50}$ ≤40 which reflects the titer level distinguishing between seropositivity and seronegativity, for Part B onwards. No subjects have been discontinued from the study due to their $\mu PRNT_{50}$ titers falling below 150.

SAEs will also be assessed up until Year 2 in all subjects. Moreover, any AESIs reported to be ongoing at the point in time the subject rolls over to study VLA1553-303 will be monitored. The clinical study design is displayed in Figure 8-1 below.

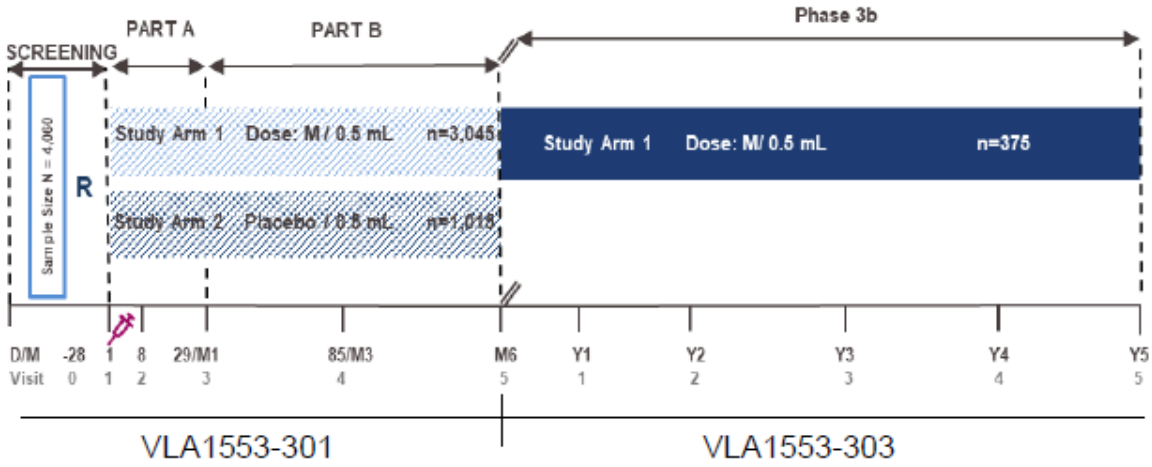

Figure 8-1 Open-label Phase 3b Study Design

The overall study duration (First Subject In - Last Subject Out) is estimated to be approximately 57 months.

Individual subject participation is approximately 54 months from enrollment to study completion unless prematurely discontinued.

8.1 Sample Size Considerations

No formal sample size calculation was performed as this is a long-term safety follow up of subjects in an existing study (VLA1553-301). Up to 375 subjects in study VLA1553-301 will participate in this study.

However, with a sample size of 375 (maximum expected patients, assuming no drop-out), two-sided exact (Clopper-Pearson) 95%-confidence intervals for an actual Seroresponse rate (SRR) of 60% will have a width of 10.2%. Confidence intervals for an SRR of 80% will have a width of 8.3%.

The expected drop-out rate within the study is likely to be higher than the initial estimate above. Hence confidence intervals will be wider than predicted. No formal re-estimation is included due to the observational nature of this study.

8.2 Randomization

Subjects in the VLA1553-301 study were allocated in a 3:1 ratio to VLA1553 (n= approximately 3,045) or placebo (n= approximately 1,015). The approximately 4,060 subjects in this study were stratified into two age strata of subjects aged 18 to 64 years (Stratum A: overall approximately 3,653 subjects) and subjects of 65 years of age or above (Stratum B: overall approximately 407 subjects). The first enrolled and randomized approximately 500 subjects constituted the initial group of subjects (i.e., the immunogenicity subset of study VLA1553-301) that will be approached to join VLA1553-303. Thereafter, additional subjects were approached in a predefined manner (see Section 7.4.1 of the study protocol) to achieve a more robust number of participants in study VLA1553-303.

9.0 Study Endpoints, Variables and Covariates

9.1 Primary Endpoints

- Proportion of subjects with seroresponse defined as $\mu PRNT_{50}$ ≥150 for baseline negative subjects at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination

9.2 Secondary Endpoints

Safety

- Frequency and relatedness of any serious adverse event (SAE) until Year 2.

Immunogenicity

- Immune response as measured by CHIKV-specific neutralizing antibody titers at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as determined by µPRNT assay;
- Proportion of subjects with seroconversion at Year 1, Year 2, Year 3, Year 4 and Year 5 as determined by µPRNT assay;
- Fold increase of CHIKV-specific neutralizing antibody titers determined by µPRNT assay at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as compared to baseline antibody titers at start of study VLA1553-301;
- 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.

