Statistical Analysis Plan

Part A, Part B and Part C Analysis

A RANDOMIZED, OBSERVER-BLINDED, DOSE RESPONSE PHASE 2 TRIAL TO ASSESS THE SAFETY AND IMMUNOGENICITY OF TWO DIFFERENT DOSE LEVELS OF A LIVE ATTENUATED CHIKUNGUNYA VIRUS VACCINE (VLA1553) IN HEALTHY CHILDREN AGED 1 TO 11 YEARS

Protocol: VLA1553-221

Confidential

Sponsor: Valneva Austria GmbH

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Document			
Trial	VLA1553-221		
Document	Statistical Analysis Plan (SAP)		
Version	FINAL 2.0		
Date	01-Apr-2025		
Revision History			
Version	Date	Reason for Revision	
FINAL 1.0	30-Oct-2024	First version;	
FINAL 2.0	01-Apr-2025	Update for Part B Analysis: new CLAR definition included; more details for analysis of viremia data added; minor changes in text;	
FINAL 3.0	09-Sep-2025	Update for Part C Analysis: additional TLFs (by serostatus/pooled trial arm) for analysis of viremia added;	
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List of Abbreviations

AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CHIKV	Chikungunya virus
CI	Confidence interval
CLAR	Chikungunya-like adverse reaction
CTR	Clinical Trial Report
DSMB	Data Safety Monitoring Board
GMFI	Geometric mean fold increase
GMT	Geometric mean titer
Ig	Immunoglobulin
IMM	Immunogenicity Analysis Population
IR	Invalid result
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
MedDRA	Medical Dictionary for Regulatory Activities
PD	Protocol deviation
PP	Per Protocol Analysis Population
PT	Preferred Term
µPRNT	Micro Plaque reduction neutralization test
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SOC	System Organ Class
TCID	Tissue Culture Infectious Dose
TMF	Trial Master File
WHO	World Health Organization

1. OVERVIEW

1.1 Trial Objectives

1.1.1 Primary Objective

- To evaluate the tolerability of the full dose and half dose formulation of the live-attenuated CHIKV vaccine (VLA1553) following vaccination in healthy children aged 1 to 11 years after a single immunization.

1.1.2 Secondary Objectives

- To assess the immunogenicity of the full dose and half dose formulation of VLA1553 in healthy children aged 1 to 11 years after a single immunization.
- To assess the safety of the full dose and half dose formulation of VLA1553 in healthy children aged 1 to 11 years after a single immunization.
- To identify the optimal dose level(s) of VLA1553 in healthy children aged 1 to 11 years.

1.1.3 Exploratory Objective

- To evaluate the efficacy of VLA1553 in pediatric participants in CHIKV endemic areas after a single immunization.

1.2 Trial Design

This is a multicenter, prospective, randomized, observer-blinded, three arm, phase 2 clinical trial evaluating the full dose formulation (target 4.0 log 10 $TCID_{50}$ per 0.5mL) of VLA1553, half dose formulation (3.7 log 10 $TCID_{50}$ per 0.5mL) of VLA1553 and control (Nimenrix, a tetravalent meningococcal vaccine - Men ACWY).

1. At least 300 male and female healthy children aged 1 to 11 years will be enrolled and the overall distribution of participants will be 2:2:1 to the two VLA1553 dose groups (n=120 each) or control (Nimenrix) (n=60).
2. These volunteers will be screened for evidence of previous CHIKV exposure. Enrolment will then be stratified by CHIKV serostatus at baseline, targeting:
   - up to 10 % CHIKV seropositive participants (i.e., IgM+/IgG+ or IgM-/IgG+)
   - at least 90% CHIKV seronegative participants (i.e., IgM-/IgG-)

Participants who are IgM+/IgG- do not qualify for participation in this trial. CHIKV IgM+/IgG- is suggestive for an early phase acute CHIKV infection. Therefore, as safety precaution, no vaccination with the attenuated CHIKV vaccine VLA1553 should be applied.

Note: All participants will be screened for CHIKV-specific IgG and IgM antibody testing using a rapid test. Only in case the rapid test is positive, confirmatory anti-Chikungunya virus IgG and IgM ELISA testing will be performed. A borderline ELISA result is considered negative. Hence, participants with ELISA results IgM borderline/IgG-, IgM-/IgG borderline, or IgM borderline/IgG borderline are mapped to seronegative group; participants with ELISA result IgM borderline/IgG+ are mapped to seropositive group; participants with ELISA result IgM+/IgG borderline are not eligible for the trial.

