valneva

Clinical Study Protocol

DRUG SUBSTANCE(S)	VLA1553
VERSION NO.	FINAL 6.0
STUDY CODE	VLA1553-301
DATE	23-Mar-2021

A MULTICENTER, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLINDED PIVOTAL STUDY TO EVALUATE SAFETY AND IMMUNOGENICITY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADULTS AGED 18 YEARS AND ABOVE

Phase 3 Study

PROTOCOL NUMBER: **VLA1553-301**

IND NUMBER: **17854**

Sponsor **Valneva Austria GmbH**
[redacted]
Campus Vienna Biocenter 3
A-1030 Vienna, Austria

1. PROTOCOL SIGNATURE PAGE

Title of Clinical Trial: **A MULTICENTER, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLINDED PIVOTAL STUDY TO EVALUATE SAFETY AND IMMUNOGENICITY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADULTS AGED 18 YEARS AND ABOVE**

Protocol Number: **VLA1553-301**

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With their signature, Investigators and Sponsor agree to conduct this study in accordance with the Protocol, International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines and with the applicable local regulatory requirements. Moreover, the site will keep all information obtained from the participation in this study confidential unless otherwise agreed in writing.

Principal Investigator

Print Name	Signature	Date

[redacted]

[redacted]
Clinical Operations
Valneva Austria GmbH

Signature Date

[redacted]

[redacted]
Clinical Strategy
Valneva Austria GmbH

Signature Date

[redacted]

[redacted]
Chief Medical Officer
Valneva Austria GmbH

Signature 26 March 2021
Date

2. STUDY PERSONNEL

Clinical Operations

[redacted]
Valneva Austria GmbH
[redacted]
e-mail: [redacted]

Sponsor's Medical / Safety Officer

[redacted]
Valneva Austria GmbH
e-mail:
[redacted]

Study Medical Monitor

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e-mail: [redacted]

2.1 Study Organization

The contact details of the organization/individuals involved in the study (e.g.; investigator(s), Sponsor's representative(s), laboratories, oversight committees [including institutional review boards (IRBs), as applicable] will be maintained by the Sponsor and provided to the Investigator.

3. SERIOUS ADVERSE EVENT REPORTING

The Investigator will comply with applicable laws/requirements for reporting serious adverse events (SAEs) to the IRB. For information on the definition and assessment of adverse events (AEs), refer to Section 17.1.

All SAEs should be reported on the SAE Report Form to the PRA Safety Desk by fax or email within 24 hours after the Investigator has become aware of the event. Under certain circumstances the initial notification could be done by phone, but nevertheless a written SAE Report Form has to be submitted within 24 hours to:

PRA Safety Desk

Fax: [redacted]

Email:

Safety Hotline: [redacted]

4. ADVERSE EVENTS OF SPECIAL INTEREST REPORTING

The Investigator will comply with requirements set forth in this protocol for reporting adverse events of special interest (AESIs). For information on the definition and assessment of adverse events of special interest (AESIs), refer to Section 17.9.

All AESIs should be reported on the AESI Report Form to the PRA Safety Desk by fax or email within 48 hours after the Investigator has become aware of the event. Under certain circumstances the initial notification could be done by phone, but nevertheless a written AESI Report Form has to be submitted within 48 hours to:

PRA Safety Desk

Fax: [redacted]

Email:

Safety Hotline: [redacted]

5. PREGNANCY REPORTING

The Investigator will comply with applicable laws/requirements for reporting pregnancies to the IRB. For information on the definition and assessment of pregnancies, refer to Section 17.10.3.

All Pregnancies* should be reported on the Pregnancy Report Form to the PRA Safety Desk by fax or email within 24 hours after the Investigator has become aware of the event.

PRA Safety Desk

Fax: [redacted]

Email:

Safety Hotline: [redacted]

* A pregnancy is not considered an SAE. If a seriousness criterion applies in addition to the pregnancy (e.g. hospitalization, congenital anomaly/birth defect) the pregnancy qualifies as an SAE. In such case a Pregnancy Report Form **and** an SAE Report Form have to be filled out.