9.3 Estimand Attributes

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

Objectives	Study Endpoint	Estimand		Study Part	Population
Immunogenicity					
Primary	Primary Endpoints	Primary Estimand			
To evaluate persistence of antibodies annually from 1 to 5 years after the single immunization with VLA1553.	Proportion of subjects with seroresponse defined as $\mu PRNT_{50}$ ≥150 at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination	• Treatment of interest: VLA1553 • Population of interest: Subjects in the per protocol (PP) analysis population, i.e. those who received a vaccination, who were CHIKV seronegative at baseline, had no major protocol deviations and have at least one non-missing post baseline immunogenicity sample in the VLA1553-301 study (within SAP visit windows for study VLA1553-301). Samples for at least one of the following timepoints are required but also several are allowed: 29d, 85d or 180d. • Variables of interest: Seroresponse ($\mu PRNT_{50}$ ≥150) status at Year 1, Year 2, Year 3, Year 4 and Year 5 post vaccination • Handling of Intercurrent Events: o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, samples collected out of visit window will be excluded from the analysis. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 will be deemed as not having a seroresponse at each of the post-discontinuation scheduled visits. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response will be excluded from the analysis. • Population level summary: Proportion of subjects with seroresponse over time, accompanied by two-sided exact (Clopper-Pearson) 95% confidence limits		Parts A, B, C, D and E	PP

Objectives	Study Endpoint	Estimand		Study Part	Population
Immunogenicity					
Primary	Primary Endpoints	Primary Estimand			

		Sensitivity Analysis of Primary Endpoint
		• As Primary, except:
		• Population of interest: Whole Sample (WS) population.
		• Handling of Intercurrent Events:
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be included in the analysis.
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5; subjects who discontinued because their μPRNT$_{50}$ titer is ≤ 40 will be deemed as not having a seroresponse at each of the post-discontinuation scheduled visits.
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are included in the analysis.
				Parts A, B, C, D and E	WS

		Additionally, to assess the robustness in the assessment of the seroresponse with regard to missing data, using the same estimand as the one used for primary analyses, missing data will be imputed using a multiple imputation strategy. This additional analysis may be carried out if relevant and will be reported for the Part E only. Further details will be included in a subsequent version of the SAP.		Part E	PP

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
Primary	Secondary	Secondary Estimand		
	Immune response as measured by CHIKV-specific neutralizing antibody titers at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as determined by µPRNT assay	• Treatment of Interest: VLA1553 • Population of interest: Subjects in the PP analysis population. • Variables of interest: Neutralizing antibody titer value at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as determined by µPRNT assay • Handling of Intercurrent Events: ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be excluded from the analysis. ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 will have their $\mu PRNT_{50}$ imputed to 10 at each of the post-discontinuation scheduled visits. ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are excluded from the analysis. • Population-level summaries: 95% confidence intervals for geometric mean titer in each strata group	Parts A, B, C, D and E	PP

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
Primary	Secondary	Secondary Estimand		

		Sensitivity Analysis of Secondary Endpoints
		• As Secondary, except:
		• Population of interest: WS population.
		• Handling of Intercurrent Events:
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be included in the analysis.
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their μPRNT$_{50}$ titer is ≤ 40 will have their μPRNT$_{50}$ imputed to 10 at each of the post-discontinuation scheduled visits.
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are included from the analysis.
			Parts A, B, C, D and E	WS

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
Primary	Secondary	Secondary Estimand		
		Additionally, to assess the robustness in the assessment of the immune with regard to missing data, using the same estimand as the one used for primary analyses of secondary endpoint but removing the single imputation strategy. This additional analysis may be carried out if relevant and will be reported for the Part E only. Further details will be included in a subsequent version of the SAP.	Part E	PP

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
Primary	Secondary	Exploratory Estimand		
		• Treatment of Interest: VLA1553 • Population of interest: Subjects in the PP analysis population. • Variables of interest: Neutralizing antibody titer value at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as determined by µPRNT assay • Handling of Intercurrent Events: o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be excluded from the analysis. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 will have their $\mu PRNT_{50}$ imputed to 10 at each of the post-discontinuation scheduled visits. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are included in the analysis. No explicit imputation will be performed for missing data or results excluded from the analysis, however, the mixed model with repeated measures model will yield unbiased results under the assumption that data are missing at random. • Population-level summaries: Estimates of annual change rate of antibody titers and associated 95% CIs, from a mixed model with repeated measures.	Parts B,C,D and E	PP

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				

		• As Exploratory, except:
		• Population of interest: Subjects in the WS analysis population.
		• Handling of Intercurrent Events:
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be included in the analysis using WS population visit mapping rules
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 will have their $\mu PRNT_{50}$ imputed to 10 at each of the post-discontinuation scheduled visits.
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are included in the analysis.
			Parts B,C,D and E	WS

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
	Secondary	Secondary Estimand		
	Proportion of subjects with seroconversion at Year 1, Year 2, Year 3, Year 4 and Year 5 as determined by μPRNT assay	• Treatment of Interest: VLA1553 • Population of interest: Subjects in the PP analysis population. • Variables of interest: Seroconversion (>4-fold increase of $\mu PRNT_{50}$ compared to baseline, defined as $\mu PRNT_{50} > 40$) status at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination. • Handling of Intercurrent Events: o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be excluded from the analysis. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 will be deemed as not having reached seroconversion at each of the post-discontinuation scheduled visits. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are excluded in the analysis. • Population-level summaries: 95% confidence intervals for seroconversion (>4-fold increase of $\mu PRNT_{50}$) rate in each strata group	Parts A, B, C, D and E	PP