Figure 1 Phase 2 Clinical Trial Design

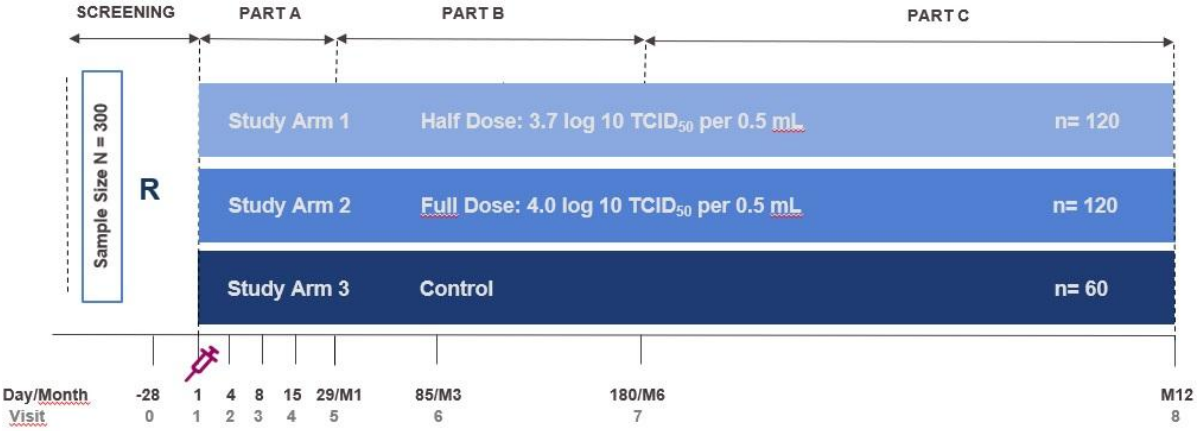


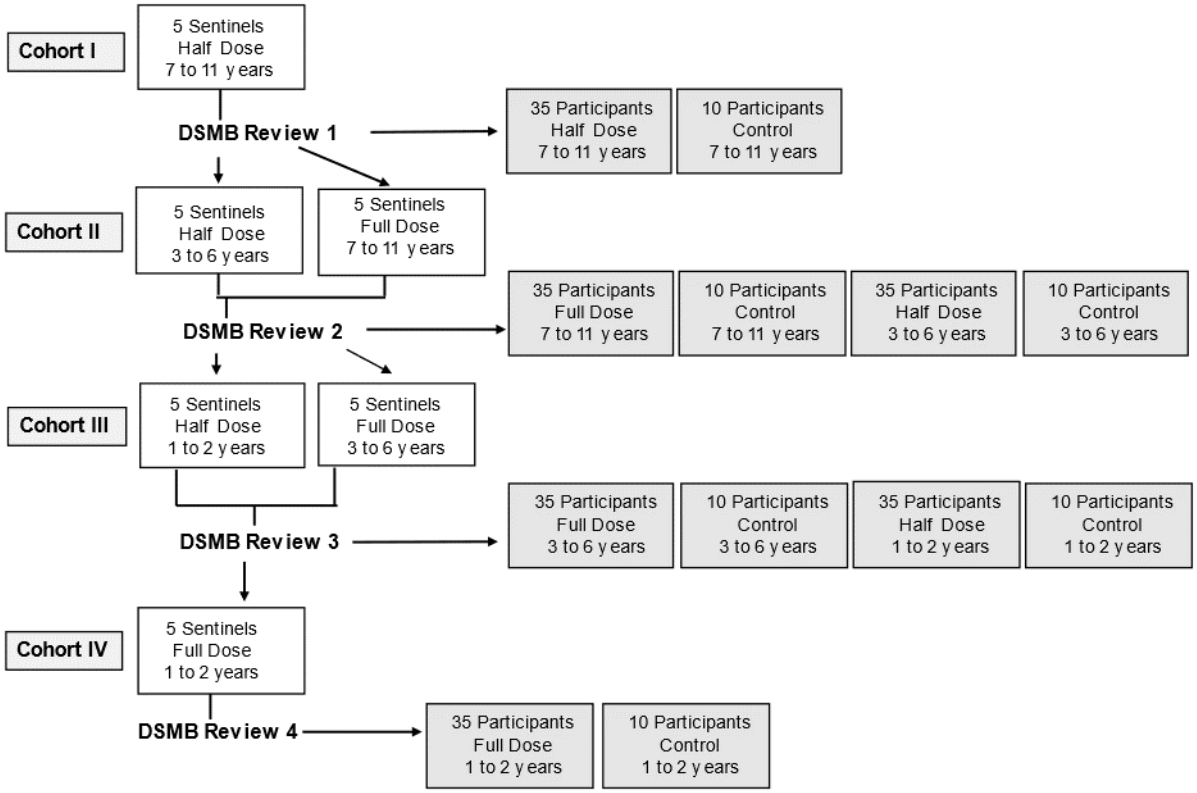
Table 1 Participant Distribution

Trial Arm	Dosage ($TCID_{50}$/dose)	Stratum	Cohort I	Cohort II	Cohort III	Cohort IV	Total N
1	VLA1553 half dose 3.7 log 10	A (7 to 11 yrs.)	5*+35	-		-	40
		B (3 to 6 yrs.)	-	5*+35	-		40
		C (1 to 2 yrs.)	-	-	5*+35	-	40
2	VLA1553 full dose 4.0 log 10	A (7 to 11 yrs.)	-	5*+35		-	40
		B (3 to 6 yrs.)	-	-	5*+35		40
		C (1 to 2 yrs.)	-	-	-	5*+35	40
3	Control	A (7 to 11 yrs.)	10	10		-	20
		B (3 to 6 yrs.)	-	10	10		20
		C (1 to 2 yrs.)	-	-	10	10	20
		Total	**50**	**100**	**100**	**50**	**300**

*Sentinel enrolment will be conducted in open-label fashion and sentinels recruited preferably at a single trial site. Sentinel vaccinations in the following cohort can be started based on the previous cohort sentinels' review by the Data Safety Monitoring Board (DSMB), DSMB's recommendation to the Sponsor and final Sponsor confirmation to proceed with the trial.

Staggered enrolment approach

Figure 2 Staggered Enrolment



As a safety precaution measure, 30 sentinel participants will be enrolled into the trial in an open-label fashion according to an age step down scheme outlined in Figure 2.

After sentinel analysis, participants will be enrolled in a blinded, randomized manner into three trial arms. Within each trial arm participants will be stratified into three age strata:

Stratum A: 7 to 11 years -children from their 7th birthday until the day before their 12th birthday.

Stratum B: 3 to 6 years - children from their 3rd birthday until the day before their 7th birthday.

Stratum C: 1 to 2 years - children from their 1st birthday until the day before their 3rd birthday.

Age strata for the VLA1553 trial arms are targeted to be equal in size, i.e., approximately 40 per age stratum.

The age step down phase of the trial will be preferably initiated at a single site to allow permanent oversight on safety data by one principal Investigator.

Details of the staggered enrolment approach are described in the Randomization Plan.

1.3 Endpoints

1.3.1 Primary Endpoint

The primary endpoint is to assess tolerability through the frequency and severity of solicited injection site and systemic reactions within 14 days post-vaccination.

1.3.2 Secondary Endpoints

Safety

- Frequency and severity of any adverse event (AE) within 28 days post-vaccination;
- Frequency and severity of unsolicited AE until Month 6 (Day 180) and Month 12 (Day 365) post-vaccination;
- Frequency and severity of any serious adverse event (SAE) until Month 6 (Day 180) and Month 12 (Day 365) post-vaccination;
- 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 trial starting 22 days post-vaccination (i.e. Day 23 – trial end);
- Assessment of viremia on Days 1, 4, 8, and 15 and beyond as applicable;

Immunogenicity

- Immune response in baseline seronegative participants as measured by CHIKV-specific neutralizing antibody titers on Day 1, Day 15, Day 29, Day 85, Day 180 and Month 12 post-vaccination as determined by µPRNT assay;
- Proportion of participants with seroconversion as compared to baseline at Day 15, Day 29, Day 85, Day 180 and Month 12 as determined by µPRNT assay;
- Proportion of participants with a seroresponse on Day 15, Day 29, Day 85, Day 180 and Month 12 post-vaccination as determined by µPRNT assay;
- Fold increase of CHIKV-specific neutralizing antibody titers determined by µPRNT assay at Days 15, 29, 85, 180 and at Month 12 post-vaccination as compared to baseline;
- Proportion of participants reaching an at least 4-fold, 8-fold, 16-fold or 64-fold increase in CHIKV-specific neutralizing antibody titers compared to baseline at Day 15, Day 29, Day 85, Day 180 and Month 12 as measured by µPRNT assay;
- Antibody titers, seroresponse, seroconversion and fold increases for CHIKV-specific neutralizing antibodies, determined by µPRNT assay at Day 15, Day 29, Day 85, Day 180 and Month 12 post-vaccination stratified by baseline serostatus (based on µPRNT), age stratum and dose.

1.3.3 Exploratory Endpoint

- Incidence of CHIKV infections with onset after 15 days post-vaccination (i.e., Day 16) as evidenced by viremia by virus specific RT-qPCR*, clinical diagnosis and seroconversion by µPRNT for the entire trial period - *measured in Stratum A and in sentinel participants of Stratum B and Stratum C.

However, this endpoint will not be analyzed for Part A, since no data are available. This endpoint might be analyzed for Part B and/ or Part C (e.g. data collected at Unscheduled Visits). See Section 7 for further details.

1.4 Sample Size Calculation

A total number of 240 children aged 1 to 11 years exposed to VLA1553 in this trial has been selected to provide a sufficient number of participants for proper safety evaluation. With 240 participants exposed, the trial will provide 95% confidence that an AE does not occur at a frequency of 1.24% or higher, if not observed in the trial.

In terms of immunogenicity, up to 240 participants exposed to VLA1553 and assuming 10% seropositive participants and among seronegatives an expected rate of 10% of participants to be excluded due to drop-outs/major protocol deviations would leave 194 participants in the analysis and would allow determination of the GMT with meaningful accuracy. I.e., with an assumed Log10-SD of 0.45 and an expected GMT of 2954 (based on pooled data from Phase 3 results in adults), the 95%CI for the GMT would be [2551, 3421] for combined VLA1553 groups and [2397, 3640] for one dose group. Furthermore, these 97 participants per dose group would allow for the detection of a difference in GMTs of 2954 vs. 1943 between the groups with a two-sided significance level of 5% and a statistical power of 80%.

1.5 Flowchart

Table 2 Schedule of Trial Procedures and Assessments

		Part A					Part B		Part C	
	Visit 0 **Screening** (Day -28 to Day 0)	**Visit 1** [j] Day 1	**Visit 2** Day 4 (+/-1d)	**Visit 3** Day 8 (+/- 1d)	**Visit 4** Day 15 (+/-1d)	**Visit 5** Day 29 M1 (+/- 4d)	**Visit 6** Day 85 M3 (+/- 7d)	**Visit 7** Day 180 M6 (+/- 14d)	**Visit 8** Day 365 M12 (+/- 14d)	**Visit ET**
Informed consent/ assent [a]	X									
Inclusion/ Exclusion criteria	X	X (Review)								
Demographics [b]	X									
Medical history [c] (including vaccination history)	X	X (Update)								
Randomization		X								
Prior / Concomitant medications	X	X	X	X	X	X	X	X	X	X
Physical examination [d]	X	X	X	X	X	X	X	X	X	X
Vital signs [e]	X	X								
CHIKV Screening sample [1.2 mL]	X									
Pregnancy test (if applicable) [f]	X	X								
Immunogenicity sample [g] [2 mL]		X			X	X	X	X	X	X
Safety sample [h] [4 mL]	X					X				X [n]
Urinalysis [l]		X	X	X	X	X	X	X	X	X
Viremia sample [i] [2 mL]		X	X	X	X	X [k]	X [m]	X [m]	X [m]	X [m]
Maximum total approximate blood volume [mL]	5.2/5.2	4.0/4.0	2.0/2.0	2.0/2.0	4.0/4.0	8.0/4.0	4.0/4.0	4.0/4.0	4.0/4.0	8.0/6.0