6. STUDY CHANGES IN RESPONSE TO SARS-COV2 PANDEMIC

The study Sponsor will continuously monitor and evaluate the development of the SARS-COV2 pandemic in the area of study sites to determine if any measures need to be implemented to mitigate undue risks to the subjects or in response to local governmental recommendations. Such measures may include, temporarily halting further recruitment, switching in-person visits to phone calls, or employing mobile teams to collect serum samples. Any measure would be communicated to the relevant Competent Authority and Institutional Review Board and documented in the CSR.

Due to the ongoing SARS-COV2 pandemic, study sites experience challenges in recruiting study participants, especially elderly people. Reasonable efforts have been made to mitigate significant recruitment delays, e.g. intensified study advertisement, and opening new study sites.

7. CLINICAL STUDY SYNOPSIS

INVESTIGATIONAL PRODUCT, DOSAGE AND MODE OF ADMINISTRATION	
Title	**A MULTICENTER, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLINDED PIVOTAL STUDY TO EVALUATE SAFETY AND IMMUNOGENICITY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADULTS AGED 18 YEARS AND ABOVE**
Name of Investigational Medicinal Product (IMP)	Live-attenuated Chikungunya (CHIKV) vaccine (VLA1553)
Name(s) of Active Ingredient(s)	Live-attenuated CHIKV vaccine strain based on the La Reunion strain (LR-CHIKV clone LR2006-OPY1) of East/ Central/ South African (ECSA) genotype (del5nsP3)

VLA1553 is a live-attenuated chikungunya virus (CHIKV) vaccine candidate designed for active immunization for the prevention of disease caused by CHIKV. The candidate vaccine is intended to prevent CHIKV infections in the general population living in endemic regions, as well as to serve as a prophylactic measure for travelers to epidemic areas or areas at risk for an upcoming outbreak or ongoing endemic transmission. The replicating CHIKV vaccine comprises a large deletion of 60 amino acids in the nsP3 gene (del5nsP3) encoding the non-structural replicase complex protein nsP3, which leads to attenuation of the virus *in vivo*.

In this pivotal study, the final dose of VLA1553 (target 1x 10E4 $TCID_{50}$ per 0.5 mL) will be evaluated for safety and immunogenicity. The study will be carried out in multiple sites in the U.S. VLA1553 and control will be administered intramuscularly (i.m.) into the deltoid muscle as a single-shot immunization on Day 1. Subjects will be followed for safety, immunogenicity and antibody persistence for 6 months. The vaccine presents in a lyophilized dosage form.

CHIKV is a small spherical RNA virus and a member of the *Alphavirus* genus in the family *Togaviridae*. The arthropod-borne virus is closely related to other viruses in Africa, South America and Australia that cause similar signs and symptoms such as Ross River virus, Mayaro- virus or o'nyong-nyong-virus. The virus is vectored by the daytime-biting *Aedes aegypti* mosquito, which also transmits yellow fever, Zika and dengue viruses. CHIKV can also be transmitted by *Aedes albopictus* mosquitoes, a more cold-tolerant mosquito that has resulted in the spread of chikungunya to more temperate areas of the world such as Italy and France.

CHIKV has been reported in over 100 countries with more than 2.2 million suspected cases in reported to the Pan American Health Organization (PAHO) in the Americas alone. CHIKV epidemics are explosive and rapidly moving, but not predictable.

An infection with CHIKV results in chronic and incapacitating arthralgia affecting all gender and age groups accompanied by an acute febrile disease with headache, muscle pain, and skin rashes. The severe, often debilitating joint pain in infected patients can persist for years, especially in adults. Individuals who are at higher risk of more serious complications include infants, the elderly and individuals with chronic medical conditions. Currently, neither specific antiviral treatment nor a vaccine is available to prevent CHIKV infection. Prevention against CHIKV infection is therefore limited to non-