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
	Secondary	Secondary Estimand		
		Sensitivity Analysis of Secondary Endpoints • As Secondary, except: • Population of interest: WS population. • Handling of Intercurrent Events: o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be included in the analysis. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their μPRNT$_{50}$ titer is ≤ 40 will be deemed as not having reached seroconversion at each of the post-discontinuation scheduled visits. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are included in the analysis.	Parts A, B, C, D and E	WS

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
	Secondary	Secondary Estimand		
	Fold increase of CHIKV-specific neutralizing antibody titers determined by µPRNT assay at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as compared to baseline antibody titers at start of study VLA1553-301	• Treatment of Interest: VLA1553 • Population of interest: Subjects in the PP analysis population. • Variables of interest: Fold-increase from baseline in the CHIKV-specific $\mu PRNT_{50}$ ($\mu PRNT_{50}$ result at nominal time point / $\mu PRNT_{50}$ result at baseline) at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination • Handling of Intercurrent Events: ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be excluded from the analysis. ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 will have their $\mu PRNT_{50}$ titer imputed to by their $\mu PRNT_{50}$ baseline titer at each of the post-discontinuation scheduled visits. ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are excluded from the analysis. • Population-level summaries: Descriptive summary of continuous fold-increase from baseline in the CHIKV-specific $\mu PRNT_{50}$ ($\mu PRNT_{50}$ result at nominal time point / $\mu PRNT_{50}$ result at baseline) values.	Parts A, B, C, D and E	PP

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
	Secondary	Secondary Estimand		
		Sensitivity Analysis of Secondary Endpoints • As Secondary, except: • Population of interest: WS population. • Handling of Intercurrent Events: o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be included in the analysis. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 will have their $\mu PRNT_{50}$ titer imputed to by their $\mu PRNT_{50}$ baseline titer at each of the post-discontinuation scheduled visits. o Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are included in the analysis.	Parts A, B, C, D and E	WS

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
	Secondary	Secondary Estimand		

	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	• Treatment of Interest: VLA1553
		• Population of interest: Subjects in the PP analysis population.
		  ○ Variables of interest: Fold-increase (NT result at nominal time point / NT result at baseline at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination
		  ○ Handling of Intercurrent Events:
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be excluded from the analysis.
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their µPRNT$_{50}$ titer is ≤ 40 will have their µPRNT$_{50}$ titer imputed to by their µPRNT$_{50}$ baseline titer at each of the post-discontinuation scheduled visits.
		  ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are excluded in the analysis.
		• Population-level summaries: Categorical summary of the rate of subjects reaching prespecified thresholds for fold-increase (µPRNT$_{50}$ result at nominal time point / µPRNT$_{50}$ result at baseline) in µPRNT$_{50}$, including 95% confidence intervals for percentages.
			Parts A, B, C, D and E	PP

Objectives	Study Endpoint	Estimand	Study Part	Population
Immunogenicity				
	Secondary	Secondary Estimand		
		Sensitivity Analysis of Secondary Endpoints • As Secondary, except: • Population of interest: WS population. • Handling of Intercurrent Events: ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5 samples collected out of visit window will be included in the analysis. ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, subjects who discontinued because their μPRNT$_{50}$ titer is ≤ 40 will have their μPRNT$_{50}$ titer imputed to by their μPRNT$_{50}$ baseline titer at each of the post-discontinuation scheduled visits. ○ Respectively for Year 1, Year 2, Year 3, Year 4 and Year 5, titers associated with events likely to interfere with immune response are included in the analysis.	Parts A, B, C, D and E	WS

Objectives	Study Endpoint	Estimand	Study Part	Population
Safety				
	Secondary	Secondary Estimand		
To evaluate long-term safety (i.e. SAEs) 6 months to 2 years after the single immunization with VLA1553	Frequency and relatedness of any serious adverse event (SAE) until Year 2.	• N/A	Parts A, B	WS

10.0 Population Sets

10.1 Whole Sample Population

The Whole Sample (WS) population contains all subjects who entered into the VLA1553-303 study and received VLA1553 in study VLA1553-301 and with post-baseline immunogenicity sample available from the VLA1553-301 study. Samples for at least one of the following timepoints are required but also several are allowed: 29d, 85d or 180d. Subjects also need a Visit 1 record on the VLA1553-303 study to be included in the WS population. This will include all subjects who had an informed consent in the VLA1553-303 study and met all inclusion/exclusion criteria to be enrolled into the study and had a Visit 1 record in VLA1553-303.

This analysis set is used for all demographic and safety reporting, as well as for sensitivity analyses of immunogenicity endpoints.

10.2 Per Protocol Population

The PP contains all subjects who were randomized and vaccinated with VLA1553 in study VLA1553-301, who were CHIKV seronegative at baseline (defined as $\mu PRNT_{50}$ titer ≤40), and have at least one or more evaluable post-baseline titer measurements of either 29d, 85d or 180d (within SAP visit windows for study VLA1553-301). Subjects with no Visit 1 performed were also excluded from the per-protocol analysis.

In addition, the subjects must have no major protocol deviations at the start of VLA1553-303 study being analyzed 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);
- Temperature excursion of the vaccine in study VLA1553-301.