Stratum A sentinels/non-sentinels Stratum B sentinels/non-sentinels Stratum C sentinels/non-sentinels	5.2/5.2 5.2/5.2	4.0/2.0 4.0/2.0	2.0/none 2.0/none	2.0/none 2.0/none	4.0/2.0 4.0/2.0	8.0/2.0 8.0/2.0	4.0/2.0 4.0/2.0	4.0/2.0 4.0/2.0	4.0/2.0 4.0/2.0	8.0/6.0 8.0/6.0
VACCINATION		X								
Participant Diary		D	R/C/D	R/C/D	R/C					R/C
Memory Aid					D	R/C/D	R/C/D	R/C/D	R/C	R/C
AE/ AESI/ SAE Assessment	X[o]	X	X	X	X	X	X	X	X	X

ET … early termination; R … review; C … collect; D … distribute

[a] Occurs at enrollment before Screening.
[b] Demographics include date of birth, age, height, weight, BMI, gender, race and ethnicity.
[c] Symptoms noted (except those qualifying as a SAE) at Visit 1 (prior to first vaccination) are not considered AEs but will be recorded as medical history. Any prior vaccination should be documented in the Medical History.
[d] A physical examination will be performed on the following body systems being described as normal or abnormal: general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological.
[e] Vital signs will include systolic and diastolic blood pressure and pulse rate while seated and at rest, and body temperature measured with infrared thermometer targeting the forehead before vaccination. In addition, after an observation period of 60 minutes (open label phase) or 30 minutes (blinded phase) following vaccination, pulse rate and blood pressure while seated and at rest will again be assessed.
[f] At the screening visit and at indicated visits (i.e., V1) a pregnancy test (in accordance with local regulations) will be performed for all female participants after onset of menarche and if involved in activities that could result in pregnancy.
[g] Immunogenicity sample [blood: 2 mL] for CHIKV-specific neutralizing antibody titer evaluation.
[h] Safety laboratory sample for hematology and coagulation panel [blood: 4 mL]. Safety sample will be taken from all participants at the screening visit; at subsequent visits (i.e., V5 or ET if earlier than V5) the safety sample will be only taken from the sentinel participants. If deemed necessary by the investigator a safety sample can be taken any time if safety concerns arise.
[i] Viremia sample for investigation of viremia by RT-qPCR [plasma: 2 mL]. Viremia sample will be taken from all participants of Stratum A and only taken from sentinel participants from Stratum B and C.
[j] All procedures/assessments (apart from Participant Diary distribution and AE assessment) occur prior to vaccination (unless stated otherwise).
[k] Visit 5 (Day 29) will be only tested if Visit 4 (Day 15) tests positive.
[l] Urinalysis (i.e., pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) - if possible.
[m] Samples should be obtained for clinically indicated retrospective investigation of viremia by RT-qPCR.
[n] Safety sample should be collected if ET is before Day 29 (Visit 5)-sentinels only.
[o] SAE(s) should be recorded from signed ICF onwards.

2. GENERAL CONSIDERATIONS

2.1 Conduct of Analysis

The following data analyses will be performed:

- Part A analysis will be performed after all participants have completed Visit 5 (Day 29).
- Part B analysis will be performed after all participants have completed Visit 7 (Day 180).
- Part C analysis will be performed after all participants have completed Visit 8 (Month 12) and will include all data documented in the clinical database.

For Part A and Part B analysis, data from scheduled visits will be included up to the respective cut-off visit, i.e. Visit 5 (Day 29) for Part A and Visit 7 (Day 180) for Part B. For other data (AEs, AESIs, concomitant medications, AESI laboratory tests, unscheduled and early termination visits) the following rules will be applied:

- AEs, AESIs, concomitant medications and healthcare events will be considered to have started up to cut-off (e.g. Day 29) if the start date is before or on trial day of the cut-off. In case of an incomplete start date where it cannot be clearly decided if the event started up to cut-off day or not, the event will be considered to have started up to the cut-off. For derivation of cut-off, the date of the respective visit as reported in the eCRF is used (e.g. date of Visit 5 for Day 29, date of Visit 7 for Day 180). In case no respective visit was performed, the event will be considered to have started up to cut-off if the trial day in relation to Visit 1 is lower than or equal to the end of the relevant visit window (e.g. trial day <=33 for Part A cut-off). For visit window details, see Section 2.7.
- AESI laboratory tests, unscheduled and early termination visits will be assigned to a specific trial visit as described in Section 2.7.2 and therefore will be handled like scheduled visits. Such visits will be included in an analysis (e.g. Part A) if assigned to a Part A visit (e.g. Day 29 visit).

Prior to each analysis, the clinical database will be cleaned as described in the Data Management Plan.
After Part A analysis, a Clinical Trial Report (CTR) will be compiled. For the Part B analysis another CTR will be compiled (integrated CTR of Part A+B). For the Part C analysis, a final CTR will be compiled (integrated CTR of Part A+B+C).

2.2 Statistical Software and Quality Control

All statistical analyses will be performed using SAS® version 9.3 or 9.4. Tables, figures and data listings will be generated in Microsoft® Word® as well as PDF® format. Additionally, selected listings will be provided in Microsoft® Excel® spreadsheets as well.

Quality control of SAS® programs will include a review of the whole process of result generation:

- Review of all analysis SAS® programs
- Review of SAS® log for errors, warnings and other notes that could indicate mistakes in the programs
- Review of all tables, listings and figures for completeness and correctness

As an additional quality control measure, independent re-programming will be performed as described in SOP SAS04.

2.3 Blinding and Randomization

Participants will be allocated to trial arms via the randomization module of the EDC system. Overall distribution of participants will be 2:2:1 to the VLA1553 half dose formulation, VLA1553 full dose formulation or control group. Within each trial arm participants will be further stratified into three age strata (stratum A: 7 to 11 years, stratum B: 3 to 6 years, stratum C: 1 to 2 years).

The trial is an observer-blinded trial, which will be conducted in a blinded manner for the trial investigators, the sponsor including laboratory personnel, and the participants. ADMB and Valneva personnel will be unblinded at the time of the Part A analysis after the respective database snapshot is performed. All other trial personnel including the investigators and other trial staff involved in general trial conduct and safety assessments as well as laboratory personnel who are performing analytical assays will remain blinded until the trial ends.

For more details on blinding and randomization refer to the Randomization Plan.

2.4 Descriptive Analyses

Descriptive analyses of continuous variables (summary statistics) will be described with the number of non-missing observations, arithmetic mean, standard deviation (±SD), median, quartiles (Q1 and Q3), minimum and maximum.

Descriptive analyses of continuous immunogenicity variables (e.g. tables for the Geometric Mean Titer) will be described with the number of non-missing observations, geometric mean, standard deviation of logarithmic values, median, quartiles (Q1 and Q3), minimum and maximum.

Categorical variables (frequency statistics) will be described with the number of non-missing observations and percentages (%). Percentages will be calculated within each stratum on the total number of non-missing observations, if not stated otherwise.

2.5 Site and Country Effect and Stratification Variables

Site and country effect will be examined in Part B and Part C analysis. Selected tables will be stratified by site and country as specified in the List of TLFs. All tables will be stratified by age group, i.e. age groups will be implemented as columns as described in Section 2.12 below. Further, selected safety and immunogenicity tables will be stratified by µPRNT baseline serostatus as specified in the List of TLFs. For safety, in addition to tables including the overall safety analysis population, separate tables filtered for µPRNT baseline seronegative (seropositive, respectively) participants will be prepared. Immunogenicity tables produced for the immunogenicity analysis population will be additionally repeated for the seronegative and seropositive immunogenicity analysis set, i.e. stratification will be covered via the use of those analysis populations.

2.6 Handling Missing Data

All statistical analysis will generally be based on observed values, missing values will not be imputed.

However, only for Part C analysis, AEs with missing causality and/or severity assessment will be analyzed as described in Section 6.1.2.

2.7 Trial Days and Visit Windowing

2.7.1 Trial Days

Trial day is defined relative to day of vaccination (Visit 1). Trial Day 1 is the day of vaccination (Visit 1). The scheduled visits along with the predefined visit window per CTP are included in the table below.

Trial Part	Visit	Trial Day (Visit Window)
A	Visit 0 Screening	Day -28 to Day 0
A	Visit 1	Day 1
A	Visit 2	Day 4 (+/-1d)
A	Visit 3	Day 8 (+/-1d)
A	Visit 4	Day 15 (+/-1d)
A	Visit 5	Day 29 M1 (+/- 4d)
B	Visit 6	Day 85 M3 (+/- 7d)
B	Visit 7	Day 180 M6 (+/- 14d)
C	Visit 8	Day 365 M12 (+/- 14d)

Data will be analyzed according to visit windowing rules below, (see Section 2.7.2) except in the case of the immunogenicity analyses on the PP (see Section 2.9). Unscheduled visits may be held at any time during the trial as necessary.