treatment interventions such as the deployment of insecticides, wearing long sleeves and pants and repellants, and other means to restrict exposure to vector mosquitos.	
CLINICAL CONDITION(S)/INDICATION(S)	
Active immunization for the prevention of disease caused by CHIKV	
STUDY PHASE	Pivotal Phase 3
PLANNED STUDY PERIOD	
Initiation	Q3 / 2020
Duration	The overall study duration (First Subject In – Last Subject Out) is estimated to be approximately 13 months. Individual subject participation is approximately 7 months from enrollment to study completion unless prematurely discontinued.
Completion	Part A (Visit 3, Day 29): planned Q2 / 2021 Part B (Visit 5, Month 6): planned Q4 / 2021 Individual study parts will be analyzed sequentially. Part A analysis will be performed on Day 29 and will comprise the primary endpoints. Part B final analysis will be performed once the last subject has completed Month 6 (Visit 5) and will be submitted for BLA filing.
STUDY OBJECTIVES	
Primary Objective ➢ To evaluate immunogenicity and safety of the final dose of the live-attenuated CHIKV vaccine candidate (VLA1553) 28 days following vaccination in a population aged 18 years and above after a single immunization. **Secondary Objective** ➢ To evaluate immunogenicity and safety of the final dose VLA1553 up to 180 days following vaccination in a population aged 18 years and above after a single immunization.	

STUDY DESIGN	
Investigator and sites	Multicenter study. The study will be conducted at approximately 44 study sites throughout the U.S.
Study participants	A total of approximately 4,060 male and female subjects aged 18 years and above will be randomized 3:1 to either the final dose of VLA1553 or control.
Study Type	Safety and Immunogenicity
Vaccine Type	Live-attenuated CHIKV in a lyophilized formulation
Control Type	Placebo (Phosphate buffered saline, PBS) in a liquid formulation
Study Indication Type	Prevention
Blinding Scheme	Blinded (double-blind, i.e. the subject as well as all study site staff/ Investigator/ Sponsor remain blinded to treatment allocation)

Study Design

This is a prospective, randomized, double-blinded, multicenter, pivotal clinical study evaluating the final dose of VLA1553 (1 x10E4 $TCID_{50}$ per 0.5 mL) in comparison to a placebo control. The final dose of VLA1553 or control will be administered as single immunization on Day 1. Overall, approximately 4,060 male and female subjects aged 18 years and above will be enrolled (i.e. ICF signed) into the study.

Subjects will be allocated in a 3:1 ratio to VLA1553 (n= approximately 3,045) or control group (n= approximately 1,015). The approximately 4,060 subjects in this study will be 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 346 subjects in Stratum A and 154 subjects in Stratum B will be included in the immunogenicity evaluation and comprise the immunogenicity subset of in total approximately 500 subjects (**Table 1**). The immunogenicity subset will be randomly enrolled at approximately 15 pre-selected study sites across the U.S. representative of the whole study population.

Due to the difficulty to enroll elderly subjects of Stratum B (≥ 65 yrs.), enrollment was opened to all subjects in December 2020 and Stratum B was filled up with either subjects of Stratum A (18 – 64 yrs.) or Stratum B (≥ 65 yrs.) changing the composition of the immunogenicity subset, but not its total number. Due to study blinding, the exact figures for study arms 1 and 2 are not available for the time being.

Table 1 below illustrates the subject distribution.

Table 1. Subject Distribution

Study Arm	Treatment	Stratum	Number of subjects (n)	Immunogenicity evaluation (n)	Dose ($TCID_{50}$/dose)	Injection Volume (mL)
1	VLA1553		3,045			0.5
		A (18 – 64 yrs.)	2,740	292*	1x 10E4	
		B (≥ 65 yrs.)	305	83*		
2	Control		1,015		n.a.	0.5
		A (18 – 64 yrs.)	913	98*		
		B (≥ 65 yrs.)	102	28*		
		Total N:	**4,060**	**501***		

Note: * with actually 111 subjects enrolled in Stratum B, we expect approximately 83 subjects receiving VLA1553 and 28 subjects receiving placebo, similarly with 390 Stratum A subjects enrolled into the immunogenicity subset we expect approximately 292 on VLA1533 and 98 on placebo (to be confirmed after unblinding of the study).