In addition, subjects with any of the following prohibited medications will be assessed to determine if their $\mu PRNT_{50}$ titers should be excluded from the analysis at specific visit(s), based on the decision of the medical team:

- Blood products or immunoglobulins 30 days prior to study visitation;
- Immunosuppressive therapies known to impact antibody levels (e.g. systemic or high dose inhaled [>800 µg/day of beclomethasone dipropionate or equivalent] corticosteroids, radiation treatment or other immunosuppressive or cytotoxic drugs);
- Investigational drugs or devices 30 days prior to study visitation;
- Subjects are requested to refrain from donation of blood, blood fractions and plasma within 30 days prior to visitation
- If the subject is on a steroid taper, the subject must be at a dose lower than 0.05 mg/kg at the time of immunogenicity sampling.

- The subject must notify the investigator if between visits they have had a non-drug therapy such as a surgery (i.e. splenectomy) known to suppress antibody levels.

This will be the primary analysis set for all immunogenicity analyses.

Protocol deviations which are deemed exclusionary from the PP at Part A will be deemed exclusionary for all future study parts. Final adjudications for protocol deviations deemed exclusionary for the PP analysis set or for the analyses at the year it is linked will be done prior to the lock for each study part. As such, the PP will not differ at each reporting part of the study.

10.2.1 Exclusion of Time Points in Per Protocol Analysis

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

Study Part	VISIT	Study Day (Visit Window)
A	Visit 1	Year 1 Month 12 (+/- 84 days)
B	Visit 2	Year 2 Month 24 (+/- 42 days)
C	Visit 3	Year 3 Month 36 (+/- 42 days)
D	Visit 4	Year 4 Month 48 (+/- 42 days)
E	Visit 5	Year 5 Month 60 (+/- 42 days)

Sample testing issues may also lead to exclusion from the analysis for particular timepoints.

10.3 Sensitivity Per Protocol Analysis Sets

A first Sensitivity Per Protocol (sPP) analysis set (sPP1) will be used for the additional sensitivity analyses of immunogenicity data at Part A, based on the updated sensitivity threshold for baseline serostatus using the cutoff of $\mu PRNT_{50} = 20$.

This analysis set is defined similarly to the PP analysis set, but using the sensitivity definition of baseline serostatus. As such, it will include all randomized and vaccinated subjects of the per protocol population who were CHIKV seronegative at baseline (defined as $\mu PRNT_{50}$ titer <20), have at least one evaluable post-baseline titer measurement of either 29d, 85d or 180d (within SAP visit windows for study VLA1553-301) and do not have any exclusionary protocol deviations (PDs) as described in section 10.2 (intercurrent events will lead the patient to be excluded from the sPP1 population).

A second sPP analysis set (sPP2) will be used for the additional sensitivity analyses of the immunogenicity data at Part A. This will also include all randomized and vaccinated subjects of the per protocol population who were CHIKV seronegative at baseline (defined as $\mu PRNT_{50}$ titer ≤40), have at least one evaluable post-baseline titer measurement of either 29d, 85d or 180d (within SAP visit windows for study VLA1553-301) The difference is the definition of intercurrent event linked to the visit window at Part A (i.e. the result outside of visit window will not be excluded from the analysis) but other intercurrent events will lead to exclusion from the sPP2 population. This new population has been defined to investigate the impact of the visit window on the results due to delays in unblinding of VLA 301, resulting in a high number of subjects which

were not able to attend Visit 1 (V1) in this study. This population will be stratified according to their time window around vaccination, including those who had their out of window (OOW) V1 1 year post-vaccination +/- 4 weeks, those with OOW V1 1 year post-vaccination +/- 8 weeks, +/- 12 weeks and +/- 16 weeks.

A third sensitivity analysis set (sPP3) will be used for a sensitivity analysis regarding the impact of a temperature excursion for the Investigational medicinal product (IMP) which took place for some subjects during the VLA1553-301 study. There are 20 subjects who were impacted by the excursion and enrolled into the VLA1553-303 study. These subjects are listed in an excel and merged in at the protocol deviation dataset level. In the standard PP population, the subjects are excluded due to these major deviations. In the sPP3, the subjects with temperature excursion will not be excluded from the population and in addition other intercurrent events will lead to exclusion from the sPP3 population. This population will be used for sensitivity analyses of the main immunogenicity datasets.

The sensitivity analysis for sPP1, sPP2 and sPP3 were carried out at Part A and showed no impact on results, and so will be omitted from future study parts (Part B onwards).

10.4 Invited Population

The invited population includes all the subjects who participated in the VLA1553-301 study and were invited to participate in the VLA1553-303 study and signed the Informed Consent (see section 7.4.1 of the protocol for more details).

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 in VLA1553-301 study. All procedures/assessments (apart from adverse event [AE] assessment) taken at Visit 1 of study VLA1553-301 are assumed to occur prior to vaccination in VLA1553-301 study.

Change from baseline is defined as:

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

11.2 Study Year and Visit Windows

Study Year is defined relative to the day of vaccination (Visit 1 in study VLA1553-301). Visit 0 for this study is planned to be equivalent to Visit 5 for VLA1553-301 study. In reality, many subjects had the visit 0 for this study later than their Visit 5 in the VLA1553-301 study, (i.e. later than Month 6) but all study year calculations are relative to the day of vaccination in VLA1553-301.