Analyses described as being presented at a trial day will include data up to the corresponding visit number. E.g. an analysis presented at Day 29 will include all data up to the participants Visit 5 time point, even if this is after trial Day 29. Similarly, endpoints analyzed up to Visit 7 (Day 180) will include all data collected on the trial, up to their Visit 7 or Early Termination (ET) visit.

2.7.2 Visit Windowing Rules

Visit windowing will be included in the analysis of planned time points on this trial. To this end, all visits (including planned, unscheduled, AESI laboratory or ET visits) or immunogenicity samples will be assigned to the visit number of the closest planned trial visit. In order to do this, the mid-point between two planned trial visits is used as the cut-off for visit windowing, according to the mapping per below. All visits will be windowed for analysis per the below.

Trial Part	Windowed Visit	Trial Day Range for Window Mappings
A	Visit 0 Screening	Day -28 to Day 0
A	Visit 1 – Day 1	Day 1
A	Visit 2 – Day 4	Day 2 – Day 6

A	Visit 3 – Day 8	Day 7 – Day 11
A	Visit 4 – Day 15	Day 12 – Day 22
A	Visit 5 – Day 29	Day 23 – Day 57
B	Visit 6 – Day 85	Day 58 – Day 132
B	Visit 7 – Day 180	Day 133 – Day 272
C	Visit 8 – Day 365	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 re-windowing, 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 immunogenicity. In more detail, the following rules will be applied:

- Viremia (RT-qPCR): worst result is higher copy number value
- µPRNT: worst result is a lower µPRNT50 titer value (e.g. a more conservative value);
- ELISA: worst result would be positive and then indetermined;
- 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 (e.g. in free-text abnormal findings fields), then the planned trial visit will be used.
- Safety labs (including AESI laboratory test): furthest out of range per analyte used;
  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 results will be 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. E.g., if there are two records of Calcium, as both high and low Calcium are 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.

2.8 Analysis Populations

2.8.1 Safety Analysis Population

The safety analysis population contains all participants who entered into the trial and received one vaccination. Participants will be analyzed as treated.

2.8.2 Immunogenicity Analysis Population

Supportive immunogenicity analysis will be based on the immunogenicity analysis population (IMM).

Statistical analyses for immunogenicity will be based on µPRNT serostatus. Detection of anti-CHIKV antibodies at screening (based on rapid test/confirmatory ELISA) will be used for enrollment at the sites only, i.e. for

randomization stratified by serostatus. The IMM is defined to include all vaccinated participants who have evaluable µPRNT antibody titer results at baseline (Visit 1/ Day 1) and at least one post-baseline titer measurement after vaccination.

Participants will be analyzed according to the planned trial arm, rather than by the actual treatment they received.

- The baseline seronegative immunogenicity analysis set (seronegative IMM) includes all IMM participants, who are seronegative ($\mu PRNT_{50} \leq 40$) at baseline (Visit 1/ Day 1).
- The baseline seropositive immunogenicity analysis set (seropositive IMM) includes all IMM participants, who are seropositive ($\mu PRNT_{50} > 40$) at baseline (Visit 1/ Day 1).

2.8.3 Per Protocol Analysis Population

The main immunogenicity analysis will be based on the per protocol analysis population (PP). The PP contains all seronegative IMM participants who have no major protocol violations on participant level that could impact immune response (i.e. deviations leading to exclusion of all samples). The per-protocol analysis will thus not be stratified by baseline serostatus.

The PP population will be defined in the Data Review Meeting (DRM) prior to the respective analysis. In case time points are affected by a major protocol deviation (PD), this will not automatically lead to an exclusion of the participants from the overall PP population but only of the respective time points (samples) from the IMM analysis. For example, a major PD may occur post Day 29, such as certain prohibited concomitant medication. Then immunogenicity results after the occurrence of the PD will be removed from the PP analysis, but the respective participant remains in the PP population (see Section 2.9 below).

2.9 Exclusion of Time Points in the Per-Protocol Analysis

In the PP analysis of immunogenicity, samples with extensive time window deviations will be excluded from the analysis, even if the participant may remain in the PP population. Time windows for the exclusion of immunogenicity samples differ from the time windows for trial visits because the time window instruction for trial sites that define the time point of the participant's return to subsequent visits is different from the time window for an immunogenicity sample to be eligible for the PP immunogenicity analysis.

Samples that are outside the following time windows will be excluded via programming:

Time Point	Time Window for Per-Protocol Analysis	
	Start date	End date
Visit 2 / Day 4	Visit 1 plus 3 days minus 1 day	Visit 1 plus 3 days plus 1 day
Visit 3 / Day 8	Visit 1 plus 7 days minus 2 days	Visit 1 plus 7 days plus 2 days
Visit 4 / Day 15	Visit 1 plus 14 days minus 2 days	Visit 1 plus 14 days plus 2 days
Visit 5 / Day 29	Visit 1 plus 28 days minus 8 days	Visit 1 plus 28 days plus 14 days
Visit 6 / Day 85	Visit 1 plus 84 days minus 16 days	Visit 1 plus 84 days plus 16 days
Visit 7 / Day 180	Visit 1 plus 179 days minus 42 days	Visit 1 plus 179 days plus 42 days
Visit 8 / Day 365	Visit 1 plus 364 days minus 42 days	Visit 1 plus 364 days plus 42 days

Further PDs, such as prohibited concomitant medication, might also lead to exclusion of samples from the immunogenicity analysis at a given time point. Details will be discussed during the DRM.

2.10 Protocol Deviations and Data Review Meeting

2.10.1 Protocol Deviations

Protocol deviations (PD) identified by the medical monitor and ADMB data management will be entered in a database at VaxTrials (see also Protocol Deviation Management Plan). The PDs will be classified as “critical”, “major” and “minor” by the CRA regarding integrity of the data or effect on participants’ rights, safety, or well-being. Prior to the DRM, the CRA classification will be adapted to “important” (i.e. critical and major severity of PDs) and “non-important” (i.e. minor severity of PDs) prior the DRM, see also PD Management Plan.

In general, DRMs will be conducted on all available data to review PDs, to discuss specific unforeseeable data issues, and to allocate the participants to the analysis populations, where all necessary information is already available. In particular, the respective per-protocol analysis populations will be defined in the DRMs. DRMs will be conducted prior to each analysis. PDs will be classified as “major” or “minor” deviations on a case-by-case decision or by PD category, based on the potential impact on the immunogenicity analysis.

Associated decisions of the DRMs will be documented and approved in the Data Review Meeting Report.

PDs classified as “major” during the DRMs and / or classified as “important” by CRA and sponsor will be described in the CTR.

Examples that may lead to exclusion from the PP are provided here:

- Participant has a known or suspected defect of the immune system that can be expected to influence the immune response to the vaccine, such as participants with congenital or acquired immunodeficiency, 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 ≥0.05 mg/kg/day within 4 weeks prior to trial entry, radiation therapy or immunosuppressive cytotoxic drugs/ monoclonal antibodies in the previous 3 years; topical and inhaled steroids are allowed (EX10);
- Participant has received the wrong (not according to randomization) or no IMP;
- Participant has received IMP not approved for use (e.g. due to temperature excursions);
- Participant is HIV positive (EX10);
- Participant has taken any of the following medications, which are not permitted if administered within the specified trial periods (unless such treatment has to be administered in an emergency situation):
    - Any blood products or immunoglobulins during the course of the trial;
    - Prophylactic administration of antipyretics or analgesics within 24 hours prior to and during the first 72 hours after vaccination; Note: Considering that low grade fever is NOT an emergency situation (if a low-grade fever gets treated with antipyretics: it is NOT prophylactic administration, but a therapeutic treatment for an elevated temperature).

These criteria for exclusion from per-protocol analysis are identified at the time of planning the trial. However, during the course of the trial unforeseen events may occur or new scientific knowledge may become available, therefore

final decisions on whether any protocol violation could impact immune response and thus lead to exclusion from the PP will be made by the sponsor on a case-by-case basis. Sample testing issues may also lead to exclusion from the PP for particular time points as described in Section 2.9 above.