All subjects will be asked to return to the study site at Day 8 (Visit 2), Day 29 (Visit 3), Day 85 (Visit 4) and Month 6 (Day 180, Visit 5) for immunogenicity sampling. However, immunogenicity analysis and evaluations were only planned to be done in the immunogenicity subset. Due to the addition of non-immunogenicity subset subjects in the Immunogenicity elderly population, some immunogenicity samples will also be evaluated and analyzed for these subjects. In addition, a clinical sample for safety laboratory evaluations will be obtained at all study visits from the immunogenicity subset only. Safety data collection will capture solicited AEs until Day 11 and unsolicited AEs up to Day 180 (Month 6, Visit 5) from all subjects. AESIs will be captured 2 to 21 days post-vaccination. Subjects presenting with acute arthralgia within this time period will be followed-up until resolution and monitored for recurrences until the end of the study. SAEs will also be assessed until the end of the study in all subjects (Month 6, Visit 5).

The clinical study design is displayed in the **Figure 1** below.

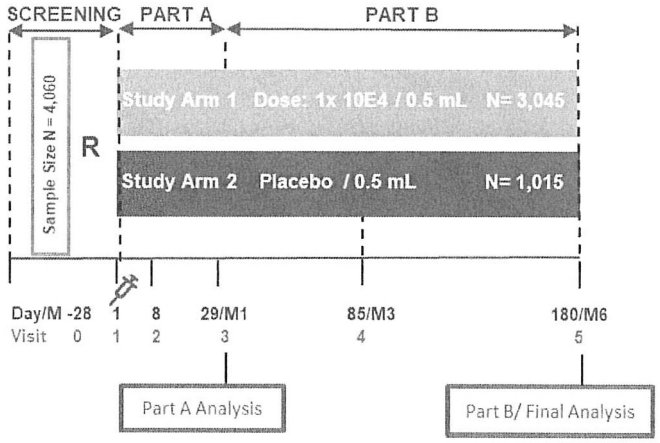


Figure 1. Pivotal Clinical Study Design.

STUDY ENDPOINTS

Primary Endpoint

- Proportion of subjects with a seroprotective CHIKV antibody level defined as [redacted] for baseline negative subjects 28 days post-vaccination.

Secondary Endpoints

Immunogenicity

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

Safety

- Frequency and severity of unsolicited AEs within 28 days post-vaccination;
- Frequency and severity of solicited injection site and systemic reactions within ten days post-

[i] Seroconversion defined as CHIKV-specific neutralizing antibody titer of ≥1:20 for baseline negative subjects and >4-fold for baseline positive subjects.

vaccination;

- Frequency and severity of any adverse event (AE) during the entire study period;
- Frequency and severity of any serious adverse event (SAE) during the entire study period;
- Frequency and severity of any adverse event of special interest (AESI) within 2 to 21 days post-vaccination.

CRITERIA FOR INCLUSION / EXCLUSION

Approximately 4,060 adults of either gender, who satisfy the inclusion and exclusion criteria listed below will be invited to participate in the study.

Inclusion Criteria

Subjects who meet **ALL** of the following criteria are eligible for this study:

1. Subject is 18 years of age or above[ii] on the Day of screening (Visit 0);
2. Subject has an understanding of the study and its procedures, agrees to its provisions, and voluntarily gives written informed consent prior to any study-related procedures;
3. Subject is **generally healthy**[iii] as determined by the Investigator's clinical judgement based on medical history, physical examination and screening laboratory tests;
4. If subject is of childbearing potential:
    a) Subject has practiced an adequate method of contraception (see below) during the 30 days before screening (Visit 0);
    b) Subject has a negative serum or urine pregnancy test at screening (Visit 0) or Visit 1, respectively;
    c) Subject agrees to employ adequate birth control measures for the first three months post-vaccination (i.e. until Day 85, Visit 4). This includes one of the following measures:
        - Hormonal contraceptives (e.g. implants, birth control pills, patches);
        - Intrauterine hormone-release systems and intrauterine device;
        - Barrier type of birth control measure (e.g. diaphragms, cervical caps);
        - Vasectomy in the male sex partner ≥ 3 months prior to first vaccination;
        - Sexual abstinence;
        - Same sex relationships.