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

Study Part	VISIT	Study Day	Per-Protocol Study Day (Visit Window)	Analysis Study Day (Visit Window)
A	Visit 1	365	Year 1 Month 12 (+/- 84 days)	Year 1 Month 12 (+/- 182 days)*
B	Visit 2	730	Year 2 Month 24 (+/- 42 days)	Year 2 Month 24 (+/- 182 days)
C	Visit 3	1095	Year 3 Month 36 (+/- 42 days)	Year 3 Month 36 (+/- 182 days)

Study Part	VISIT	Study Day	Per-Protocol Study Day (Visit Window)	Analysis Study Day (Visit Window)
D	Visit 4	1460	Year 4 Month 48 (+/- 42 days)	Year 4 Month 36 (+/- 182 days)
E	Visit 5	1825	Year 5 Month 60 (+/- 42 days)	Year 5 Month 60 (+/- 182 days)

*For immunogenicity, the visit window at Year 1 lower bound will only be relevant for data that is reported as a VLA1553-303 visit.

Subjects who withdraw from the study prior to completion of the study will attend an End of Study (EOS) Visit where possible. Unscheduled visits will also be mapped to the closest visit per the above Table. For the purposes of analysis and reporting, each EOS visit will be assigned to one of the five study parts.

For the PP analysis the $\mu PRNT_{50}$ titers collected within the visit closest to the target study day and within Per-Protocol visit windowing (as defined in section 10.2.1) will be included in the analysis.

For WSP analysis (Part B onwards), analysis visit windowing is used as defined above. The non-missing result closest to the target date will be included in the analysis. If multiple results are non-missing and equally distant to the target date, the result from the scheduled visit will be included in the analysis. For EOS visits, if a subject has both a scheduled and EOS visit equidistant to the same target date, the result from the scheduled visit will be included in the analysis.

Unscheduled visits may be held at any time during the study if deemed necessary by the Investigator. During the unscheduled visit the investigator should query what concomitant medications have been taken by the subject and if there are any updates on ongoing SAEs or if any new SAEs have occurred since their last visit. Immunogenicity samples will be collected at all unscheduled visits throughout the study unless the subjects has had a sample taken at their previous regular VLA1553-303 study visit.

Analyses described as being presented at a study year will include all data up to the corresponding visit number. For example, an analysis presented at Year 2 will include all data up to the subject's visit 2 timepoint.

11.2.1 Visit Windowing

Visit windowing will be included in the analysis of planned timepoints on this study as described above. To this end, visits (including planned, unscheduled or EOS) or immunogenicity samples will be assigned to the Visit number of the window they fall into. Hence, any unscheduled visits which fall into the allowed time window for a scheduled visit would be analyzed under that visit. If there are multiple results for a specific endpoint falling into one visit after re-windowing, then the visit closest to the planned visit day will be used in the first instance. Refer to Section 11.2 for more details.

11.3 Immunogenicity Endpoints

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

Any $\mu PRNT_{50}$ value less than 40 will be imputed as 10. 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. The upper limit of quantification was determined as 7217. However, values above 7217 will be reported in the data, and the reported values will be used in the analysis as they are.

If subjects titers fall below $\mu PRNT_{50} \leq 40$ during immunogenicity sampling they will have an end of study visit at the next scheduled visit in the following study part. At all visits up to and including their end of study visit, the subject's actual $\mu PRNT_{50}$ value will be used for analysis. Although no longer attending future visits, these subjects will then remain in the analysis at future study parts, but with an imputed $\mu PRNT_{50}$ value of

10, i.e. those subjects will be considered as not having a seroconversion nor a seroresponse, their fold-increase will be assumed to be 1 (back to the baseline $\mu PRNT_{50}$ titer).

For the purpose of a sensitivity analysis using a different cutoff value, a secondary definition of baseline negative or positive is defined. In this case, any subject with a baseline $\mu PRNT_{50}$ <20 will be classified as sensitivity baseline negative; subjects with baseline $\mu PRNT_{50}$ ≥20 are classed as sensitivity baseline positive. This is kept to align with the VLA1553-301 primary analysis endpoint. In this case, any $\mu PRNT_{50}$ value less than 20 will be imputed as 10.

This sensitivity analysis was carried out at Part A and showed no impact on results, and so will be omitted from future study parts (Part B onwards).

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 of the log-transformed titer. The 95% confidence interval (CI) will be calculated as the anti-logarithm transformation of the upper and lower limits for a two-sided CI for the mean of the log-transformed titers.

11.3.2 Seroconversion

Seroconversion is defined as a >4-fold increase of NT compared to baseline (Day 1) for baseline negative subjects and baseline positive subjects.

For sensitivity analysis, a second definition of seroconversion is used. The sensitivity seroconversion is defined as a CHIKV-specific $\mu PRNT_{50}$ of ≥20 for baseline negative subjects. Seroconversion for baseline positive subjects is defined as a >4-fold increase over baseline, according to the sensitivity definition of baseline serostatus. The seroconversion rate (SCR) is defined as the proportion of subjects meeting the criteria for seroconversion at the relevant study timepoint.