2.10.2 Safety Relevant Topics

The following AESI-relevant topics will be discussed during the DRM:

- Listing containing potential AESIs according to regulatory agencies definition as described in Section 6.1.2
- Listing containing potential AESIs as adjudicated by the Subject Matter Expert
- Listing containing AEs will be reviewed for classification into the following categories: immediate AE, non-solicited reactogenicity event, solicited event delayed onset

2.11 Participant Data Listings

All participants will be included in listings with information on participant enrollment, disposition, screening failures, inclusion/exclusion criteria and trial vaccination details. All other listings will be limited to vaccinated participants. Data listings will include the participant number as identifier, the trial arm, the age group, a column indicating if a participant is a sentinel or not (and parameter and/or visit if available) as well as baseline serostatus by µPRNT and will be sorted by participant ID (and parameter and/or visit if available). In listings including visits, both the visit name as reported in the eCRF, and the windowed visit will be displayed, with an indication of which results are used in the analysis for each windowed visit (i.e. some duplicate data might be shown). Further, the trial day will be included to give an overview on the actual visit window. In immunogenicity listings, columns that indicate if a participant is in the PP and/or if the respective sample is in the PP analysis will be shown.

In general, listings will include at least all columns specified in the dummy analysis of the respective analysis part (see Section 9).

2.12 Columns in Tables

In general, tables will include the following separate columns for each combination of treatment (half dose, full dose, control) and age group (7-11 years, 3-6 years, 1-2 years) and one column including all participants of the respective analysis population: half dose 7-11 yrs., full dose 7-11 yrs., control 7-11 yrs., half dose 3-6 yrs., full dose 3-6 yrs., control 3-6 yrs., full dose 1-2 yrs., half dose 1-2 yrs., control 1-2 yrs., total population.
Selected tables will be repeated showing the following pooled trial arm columns: half dose, full dose, control, total population. Details are available in the List of TLFs.

2.13 Medical Coding

After data entry, AEs and medical history will be coded according to the latest MedDRA version. The same MedDRA version will be applied to all trial parts. Previous and concomitant medication and vaccines will be coded according to the latest version of the WHO Drug Reference List and Anatomical Therapeutic Chemical (ATC) Classification System. For details see also the Coding Guideline attached to the Data Management Plan.

2.14 Programming According to CDISC Standards

For the VLA1553-221 trial, CDISC standards like the Study Data Tabulation Model (SDTM) and Analysis Data Model (ADaM) will be applied in order to comply with submission regulations. Hence, ADaM datasets will be generated based on SDTM datasets. However, for specific data which will only be listed, and no table or figure will be generated, and no SDTM dataset is available, non-standardized datasets will be generated. This includes e.g. vaccination delay criteria.

2.15 Changes in the Conduct of the Trial or Planned Analysis

The protocol states that confidence intervals (CI) for AE rates should be calculated according to Altman (i.e. 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 to other VLA1553 trial analyses.

In the protocol a table for solicited AEs by diary day is mentioned. However, the sponsor decided during SAP review that it is sufficient to only list this information.

In case any statistical analysis deviates from the SAP, all changes are described and justified in the CTR.

3. TRIAL DISPOSITION

Analyses will be performed for the respective safety analysis population, IMM and PP, if not stated otherwise.

3.1 Data Points

The following information will be analyzed descriptively and corresponding details on the participant level will be provided in data listings:

- Participant enrollment (including details on analysis populations)
- Screening failures and reasons
- Participant disposition (including details on end of trial)
- Visit details (separately for visits as collected in the eCRF and windowed visits)
- Protocol deviations

The following information will be listed only:

- Informed consent
- Randomization
- Violated inclusion/exclusion criteria
- Trial vaccination details

Specifications on tables and listings are provided in the List of TLFs, which is provided as appendix to this document.

3.2 Derivations and Definitions

For details on the data cut-off see Section 2.1.

4. BASELINE EVALUATION

In general, analyses will be performed for the safety analysis population. Only demographic information will be repeated for the IMM, seronegative and seropositive IMM and the PP.

4.1 Data Points

The following information will be analyzed descriptively and corresponding details on the participant level will be provided in data listings:

- Demographic information
- Medical history
- Vaccination history
- Prior/concomitant medications

The following information will be listed only:

- Chikungunya virus antibodies at screening

4.2 Derivations and Definitions

- Body Mass Index (BMI) categories will be assigned based on BMI-for-age percentiles. These will be calculated using WHO growth charts for participants aged 1-2 years and CDC growth charts for participants aged 3-11 years. Therefore, the programs cdc-source-code.sas (2022 revision, downloaded October 2024) and who-source-code.sas (no version given, downloaded October 2024) as well as the datasets CDCref_d.sas7bdat and WHOref_d.sas7bdat will be used as provided on the WHO and CDC websites https://www.cdc.gov/growth-chart-training/hcp/computer-programs/sas.html and https://www.cdc.gov/growth-chart-training/hcp/computer-programs/sas-who.html).
  The following categorization will be applied for non-missing values:
  - Underweight: BMI-for-age percentile<5
  - Healthy weight: 5<=BMI-for-age percentile<85
  - Overweight: 85<=BMI-for-age percentile<95
  - Obesity: 95<=BMI-for-age percentile
- Medications stopped prior to Day 1 (Visit 1) will be considered prior medications, all other medications will be considered concomitant. Medications with a missing or incomplete end date, where it cannot clearly be decided, if the medication stopped prior Day 1, will be considered concomitant.
- For details on the cut-off for medications see Section 2.1.
- Medical history not stopped prior to informed consent will be considered ongoing at trial entry. Entries with a missing or incomplete stop date, where it cannot clearly be decided, if the event stopped prior to informed consent, will be considered ongoing at trial entry. Medical history started at or after informed consent will be considered as not ongoing at trial entry.
- For data protection reasons, the individual participant dates of birth as captured in the eCRF will not be shown in the TLFs. However, since the date of birth of a participant is needed to calculate the BMI categories according to WHO/CDC, it will be included in the underlying datasets (SDTM domain DM and ADaM domain ADSL).

5. IMMUNOGENICITY ANALYSIS

Main analyses of immunogenicity will be performed on the PP and selected tables and figures will be repeated as supportive analyses on the IMM as well as on the seronegative and seropositive IMM.

All immunogenicity analyses will contain all available time points, i.e. from Part B analysis onwards all time points from previous analyses will be covered. E.g., for Part B analyses all time points which were already analyzed in previous Part A analyses will be repeated. For Part C analyses all time points analyzed in Part A and Part B will be repeated.

5.1 Data Points

5.1.1 Tables and Listings

The following information will be analyzed descriptively and corresponding details on participant level will be provided in data listings.

Summary tables for categorical immunogenicity variables:

- Seroconversion rate (SCR) as compared to baseline (Day 1) by visit
- Seroresponse rate by visit
- Rate of participants reaching an at least 4-fold, 8-fold, 16-fold or 64-fold increase in CHIKV-specific neutralizing antibody titers compared to baseline (Day 1) by visit

Summary tables for continuous immunogenicity variables:

- Geometric mean titer (GMT) of CHIKV-specific neutralizing antibodies by visit
- Geometric mean fold increase (GMFI) compared to baseline (Day 1) in CHIKV-specific neutralizing antibody titers by visit

5.1.2 Figures

- Line chart: GMTs for CHIKV-specific neutralizing antibodies after vaccination by trial day by trial arm and age group, using logarithmic scale and including 95% CIs (x-axis: trial visit by trial arm, y-axis: GMT)
- Overlaying scatter plot over boxplot: CHIKV-specific neutralizing antibodies after vaccination by trial visit by trial arm and age group (x-axis: trial visit by trial arm, y-axis: GMT)
- Reverse cumulative distribution curve: CHIKV-specific neutralizing antibodies at specific time points as specified in List of TLFs (x-axis: CHIKV-specific neutralizing antibodies, y-axis: percentage of participants)
- Bar chart: SCR for CHIKV-specific neutralizing antibodies after vaccination by trial visit by trial arm and age group, including 95% CIs (x-axis: trial visit by trial arm, y-axis: percentage of participants)
- Bar chart: Seroresponse for CHIKV-specific neutralizing antibodies after vaccination by trial visit by trial arm and age group, including 95% CIs (x-axis: trial visit by trial arm, y-axis: percentage of participants)
- Selected figures will be repeated stratified by µPRNT baseline serostatus and with organization of all charts on one page as specified in the List of TLFs.