Exclusion Criteria

Subjects who meet **ANY** of the following criteria are **NOT** eligible for this study:

1. Subject has had a CHIKV infection in the past, including suspected CHIKV infection; is taking medication or other treatment for unresolved symptoms attributed to a previous CHIKV infection; or has participated in a clinical study involving an investigational CHIKV vaccine;

[ii] From the 18th birthday or above.

[iii] Subjects are considered **generally healthy** if (1) any chronic illness/condition, e.g. hypertension, type 2 diabetes mellitus, or hyperlipidemia is stable and well-controlled on therapy for the past 6 months, and (2) they do not have a disease that is identified as an exclusion criterion.

2. Subject has an acute or recent infection (and who is not symptom-free in the week prior to the Screening Visit (Visit 0);
3. Subject tests positive for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV);
4. Subject has received another live virus vaccine within 28 days or inactivated vaccine within 14 days prior to vaccination in this study or plans to receive a vaccine within 28 days or 14 days after vaccination, respectively;
5. Subject has abnormal findings in any required study investigations (including medical history, physical examination, and clinical laboratory) considered clinically relevant by the Investigator which pose a risk for participation in the study based on his/her judgement;
6. Subject has a medical history of or currently has acute or progressive, unstable or uncontrolled clinical conditions (e.g. cardiovascular, respiratory, neurologic, psychiatric, or rheumatologic conditions) that poses a risk for participation in the study, based on Investigators clinical judgement. Examples include individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment, or presence of high risk comorbidities (e.g. significant cardiopulmonary disease);
7. Subject has a history of immune-mediated or clinically relevant arthritis/arthralgia;
8. Subject has a history of malignancy in the past 5 years other than squamous cell or basal cell skin cancer. If there has been surgical excision or treatment more than 5 years ago that is considered to have achieved a cure, the subject may be enrolled. A history of hematologic malignancy is a permanent exclusion. Subjects with a history of skin cancer must not be vaccinated at the previous tumor site;
9. Subject has a known or suspected defect of the immune system, such as subjects with congenital or acquired immunodeficiency, including infection with 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 ≥ 0.05 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.
10. Subject has a history of any vaccine related contraindicating event (e.g., anaphylaxis, allergy to components of the candidate vaccine, other known contraindications);
11. Subject presents with clinical conditions representing a contraindication to intramuscular vaccination and blood draws;
12. Subject is pregnant (positive serum or urine pregnancy test at screening or Visit 1, respectively), has plans to become pregnant during the first three months post-vaccination or lactating at the time of enrollment;
13. Subject has donated blood, blood fractions or plasma within 30 days or received blood-derived products (e.g. plasma) within 90 days prior to vaccination in this study or plans to donate blood or use blood products until Day 180 of the study;
14. Subject has a rash, dermatological condition or tattoos that would, in the opinion of the

Investigator, interfere with injection site reaction rating;

15. Subject has a known or suspected problem with alcohol or drug abuse as determined by the Investigator;
16. Subject has any condition that, in the opinion of the Investigator, may compromise the subjects well-being, might interfere with evaluation of study endpoints, or would limit the subject's ability to complete the study;
17. Subject is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
18. Subject has participated in another clinical study involving an investigational medicinal product (IMP) or device within 30 days prior to study enrollment or is scheduled to participate in another clinical study involving an IMP, or device during the course of this study;
19. Subject is a member of the team conducting the study or in a dependent relationship with one of the study team members. Dependent relationships include close relatives (i.e., children, partner/spouse, siblings, parents) as well as employees of the Investigator or site personnel conducting the study.

Delay Criteria

Vaccination will be delayed if:

1. Subject has an acute febrile infection within 72 hours prior to vaccination or oral temperature greater than or equal 38.0 °C/ 100.4 °F on the day of vaccination (subject may be rescheduled within the screening visit window until subject has completed 72 hours of no fever);
2. Subject has received antipyretics within 4 hours prior to the scheduled time of vaccination (subject may be rescheduled within the screening visit window).
3. Subject has received any live or inactivated vaccine within 28 days or 14 days prior to vaccination, respectively.

In addition, for a rescheduled vaccination all inclusion and none of the exclusion criteria must be met; in case not all of these criteria are met, the subject will be excluded from the study.