This analysis was carried out at Part A and showed no impact on results, and so will be omitted from future study parts (Part B onwards).

11.3.3 Seroresponse

Seroresponse is defined as $\mu PRNT_{50}$ ≥150 at any post-baseline timepoint for baseline negative subjects.

The seroresponse rate (SRR) is defined as the proportion of baseline negative 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 NT 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 in $\mu PRNT_{50}$ compared to baseline will be summarized as the number and percentage of subjects in each category.

11.4 Adverse Events

In this study only Serious Adverse Events (SAE) and ongoing SAEs and AESIs from VLA1553-301 will be reported.

11.4.1 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.

In case of 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 have been reported in the medical history section of the eCRF in VLA1553-301 study and the corresponding medical procedure should be documented as a comment to the underlying diagnosis or condition in the eCRF's medical history section.

Only new information on ongoing SAEs from the precursor study VLA1553-301 and new SAEs since the end of the VLA1553-301 study have to be captured. AEs which do not fulfil the criteria for an SAE do not have to be captured.

SAE assessment will be performed from Visit 0 until Year 2. Furthermore, the first SAE Assessment in VLA1553-303 will be performed for some subjects at Visit 5 of VLA1553-301. However due to additional recruitment please note that for other subjects V0 will not occur at Visit 5 of VLA1553-301. Therefore, for these subjects, SAEs which occurred after VLA1553-301 Visit 5 must be retrospectively captured if there is a gap between the end of the VLA1553-301 and the subjects' start in VLA1553-303. These SAEs, which have to be captured retrospectively, are not considered a medical history event.

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

11.4.2 Causality

For SAEs, the Investigator will assess the causal relationship between the IMP and the SAE using his/her clinical expertise and judgement. Causality will be recorded as Probable, Possible, Unlikely or Not related.

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

11.4.3 Ongoing Adverse Events of Special Interest

An AESI is an event of scientific and medical concern specific to the Sponsor's product. In addition to nonspecific transient muscle pain and joint pain which may occur after any vaccination, the AESI for VLA1553 include signs and symptoms suggesting an acute stage CHIKV-associated event, as described in the VLA1553-301 study protocol.

Only those AEs identified as ongoing AESIs from VLA1553-301 study were planned to be followed-up during VLA1553-303 and reported in this study. In this study any AESI that is still ongoing at the time of study entry (Month 6) will be monitored until resolution or until patient finishes in the study.

All AESIs will be adjudicated by the Data Safety Monitoring Board (DSMB) to see if the board agrees with the investigator decision. Adjudication will be a Y/N flag from the DSMB for each AESI case. This will be added to the database and used for additional reporting.

No ongoing AESIs were seen from the VLA1553-301 study so no reporting is included in this study.

11.5 Missing Data

All statistical analysis will generally be based on observed values, missing values will be imputed as follow:

- For all the summaries and analyses, the post-discontinuation $\mu PRNT_{50}$ titers will be imputed to 10 for the subjects who discontinued because their $\mu PRNT_{50}$ titer is ≤ 40 prior to discontinuation; which means that the subjects will be deemed as not having seroconversion nor seroresponse post-discontinuation, and the fold-increase will be assumed to be 1 (so back to the baseline value).
- For the PP population and the analysis of the annual change rate: No explicit imputation will be performed for missing data or results excluded from the analysis, however, the mixed model with repeated measures model will yield unbiased results under the assumption that data are missing at random.

Finally, an additional analysis using multiple imputation approach may be conducted if deemed relevant, further details will be provided in a subsequent version on the SAP.

Missing causality for SAEs will be handled as described in section 11.4.2.

11.6 Handling of Missing or Incomplete Dates

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

Untoward medical occurrences with missing or partial start dates will be considered SAEs occurring during study unless the partial start date indicates that the event began prior to vaccination with VLA1553, e.g. if the month and/or year are before the month of Visit 1 in study VLA1553-301.

All medications with missing or incomplete start dates will be considered concomitant.

11.7 Prior and Concomitant Medications

Prior medications are those taken before vaccination with VLA1553, e.g. before Visit 1 of the VLA1553-301 study. These are pulled into the VLA1553-301 study CRF from the VLA1553-301 database.

Concomitant medications are those medications taken at any time after the end of the VLA1553-301 study, ie those with a start or end date after the date of Visit 5 in the VLA1553-301 study. Concomitant medications with a known impact on the immune system taken after the end of the VLA1553-301 study up to the beginning of the VLA1553-303 study were reported during this study, along with any new medications after VLA1553-303 Visit 0. In this study only concomitant medications with known impact on the immune system or needed during SAE management are collected.

11.8 Body Mass Index

Body Mass Index (BMI) is calculated for all subjects using demographics from the start of the VLA1553-301 study, and is calculated as: BMI (kg/m^2) = weight (kg) / [height $(m)^2$]

12.0 Analyses Part A, Part B, Part C, Part D and Part E

12.1 Planned Analysis Timepoints

There are 5 planned data analyses on this study:

- Part A includes safety and immunogenicity data after all subjects have completed Visit 1 (Year 1).