5.2 Derivations and Definitions

- Baseline for immunogenicity is defined as Visit 1 (Day 1).
- Baseline seronegative is defined as $\mu PRNT_{50} \leq 40$
- Baseline seropositive is defined as $\mu PRNT_{50} > 40$
- Titers below the quantitation limit of $\mu PRNT_{50} < 20$ will be set to 10 (LLOQ/2) for statistical analysis.
- Participants with baseline titer values from 20-40 will not be imputed to 10 and the reported value will be used.
- The upper limit of quantification was determined as 13498 (see Supplement Validation Report for CHIKV Micro-PRNT in Human Serum, V02). However, values above 13498 will be reported in the data, and the reported values will be used in the analysis as they are.
- Fold-increase from baseline (Day 1) at a certain visit will be calculated by dividing the titer of the respective visit by the titer at Day 1. If one of the two titers or both are missing, the fold-increase is set to missing.
- A participant reaches a 4-fold increase, if the value at a certain visit after Day 1 is 4-times higher than the value at Day 1. 8-, 16- and 64-fold increase will be derived analogously.
- Seroconversion is defined as >4-fold increase of $\mu PRNT_{50}$ compared to baseline $\mu PRNT_{50}$ titer at Day 1 for baseline seronegative and seropositive participants.
- Seroresponse is defined as $\mu PRNT_{50} \geq 150$ for baseline seronegative and seropositive participants.
- Seroconversion/Seroresponse rate is defined as the number of participants with seroconversion/seroresponse, where the percentages are based on participants with an available result at the respective time point.

5.3 Inferential Statistics

- GMTs in the PP will be compared between VLA1553 trial arms and control by ANOVA, using the factors trial arm and age group. Further, an ANOVA with factor trial arm will also be performed for all age groups separately. In addition, Tukey's HSD test for pairwise comparisons between VLA1553 trial arms will be applied. The models will be applied on logarithmic values and estimates and two-sided 95% CIs for GMTs and GMT ratios will be obtained by back-transformation.
- GMFI's will be analyzed analogously.
- Seroresponse and seroconversion rates will be compared between VLA1553 trial arms and control by overall Fisher-Freeman-Halton test, amended by Fisher's exact test for pairwise comparisons in case of a significant overall p-value, and two-sided 95% CIs will be calculated according to Clopper-Pearson.

6. SAFETY ANALYSIS

Analyses will be performed for the safety analysis population. Selected tables will be stratified by baseline serostatus as specified in the List of TLFs.

6.1 Adverse Events

6.1.1 Data Points

6.1.1.1 Tables and Listings

The following information will be analyzed descriptively and corresponding details on the participant level will be provided in data listing:

General (solicited and unsolicited AEs):

- AE overview
    - Any AE
    - Any related AE
    - Any severe AE
    - Any related severe AE
    - Any medically attended AE
    - Any related medically attended AE
    - Any AE with fatal outcome
    - Any SAE
    - Any related SAE
    - Any AE leading to withdrawal from trial
    - Any AESI (protocol definition)
    - Any early onset AESI (protocol definition)
    - Any late onset AESI (protocol definition)
    - Any CLAR (AESI by regulatory agencies definition)
    - Any prolonged CLAR (AESI by regulatory agencies definition)
    - Any AESI adjudicated
    - Any AE with missing seriousness assessment
    - Any AE with missing severity assessment
    - Any AE with missing causality assessment
    - Any solicited AE
    - Any related solicited AE
    - Any severe solicited AE
    - Any related severe solicited AE
    - Any serious solicited AE
    - Any related serious solicited AE
    - Any solicited injection site AE

  - Any related solicited injection site AE
  - Any severe solicited injection site AE
  - Any related severe solicited injection site AE
  - Any solicited systemic AE
  - Any related solicited systemic AE
  - Any severe solicited systemic AE
  - Any related severe solicited systemic AE
  - Any solicited AE ongoing after Day 15
  - Any unsolicited AE
  - Any related unsolicited AE
  - Any severe unsolicited AE
  - Any related severe unsolicited AE
  - Any serious unsolicited AE
  - Any related serious unsolicited AE
  - Any medically attended unsolicited AE
  - Any related medically attended unsolicited AE
- Serious AEs by SOC and PT
- Related serious AEs by SOC and PT
- Medically attended AEs by SOC and PT
- Related medically attended AEs by SOC and PT
- AEs leading to withdrawal from trial by SOC and PT
- AEs by SOC and PT for PTs with frequency >= 10% in any trial arm
- AEs by SOC and PT for PTs with frequency >= 5% in any trial arm
- Non-serious AEs by SOC and PT for PTs with frequency >= 5% in any trial arm

Solicited AE tables:

- Solicited injection site AEs overall and by symptom
- Related solicited injection site AEs overall and by symptom
- Solicited systemic AEs overall and by symptom
- Related solicited systemic AEs overall and by symptom
- Severe solicited injection site AEs by symptom
- Severe solicited systemic AEs by symptom
- Related severe solicited injection site AEs by symptom
- Related severe solicited systemic AEs by symptom
- Serious solicited injection site AEs by symptom
- Serious solicited systemic AEs by symptom
- Related serious solicited injection site AEs by symptom
- Related serious solicited systemic AEs by symptom

- Medically attended solicited injection site AEs by symptom
- Medically attended solicited systemic AEs by symptom
- Related medically attended solicited injection site AEs by symptom
- Related medically attended solicited systemic AEs by symptom
- Solicited AEs overall by maximum severity
- Related solicited AEs overall by maximum severity
- Solicited injection site AEs by severity
- Related solicited injection site AEs by severity
- Solicited systemic AEs by severity
- Related solicited systemic AEs by severity
- Mean duration (in days) of solicited injection site AEs
- Mean duration (in days) of solicited systemic AEs
- Greatest single diameter (in cm) for erythema, swelling and induration
- Maximum body temperature (in °C) for case of fever
- Number of participants with prolonged joint pain

Unsolicited AE tables:

- Unsolicited AEs by SOC and PT
- Related unsolicited AEs by SOC and PT
- Serious unsolicited AEs by SOC and PT
- Related serious unsolicited AEs by SOC and PT
- Severe unsolicited AEs by SOC and PT
- Related severe unsolicited AEs by SOC and PT
- Medically attended unsolicited AEs by SOC and PT
- Related medically attended unsolicited AEs by SOC and PT
- Unsolicited AEs by maximum severity
- Medically attended unsolicited AEs by maximum severity
- Unsolicited AEs by highest causality
- Medically attended unsolicited AEs by highest causality

AESI Tables

- AESIs (protocol definition) by SOC and PT
- Early onset AESIs (protocol definition) by SOC and PT
- Late onset AESIs (protocol definition) by SOC and PT
- CLARs (AESIs by regulatory agencies definition) by SOC and PT
- Prolonged CLARs (AESIs by regulatory agencies definition) by SOC and PT
- AESIs (protocol definition) by maximum severity
- Early onset AESIs (protocol definition) by maximum severity

- Late onset AESIs (protocol definition) by maximum severity
- CLARs (AESIs by regulatory agencies definition) by maximum severity
- AESIs (protocol definition) by highest causality
- Early onset AESIs (protocol definition) by highest causality
- Late onset AESIs (protocol definition) by highest causality
- CLARs (AESIs by regulatory agencies definition) by highest causality
- Number of participants with prolonged CLARs (AESIs by regulatory agencies definition)

6.1.1.2 Figures

- Bar chart: number and percentage of participants with solicited injection site AEs overall and by symptom, by trial arm, severity and age group (x-axis: symptoms by trial arm, y-axis: percentage of participants with AEs by severity, age groups in separate charts)
- Bar Chart: number and percentage of participants with solicited systemic AEs overall and by symptom, by trial arm, severity and age group (x-axis: symptoms by trial arm, y-axis: percentage of participants with AEs by severity, age groups in separate charts)
- Bar chart: duration of solicited fever events by trial arm, severity and age group (x-axis: duration category (1-5 days; 6-10 days; 11-15 days) by trial arm, y-axis: number of participants in duration category by severity, age groups in separate charts)
- Selected figures will be repeated stratified by µPRNT baseline serostatus and with organization of all charts on one page as specified in the List of TLFs.

6.1.2 Derivations and Definitions

Sources for AEs:

- In general, there are four sources for AEs in the eCRF: “Assessments after Vaccination”, “AE Log” and “Participant Diary” and "AESI Log". Unsolicited AEs are documented only in the eCRF section “AE Log” and will be taken from there for analysis. Information of solicited AEs will be taken from the eCRF sections “Assessments after Vaccination” and “Participant Diary”. AESI case information is documented in the “AESI Log”, with links to the unsolicited AESI symptoms in the “AE Log” and/or to solicited AEIS symptoms in the “Participant Diary”.
- For presentations of solicited AEs on symptom level, diary days need to be collapsed to one event. Therefore, solicited AEs from the sources “Assessment after Vaccination” and “Participant Diary” are combined into one line per participant per symptom. To this end, “Assessments after Vaccination” will be handled like an additional diary day entry. Information from all diary days (Assessment after vaccination, Day 1 to Day 15) considering also events ongoing after Day 15, are summarized using the following rules:
    - Presence: A symptom will be counted as present, if reported as present on at least one diary day.
    - For combination of AE assessments (i.e. impact on daily life, severity/causality by investigator, outcome) the following rules apply:

- In general, the worst assessment out of all diary days will be the taken (maximum severity, highest causality).
- For combination of outcome assessments the worst assessment will be determined by the following order: fatal > not recovered/not resolved > unknown > recovering/resolving > recovered/resolved with sequelae > recovered/resolved
- Only in case assessments are missing at all diary days, the overall assessment will be set to missing.