The rescheduled visit should be within the specified time window for the vaccination visit. In case the time window for the rescheduled visit cannot be met, the subject might be invited for a re-screening.

STATISTICAL ANALYSIS

Sample Size Justification

The sample size for this study is selected in order to provide a comprehensive safety profile with regards to rare AEs and SAEs. A number of approximately 3,000 VLA1553-vaccinated subjects will allow for the detection of at least one vaccine-attributable rare event (incidence rate 1/1000) with a probability of 94% in this study.

The immunogenicity subset of 375 VLA1553-vaccinated subjects will allow for sufficient statistical

power when applying a one-sided exact binomial test with a significance level of 2.5% against a non-acceptance threshold of 70% on the proportion of subjects with a seroprotective level (defined as [redacted] for baseline negative subjects) at Day 29. A seroprotection rate (SPR) of 80% is assumed, and 200 VLA1553-vaccinated subjects would thus be necessary for a statistical power of 90%. With an expected drop-out/major protocol deviations rate of approximately 10%, 225 subjects vaccinated with VLA1553 need to be allocated to the immunogenicity subset. In order to account for placebo subjects, to achieve a meaningful number of subjects in both age strata, and to enroll sufficient numbers of subjects for a long-term follow-up in a potential subsequent trial, a total of 500 subjects will be enrolled into the immunogenicity sub-set.

Statistical Methods

A statistical analysis plan will be prepared before database closure/snapshot.

All analyses of immunogenicity data will be performed primarily on the PP population and secondarily on the IMM population.

The primary immunogenicity analysis will be a comparison of the observed proportion of subjects with a seroprotective CHIKV antibody level (defined as [redacted] for baseline negative subjects) at Day 29 (i.e. 28 days post-vaccination) against a non-acceptance threshold of 70%. An exact binomial test for the null-hypothesis H0: SPR ≤70% against the alternative H1: SPR >70% with a one-sided significance level of 2.5% will be applied and exact (Clopper-Pearson) two-sided 95% confidence limits will be calculated.

Secondary immunogenicity analysis will include the comparison of the GMTs between the VLA1553 and placebo groups at Day 29 (i.e. 28 days post-vaccination) by ANOVA (study group, covariate study site and age stratum as factors), two-sided 95% confidence intervals will be calculated for the GMT.

In addition, the seroprotection and seroconversion rates for various study days, and when applying other threshold titers for defining seroconversion or seroprotection thresholds will be compared between the study arms by Fisher's exact test and 95% confidence intervals will be calculated. Immunogenicity analyses will also be generated stratified by age stratum.

Even if recruitment of elderly subjects (Stratum B) in the IMM population does not achieve the envisaged total of 154 subjects, the analysis of the IMM population will proceed as defined above. In addition, missing subjects of Stratum B in the immunogenicity subset will be filled up with randomly selected subjects of Stratum B from the safety analysis population and an additional analysis will be performed for immunogenicity evaluations in the newly defined IMM elderly population.

All subjects entered into the study, who receive the single vaccination, will be included in the safety analysis.

Safety tabulations will generally be provided separately for solicited AEs and unsolicited AEs, and for both types of AEs combined. 95% confidence intervals according to Altman will generally be provided for all AE rates and differences between the study arms will be assessed for significance using Fisher's exact test and will be assessed regarding the clinical relevance by the DSMB during the ongoing study and discussed in the CSR.

The number and percentage of subjects with any AE, any solicited AE, unsolicited AE, any related unsolicited AE, any related severe AE, any SAEs, any related SAEs, any AESI, any related AESI, any medically attended AE, any AE leading to withdrawal from study, and any AE occurring at a frequency of at least 10% and at least 1% in at least one study arm, up to Day 29, and up to Month 6,

will be presented for each study arm, overall and by system organ class/preferred term.

Data Analysis

The following data analyses will be performed:

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

Individual study parts will be analyzed sequentially.

Final Report

The Part A analysis will be performed once the last subject has completed the study Visit 3, i.e. Day 29. Part B analysis will be performed once the last subject has completed the study Visit 5, i.e. Month 6. Part A will be unblinded (blind will be maintained for study sites) and Part B final report will be submitted for BLA filing.