- Part B includes safety and immunogenicity data after all subjects have completed Visit 2 (Year 2).
- Part C includes immunogenicity data after all subjects have completed Visit 3 (Year 3).
- Part D includes immunogenicity data after all subjects have completed Visit 4 (Year 4).
- Part E includes immunogenicity data after all subjects have completed Visit 5 (Year 5).

Individual study parts will be analyzed sequentially.

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

For the Part A - D analysis, data up to and including respective Visit 1 - 4 (Year 1 - 4) or EOS if earlier will be kept for all subjects, for the Part E analysis, all data captured throughout the study will be included.

All SAEs and concomitant medications known to suppress the immune system 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, Part B, Part C, Part D and Part E the end date will be imputed to date of Visit 1, Visit 2, Visit 3, Visit 4 and Visit 5 respectively. In cases where targeted Visit is missing and an EOS visit is earlier, this is used for the imputation date for end dates. In a case where there is no EOS visit or targeted Visit, the defined cutoff date will be used for each respective Part.

12.2 Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) will be utilized for this study. The DSMB will consist of four voting members who will be selected based upon their expertise with and understanding of infectious diseases, vaccines, clinical research and/or skills and knowledge in clinical medicine. The DSMB will review accruing safety information on an ad-hoc basis throughout the study until Year 2, as applicable. A meeting 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 review and evaluate all SAE reports for: (i) increases in frequency of SAEs and study discontinuations within the study; and (ii) SAEs in the study sample compared with the population based on what is known from the literature.

A DSMB charter including a detailed description has been prepared separately.

13.0 Interim Analyses

No interim analysis is planned for this study. Instead there are five separate study parts (A-E) as described in section 12 which will be reported separately.

14.0 Statistical Methods

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

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

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

All statistical analysis will generally be based on observed values, missing values may be imputed as detailed in Section 11.5.

For the analyses of each study part, all available data will be included up to a cutoff for each study part, regardless of the subject status during that part of the study. For each study part, the individual cutoff will be either the date of the latest study timepoint, or if not available, the End of Study visit date, or end of visit window for the latest study timepoint on the study; whichever is earlier. If a subject has the last Visit for the study Part (eg Visit 1 for Part A), then all data up to this date will be included, regardless of whether this was OOW. Data which maps to the relevant study visit is also included (eg immunogenicity data within the visit window which may have been taken after the initial visit date). For example, if a subject discontinued the study in Part A, they would still be included in any summaries of Part A timepoints in Part B, Part C, Part D and Part E. Events ongoing at each study part cutoff will be imputed as ongoing at the relevant summary, even if an end date is entered after the study cutoff point. For example, if an SAE was ongoing for a subject at Visit 1 (Year 1), but ended prior to Visit 2, it would be deemed ongoing for Part A analysis, even if the end date was present prior to the database extract for Part A.

In general, all immunogenicity and safety tables will be presented by age strata (18-64 years and ≥65 years) and total.

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.

14.1 Subject Disposition

The number and percentage of subjects vaccinated in study VLA1153-301 who were invited to this study, plus the number entered into this study will be presented, together with the number and percentage of subjects who withdrew from the study prematurely during each part and overall and a breakdown of the corresponding reasons 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.

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

14.2 Protocol Deviations

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

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

Protocol deviations will be classified into major or minor protocol deviations based on their possible impact on the study results in a blind data review meeting prior to the database snapshots at each study part. Major deviations are those which will lead to exclusion from the Per Protocol analysis set. These have been defined at Part A of study VLA1153-301.

A tabulation of the number and percentage of protocol deviations by deviation types and deviation category will be provided at each Part. A by-subject listing of all protocol deviations will be presented.

In addition, a separate table and listing of COVID-19 specific protocol deviations will be produced for Part A and B only.

14.3 Prior and Concomitant Medications

Prior medications and concomitant medications with known impact on the immune system and those needed during SAE management, categorized by ATC level 2 and preferred term (PT) according to

WHODrug (Version B3 Sep2020 or later), will be summarized separately. The number and percentage of subjects using each medication will be displayed together with the number and percentage of subjects using at least one medication within each medication group and subgroup. Each concomitant medication will be coded to a single ATC code, taking into account the indication for the use of the medication as documented on the CRF.

Prior medications are those reported as taken prior to vaccination in the VLA1553-301 study and integrated into this study's CRF.

The concomitant medications with known impact on the immune system and those needed during SAE management 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.

14.4 Demographic Characteristics

Demographic characteristics to be summarized will include gender, ethnicity, race, age (years), age group (18 – 64 years, ≥65 years), height (cm), weight (kg) and body mass index (BMI) (kg/m2).

The summary of demographic characteristics will be presented for the WS and PP populations, stratified by age stratum.

Medical history (collected at the start of the VLA1553-301 study) will be summarized by system organ class (SOC) and PT using the current version of MedDRA.

All demographic characteristics and medical history will be listed.

14.5 Immunogenicity Analyses

14.5.1 Hypothesis Testing Strategy and Multiplicity

No formal hypothesis testing is planned, and no adjustments are made for multiplicity in this trial.