- For summaries including unsolicited and solicited AEs, collapsed solicited AEs as described above will be combined with the "AE Log". In case of duplicate information (i.e. solicited SAE in the "AE Log" and the diary), the information from the "AE Log" prevails.

General principles:

- Tables showing "severe" events will include events with grade 3 or 4 for analyses up to Part B. For Part C analysis also AEs with missing severity will be analyzed as "severe".
- AEs will be considered related for analyses up to Part B, if the causality to IMP is reported as "probably related" or "possibly related". For Part C analysis also missing causality will be regarded as related.
- Tables that are not presented by symptom may include several events per participant and thus will be shown by maximum severity/highest causality per participant. Participants will be only counted once in highest grading category and events are counted in each reported grading category.
- For details on the cut-off for AEs see Section 2.1.
- AEs in the AE log will be considered as "leading to withdrawal from trial" if in section "other action taken" the question "withdrawn from trial" is answered with "yes".
- SAEs will be captured starting from ICF signed whereas other AEs will only be captured starting at time of vaccination. However, for the descriptive analyses only SAEs with SAE onset date at or after date of vaccination will be considered. SAEs occurring during screening or after screening but prior vaccination will be captured in a listing.

Definition of AESIs:

AESIs will be analyzed according to the following two definitions:

- AESIs by protocol definition: Early onset AESIs are AESIs reported on the respective eCRF page with onset 2 to 21 days after vaccination (i.e. Day 3 to Day 22). AESIs starting 22 or more days after vaccination (i.e., Day 23 or later) until trial end will be considered as late onset AESIs. However, AESIs with first symptom(s) occurring until 21 days after vaccination (Day 22) and additional symptom(s) occurring afterwards will still be classified as early onset AESIs (see also CTP, Section 17.10).
- CLARs (AESIs by regulatory agencies definition): CLARs are defined as follows: fever (≥38 °C / 100.4 °F) and any single of the following events or symptoms: arthralgia, arthritis, acute (poly)arthralgia/arthritis, fatigue, chills, pain, oedema peripheral, headache, dizziness, paraesthesia, myalgia, back pain, rash, hyperhidrosis, eye disorders (e.g. conjunctivitis, retinitis, uveitis, optic neuritis), cardiac events (e.g. tachycardia, arrythmia, myocarditis, pericarditis, chest pain, palpitation, dyspnea and other cardiac

complications), neurological symptoms (e.g. confusion, meningoencephalitis, polyneuropathy), macular to maculopapular rash (sometimes with cutaneous pruritus (foot arch), edema of the face and extremities), polyadenopathies occurring within 30 days after the vaccination, regardless of the order of their onset and duration.

- Detection of CLARs: Since the symptoms described above can not be identified in a fully automatic way the following steps will be followed:
    1. The AE dataset will be filtered for the following SOCs/PTs occurring within 30 days after vaccination (or have an incomplete AE start date):
        - SOC General disorders and administration site conditions: all PTs will be included with the exception of injection/vaccination site reactions including but not limited to injection site reactions defined per protocol as solicited AEs (injection site pain, injection site tenderness, injection site erythema/redness, injection site swelling, injection site induration/hardening,. Further, the PTs vaccination site pain and nodule will be excluded based on prior medical review for Part A Analysis.
        - SOC Cardiac disorders: all PTs will be included
        - SOC Infections and infestations: only the following PTs will be included: encephalitis, meningoencephalitis, meningitis, acute meningoencephalitis, meningoencephalitis NOS, autoimmune meningoencephalitis, potentially viral meningoencephalitis, bacterial meningoencephalitis, post-infectious meningoencephalitis
        - SOC Nervous system disorders: all PTs will be included except the PT sciatica, which was excluded based on prior medical review for Part A Analysis.
        - SOC Psychiatric disorder: only the following PTs will be included: confusional state, disorientation, altered mental state, delirium, mental state changes, cognitive disorder
        - SOC Skin and subcutaneous tissue disorders: all PTs will be included except the PTs dermatitis contact and sensitive skin, which were excluded based on prior medical review for Part A Analysis.
        - SOC Blood and lymphatic system disorders: all PTs will be included except the following: leukopenia, lymphopenia, neutropenia, lymphocytosis
        - SOC Musculoskeletal and connective tissue disorders: only the following PTs will be included: arthralgia, temporomandibular pain and dysfunction syndrome, acute aseptic arthritis, arthritis, arthritis allergic, arthritis bacterial, arthritis climacteric, arthritis enteropathic, arthritis infective, arthritis reactive, arthritis viral, arthritis-dermatitis syndrome, autoimmune arthritis, axial spondyloarthritis, epidemic polyarthritis, immune-mediated arthritis, interspinous osteoarthritis, juvenile idiopathic arthritis, juvenile psoriatic arthritis, juvenile spondyloarthritis, laryngeal rheumatoid arthritis, oligoarthritis, osteoarthritis, paradoxical psoriatic arthritis, periarthritis, peripheral spondyloarthritis, polyarthritis, rheumatoid arthritis, seronegative arthritis, SLE arthritis, spinal osteoarthritis, undifferentiated spondyloarthritis, epidemic polyarthritis, juvenile

idiopathic arthritis, polyarthritis, polymyalgia rheumatica, rheumatic fever, rheumatoid arthritis, back pain, arthralgia, fibromyalgia, myalgia, myalgia intercostal, myositis, polymyalgia rheumatica

- SOC Eye disorders: only the following PTs will be included: conjunctivitis, conjunctivitis allergic, conjunctivitis viral, noninfective conjunctivitis, vernal keratoconjunctivitis, retinitis, chorioretinitis, necrotizing retinitis, noninfective chorioretinitis, noninfective retinitis, retinitis viral, uveitis, autoimmune uveitis, immune recovery uveitis, immune-mediated uveitis, infective uveitis, keratouveitis, tubulointerstitial nephritis and uveitis syndrome, viral keratouveitis, viral uveitis, optic neuritis, immune-mediated optic neuritis, optic perineuritis

2. The list of participants who experienced at least one of the selected PTs described above within 30 days after vaccination will be narrowed down to present only those symptoms that were present in participants with fever within 30 days after vaccination.
3. A medical review will be performed to exclude those symptoms that are not known to be associated with a CHIKV acute infection. This review will be documented in the Data Review Meeting.
4. The resulting list of symptoms falling under the requested CLAR definition will then be summarized to show one entry per participant. Therefore, the worst severity of included symptoms will be taken as CLAR severity and earliest start/latest symptom end date will be used as CLAR dates.

- Prolonged CLAR: A CLAR case will be considered prolonged, if at least one symptom of the CLAR case has a duration of or greater than 30 days.

Principles for solicited AEs:

- Solicited AEs comprise reactions at the injection site or systemic reactions that are typical for vaccinations. Possible systemic reactions will further depend on the age group the participant belongs to.
  - Solicited injection site reactions: injection site pain, tenderness, erythema/redness, induration/swelling. In summaries, there will be a separate row for pain and tenderness pooled.
  - Systemic reactions:
    - Age groups A and B: nausea/vomiting, headache, fatigue, myalgia (muscle pain), arthralgia (joint pain), rash and fever
    - Age group C: nausea/vomiting, inconsolable or excessive crying, disturbed sleep, loss of appetite, drowsiness, rash and fever
- The symptom fever will be counted as present when the body temperatures is reported as >=38°C.
- For the derivation of the duration of solicited AEs by symptom the calculated duration in the corresponding SDTM dataset will not be used. For the duration the difference of the first occurrence and the last occurrence of the concerning event will be taken, no matter if the event is continuous or not. Therefore, also the end date if ongoing after Day 15 will be considered. If the AE was ongoing after Day 15, but has a missing or incomplete end date, the date of last attended visit will be used as end date.
- Prolonged joint pain is defined as joint pain with a duration >= 30 days.