8. TABLE OF CONTENTS

9. LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
AESI	Adverse Event of Special Interest
ANOVA	Analysis of variance
ALT	Alanine aminotransferase
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
BMI	Body Mass Index
CDC	Centers of Disease Control and Prevention
CHIKV	chikungunya virus
CI	Confidence interval
CRA	Clinical research associate
CRO	Contract Research Organization
CRP	C-reactive protein
CSR	Clinical Study Report
DSMB	Data and Safety Monitoring Board
eCRF	electronic Case Report Form
e.g.	for example
elMM	Immunogenicity population elderly
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
GMFI	Geometric mean fold increase
GMT	Geometric mean titer
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
Hct	Hematocrit
HIV	Human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
i.e.	that is
i.m.	Intra - muscular

Abbreviation	Definition
IMM	Immunogenicity population
IMP	Investigational medicinal product
IRB	Intitutional Review Board
IXRS	Interactive Voice/Web Response System
kDa	kilo Dalton
MedDRA	Medical Dictionary for Regulatory Activities
µL	microliter
mL	Milliliter(s)
n.a.	not applicable
NT	Neutralization Test
NT50	Antibody titer at 50% virus neutralization
PCR	Polymerase chain reaction
pH	Potential of hydrogen
PP	Per-protocol population
µPRNT	Micro Plaque Reduction Neutralization Test
RBC	Red blood cells
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
s.c.	subcutaneous
SPR	Seroprotection Rate
WBC	White blood cell

10. INTRODUCTION

10.1 Valneva's Candidate Vaccine

Valneva has developed a live-attenuated chikungunya virus (CHIKV) vaccine candidate VLA1553 for the active prevention of chikungunya infection for use in endemic regions and for travelers to epidemic areas or high-risk areas. VLA1553 is based on the chikungunya La Reunion strain (LR-CHIKV clone LR2006-OPY1) of East/ Central/ South African (ECSA) genotype and is characterized by a genetically-engineered 60 amino acid deletion in its nsP3 viral replicase complex gene which leads to an attenuation of the virus *in vivo.* VLA1553 is genetically stable and passaged three times on African green monkey kidney (VERO) cells and contains no adjuvant. VLA1553 is intended to facilitate a rapid development of a protective antibody titer.

VLA1553 investigation was designated as a fast track development program by the United States (US) Food and Drug Administration (FDA) in 2019.

For further details please refer to the Investigator's Brochure.

10.2 Clinical Condition/Indication

10.2.1 Transmission, Disease and Diagnosis

CHIKV is a mosquito-borne virus which was first isolated in 1953, during an epidemic of polyarthralgia in Tanzania (Robinson 1955). The word 'chikungunya' means 'that which bends up' in Swahili, in reference to the stooped posture of patients afflicted with the severe joint pain associated with this disease. Since its first isolation CHIKV was found to occasionally cause smaller outbreaks during the following decades in Africa, India, the Indian Ocean Islands, and Southeast Asia (Suhrbier, Jaffar-Bandjee et al. 2012; Simon, Javelle et al. 2015).

Within the human population, CHIKV is maintained by a human-mosquito-human transmission probably following a dengue-like model characterized by the absence of an animal reservoir and the ability to spread rapidly among human beings via domestic and peridomestic mosquitoes (Pialoux, Gauzere et al. 2007; Her, Kam et al. 2009). CHIKV was typically transmitted only by *Aedes aegypti* mosquitos, however coinciding with an adaptation enabling unusually efficient transmission by *Aedes albopictus* mosquitos, the virus re-merged in 2004 and rapidly spread over Africa, Asia and locally also in Europe (Delisle, Rousseau et al. 2015). More recently CHIKV also has spread across the Americas with millions of people becoming infected (Rezza, Weaver et al. 2019).