14.5.2 Primary Immunogenicity Analysis

14.5.2.1 Seroresponse Rate

The number and percentage of subjects meeting the criteria for seroresponse (CHIKV antibody level defined as $\mu PRNT_{50}$ ≥150 for baseline negative subjects) will be presented for each study timepoint by age strata. The denominator for the percentage will be the number of baseline negative subjects with non-missing NT values at each timepoint. Two-sided exact (Clopper-Pearson) 95% CIs for the seroresponse rate (SRR) will be presented to provide a descriptive analysis of the change in rate of seroresponse decline over time. This will be presented for the PP population.

Bar charts of the percentage of subjects meeting the seroresponse criteria will be produced by study visit and age strata.

14.5.2.2 Sensitivity Analyses

All primary and secondary immunogenicity analyses will be repeated on the WS population.

14.5.2.2.1 **Baseline Threshold $\mu PRNT_{50}$ <20**

At Part A, a set of sensitivity analyses will be produced using the titer threshold value of 20 and positive if $\mu PRNT_{50}$ ≥20) and the two Sensitivity Per Protocol (sPP) analysis sets (sPP1 and sPP2) based on this definition. Seroconversion is defined as CHIKV-specific neutralizing antibody titer of ≥20 for baseline negative subjects and > 4-fold increase over baseline for baseline positive subjects.

The following summaries from the above and below sections will be repeated using the updated definitions: seroresponse rate at Part A (Visit 1, Year 1); seroresponse rate by visit; analysis of GMT by visit; seroconversion rate by visit and the fold increase of CHIKV neutralizing titers by visit.

14.5.3 Secondary Immunogenicity Endpoints

14.5.3.1 Geometric Mean Titer

A summary of the NTs will be presented at all timepoints (Year 1, Year 2, Year 3, Year 4 and Year 5) for the PP and WS populations. Two-sided 95% CIs will be presented for the GMT in each age strata and total at each timepoint.

At each study part (Part A to E), a mixed model with repeated measures (MMRM) will be applied to the log-transformed $\mu PRNT_{50}$ values considering natural log-transformed $\mu PRNT_{50}$ baseline value, age stratum and time (year) as fixed effects whereas intercept as random effect. Only the $\mu PRNT_{50}$ values from the samples collected in the VLA1553-303 study were included in the model. An unstructured covariance matrix will be used to account for within-subject variability; Kenward-Rogers degrees of freedom will also be used. Model convergence will be checked. If the MMRM with unstructured covariance matrix fails to converge the following steps will be implemented (in the order listed) until convergence is reached.

1. The Fisher scoring algorithm will be used to start the first iteration to obtain the initial values of the covariate parameters (via the SCORING option of the PROC MIXED statement).
2. The no-diagonal factor analytic structure (via the TYPE=FA0(T) option of the REPEATED statement, where T is the total number of time points), which effectively performs the Cholesky decomposition of the covariance matrix and is numerically more stable,
3. Covariance matrix of ARH(1) (Heterogeneous auto-regressive 1), and CS (compound symmetry) with the sandwich variance estimator will be used in that order (for the sandwich variance estimator Kenward-Rogers degrees of freedom will be replaced by the EMPIRICAL option).

The anti-log of the estimates and the 95% CIs for annual change rate of antibody titers will be presented on the PP population and the WS population.

A line plot of the GMT at each study timepoint will be produced by age strata in the PP population.

A reverse cumulative distribution plot of the proportion of subjects by titer value will be produced for each timepoint in the study on the PP population.

14.5.3.2 Seroconversion Rate

The SCR will be summarized similarly to the SRR.

14.5.3.3 Fold-Increase in Neutralizing Antibody Titer

A descriptive summary of the continuous fold-increase in CHIKV-specific NT 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.

14.6 Safety Analyses

14.6.1 Adverse Events

Cumulative summaries of SAEs and related SAEs categorized by System Organ Class (SOC) and Preferred Term (PT) coded according to the MedDRA dictionary will be produced in Part A and Part B. Within these summaries counting will be by subject not event and subjects are only counted once within each SOC or PT. Two-sided exact (Clopper-Pearson) 95% CIs will be provided for rates of adverse events, by SOC and PT. SAEs will also be listed.

In summaries of SAEs which are categorized by relationship to IMP, SOC and PT, SAEs 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.

AESIs that are ongoing from study VLA1153-301 will be listed.

All AE tables will be produced by age strata and total.

15.0 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 ".

16.0 Glossary of Abbreviations

Glossary of Abbreviations:	
AE	Adverse event
AESI	Adverse Event of Special Interest
ATC	Anatomic Therapeutic Classification
BMI	Body Mass Index
CI	Confidence Interval
CHIKV	Chikungunya Virus
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
EOS	End of Study
FDA	Food and Drug Administration
GMT	Geometric Mean Titer
GSD	Geometric Standard Deviation
ICF	Informed Consent Form
IMM	Immunogenicity
IMP	Investigational Medicinal Product
IXRS	Interactive Voice/Web Response System
MMRM	Mixed Model with Repeated Measures
MedDRA	Medical Dictionary for Regulatory Activities
NT	Neutralizing Titer
PP	Per Protocol
PT	Preferred Term
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SCR	Seroconversion Rate
SD	Standard Deviation

SOC	System Organ Class
SRR	Seroresponse Rate
WHODrug	World Health Organization Drug Dictionary
µPRNT	Micro Plaque Reduction Neutralization Test