- For swelling, redness and induration, the maximum diameter in cm will be derived as taking the maximum of all diameters reported for that symptom for each participant.
- The maximum body temperature for present symptom fever will be derived as taking the maximum fever temperature of all temperatures reported for each participant.
- AEs from the Participant Diary will be coded according to the table below:

Event	SOC name	SOC code	PT name	PT code
Disturbed Sleep	Psychiatric disorders	10037175	Sleep disorder	10040984
Drowsiness	Nervous system disorders	10029205	Somnolence	10041349
Fatigue	General disorders and administration site conditions	10018065	Fatigue	10016256
Fever	General disorders and administration site conditions	10018065	Pyrexia	10037660
Headache	Nervous system disorders	10029205	Headache	10019211
Inconsolable or excessive crying	General disorders and administration site conditions	10018065	Crying	10011469
Injection site erythema/redness	General disorders and administration site conditions	10018065	Injection site erythema	10022061
Injection site induration/hardening	General disorders and administration site conditions	10018065	Injection site induration	10022075
Injection site pain	General disorders and administration site conditions	10018065	Injection site pain	10022086
Injection site swelling	General disorders and administration site conditions	10018065	Injection site swelling	10053425
Injection site tenderness	General disorders and administration site conditions	10018065	Injection site pain	10022086
Joint pain	Musculoskeletal and connective tissue disorders	10028395	Arthralgia	10003239
Loss of appetite	Metabolism and nutrition disorders	10027433	Decreased appetite	10061428
Muscle pain	Musculoskeletal and connective tissue disorders	10028395	Myalgia	10028411
Nausea	Gastrointestinal disorders	10017947	Nausea	10028813
Rash	Skin and subcutaneous tissue disorders	10040785	Rash	10037844
Vomiting	Gastrointestinal disorders	10017947	Vomiting	10047700

6.1.3 Inferential Statistics

- For primary endpoint analysis, groups will be compared using Fisher's exact test (Fisher-Freeman-Halton test).
- 95% confidence intervals according to Clopper-Pearson will generally be provided for all AE rates.
- Selected frequencies will be compared using Fisher's exact test (Fisher-Freeman-Halton test) as specified in the List of TLFs.

6.2 Laboratory Parameters

Laboratory data from hematology and coagulation panels as well as urinalysis results will be analyzed descriptively and corresponding details on participant level will be provided in data listings. Data from AESI laboratory tests will only be listed.

6.2.1 Data Points

The following parameters are assessed in the trial and will be included in the statistical analysis:

Hematology parameters:

- Hemoglobin
- Hematocrit
- Erythrocyte count
- White blood cell count
- Differential white blood cell count (basophils, eosinophils, lymphocytes, monocytes, neutrophils)
- Platelets
- ESR

Coagulation factors:

- Prothrombin time
- Activated partial thromboplastin time
- Fibrinogen

Urinalysis:

- pH value
- Specific gravity
- Leucocytes
- Nitrite
- Protein
- Glucose
- Ketones
- Urobilinogen
- Bilirubin
- Erythrocytes

AESI laboratory test parameters:

- Rheumatoid factor
- Anticitrullinated protein antibody (ACPA)
- Ferritin
- C-reactive protein (CRP)

The following variables will be analyzed descriptively by visit:

- Absolute values (summary statistics for hematology and coagulation)
- Absolute change from screening (summary statistics for hematology and coagulation)
- Number of participants with values above/ below normal range (for hematology and coagulation)
- Urinalysis results:
    - Number of participants with normal/ abnormal values
- Participants with values reaching grading by severity (for parameters with grading defined in Section 6.2.2 below)

6.2.2 Derivations and Definitions

Severity grading:

For statistical analysis, laboratory assessments will be graded according to the grading scale provided in the CTP, Section 17.8.3 Assessment of Laboratory Values .In particular, all values lying in the range of the severity grades will be assigned to the respective grade and all abnormal values as per site's normal ranges that do not fulfill the criteria for at least grade 1 will be labelled "Grade 0". All values that neither lie in the range of a severity grade nor are considered abnormal based on site's normal ranges will not be assigned a grade.

6.3 Other Safety Parameters

6.3.1 Vital Signs

Vital signs will be summarized descriptively at each trial time point 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 time point. Corresponding details on the participant level will be provided in data listings.

Vital signs parameters to be summarized include:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Pulse rate (bpm)
- Body temperature (°C)

6.3.2 Viremia Sample

Analysis of viremia will be performed for the safety analysis population restricted to the viremia subset. Analyses will only be performed for Part B and C.

The following will be tabulated by visit:

- Number, mean, minimum and maximum of viremic quantifiable results
- Results by type (e.g. viremic, non-viremic, inconclusive)

Analyses will be repeated, pooled by trial arm, and stratified by µPRNT baseline serostatus. Corresponding details on the participant level will be provided in data listings.

The following figures will be produced:

- Bar chart: percentage of participants with viremic results by trial visit, trial arm and age group (x-axis: trial visits by trial arm, y-axis: percentage of participants with viremic results, age group in separate charts as well as panelled)
- Overlaying scatter plot over boxplot: viral load in GCE/ml by trial visit and trial arm (x-axis: trial visit by trial arm, y-axis: GCE/ml, age group in separate charts as well as panelled)
- Line chart: mean viral load in GCE/ml by trial visit by trial arm and age group (x-axis: trial day by trial arm, y-axis: mean GCE/ml)

6.3.2.1 Derivations and Definitions

- Categorization of results will be based on the data format as described in the respective Data Transfer Specifications and will be described in respective table footnotes. The following categories will be included:
  - Viremic (quantifiable results and <LLOQ)
  - Non-viremic (not detected and <LOD)
  - Inconclusive (invalid result, IR)
- All non-numerical values (except IR) will be imputed for statistical analysis as follows:
  - Results <LLOQ will be imputed with LLOQ/2 (3214,4 /2 =1607,2)
  - Results <LOD and not detected results will be imputed with LOD/2 (1071,4 / 2 = 535,7)

6.3.3 Physical Examination

Physical examination results will be tabulated by visit and corresponding details on the participant level will be provided in data listings.

6.3.4 Pregnancy Test

Pregnancy test results will only be listed.

6.3.5 Healthcare Events

Healthcare event data will only be listed.

7. EXPLORATORY ANALYSIS OF NATURAL CHIKV INFECTIONS

An exploratory analysis of natural CHIKV infections will be performed for the safety analysis population.

An excel workbook including all information to detect potential natural CHIKV cases will be prepared as described below and sent to the sponsor prior Part C analysis. The sponsor will then perform a classification of natural CHIKV cases based on these listings. Descriptive analysis of natural CHIKV cases will therefore only be performed for Part C based on data availability.

The following information will be provided in an excel workbook prior Part C analysis:

- CHIKV disease signs and symptoms (same list of SOCs and PTs as used for CLARs described in Section 6.1.2 above will be considered, without applying time restriction)
- Local diagnostic results (if available)
- Immunogenicity data (seroconversion by µPRNT in the control arm or in the VLA1553 treatment arm at various time points, if available)
- Viremia data (if available from Nexelis i.e., RT-qPCR)

8. LIST OF TABLES, DATA LISTINGS AND FIGURES

In general, the numbering consists of the following components in the given order:

Description	Values
CTR section	14 = Tables and figures 16.2 = Listings
Analysis section	1 = Trial disposition tables / listings 2 = Baseline tables / listings 3 = Immunogenicity tables / listings 4 = Adverse event tables / listings 5 = Other safety information tables / listings 6 = Immunogenicity figures 7 = Safety figures
Analysis population and label for TLF headers	Safety analysis population Immunogenicity analysis population Baseline seronegative immunogenicity analysis set Baseline seropositive immunogenicity analysis set Per-protocol analysis population
TLF ID	1 2 …

The population digit is used only in case TLFs are produced for more than one analysis population. Further digits can be introduced during the programming of the analysis, if necessary (e.g. introducing sub-continuous numbering in case Listing 16.2.1.1 has to be divided in 16.2.1.1.1 and 16.2.1.1.2).
A list of TLFs will be provided in an appendix to this SAP.

9. SHELLS OF TABLES, DATA LISTINGS AND FIGURES

For this analysis, shells of tables, listings and figures will be generated. The table shells will serve as a basis for programming. The shells will be produced on dummy treatment allocation and dummy immunogenicity analysis results. Further, dummy data might be generated, in case data not available at the time point of the shell programming. These table shells will be finalized and will serve as a basis for the actual analyses.