CHIKV is an enveloped alphavirus of the family *Togaviridae*. It carries a single-stranded positive sense genomic RNA of around 12 kb containing two open reading frames that

encode four nonstructural replicase proteins (nsP1-4) and a structural polyprotein consisting of the capsid protein (C) and the envelope proteins (E3-E2-6K/TF-E1), respectively. The replicase complex serves two functions: it replicates the genomic RNA for incorporation into new virus particles and it also has transcriptase activity to produce a mRNA from a subgenomic promoter that encodes the structural proteins (Simizu, Yamamoto et al. 1984). Mature virions that bud from the plasma membrane of infected cells carry 240 copies of E2-E1 glycoproteins arranged into 80 heterotrimeric spike complexes. The E2 protein is the receptor-binding moiety, whereas the E1 protein is involved in fusion of the virion envelope with the target cell endosomal membrane (Voss, Vaney et al. 2010). Accordingly, it is the spike complex and in particular the E2 envelope protein that is the target for neutralizing antibodies.

An infection with CHIKV results in chronic and incapacitating arthralgia affecting all gender and age groups (Couderc, Khandoudi et al. 2009; Suhrbier, Jaffar-Bandjee et al. 2012; Simon, Javelle et al. 2015). CHIKV clinical symptoms are presented in three stages that differ in clinical features and treatment. During the acute stage, clinical symptoms manifest with common features such as fever, transient rash and multiple arthralgia/arthritis episodes. This is followed by a multi-morbid post-acute and chronic stage characterized by persisting rheumatic symptoms up to months and years after infection and impaired quality-of-life (Marimoutou, Ferraro et al. 2015; Simon, Javelle et al. 2015). Symptoms of CHIKV disease usually appear 4–7 days post-infection and include rapid onset of fever, viremia, severe joint pain, recurring mild joint pain, maculopapular rash, and may lead to death (Simon, Javelle et al. 2015).

Currently, neither specific antiviral treatment nor a vaccine is available to prevent CHIKV infection. Prevention against CHIKV infection is therefore limited to non-treatment interventions such as the employment of insecticides, wearing long sleeves and pants and repellents, and other means to restrict exposure to vector mosquitos. The treatment of CHIKV disease is mainly supportive such as bed rest, adequate fluids, and symptomatic relief by using analgesics, antipyretics like paracetamol, or anti-inflammatory drugs like ibuprofen and naproxen for the control of fever and joint pain. Persons who have persistent joint pain may require analgesic or long-term anti-inflammatory therapy (Simon, Javelle et al. 2015).

For detailed information on the disease and epidemiology please refer to the Investigator's Brochure.

10.2.2 Prospects for Vaccine Development

A number of CHIKV vaccine candidates is currently under preclinical and clinical development, ranging from inactivated whole virus vaccine compositions, over virus-like-particle approaches to RNA and DNA vaccine candidates (Schrauf et al. 2020).

A former CHIKV vaccine candidate was a serially passaged, live-attenuated CHIKV vaccine (TSI-GSD-218 or 181/clone 25) developed by the Walter Reed Army Institute of Research (WRAIR, US). The TSI-GSD-218 CHIKV isolate was derived from a patient during the Thailand outbreak in 1962 and was subsequently serially passaged on human lung cell cultures. The live-attenuated vaccine candidate was investigated in numerous Phase 1 and Phase 2 trials with a dose of $3x10^4$ PFUs/mL. The WRAIR vaccine was administered i.m. as well as subcutaneous (s.c.) as a single vaccination offering long-term protection (McClain, Pittman et al. 1998 and Edelman, Tacket et al. 2000). However, clinical development efforts were terminated in 1998 (Hoke, Pace-Templeton et al. 2012).

In addition, a measles-virus-based CHIKV vaccine developed by Themis, AT, has completed Phase 1 and 2 studies. Published results of a Phase 2 clinical trial investigating two immunizations of escalating dose levels at four sites in Austria and Germany showed that the vaccine was found to be safe and well-tolerated with a good immunogenicity profile (Ramsauer, Schwameis et al. 2015, Reisinger et al. 2018).

Furthermore, the National Institutes of Allergy and Infectious Disease (NIAID), later acquired by PaxVax, now Emergent BioSolutions, developed a Virus-like particle (VLP) vaccine for prophylaxis of CHIKV. Fist-in-human and Phase 2 studies demonstrated in non-endemic and endemic regions of the Caribbean that the VLP vaccine candidate is safe, well-tolerated and immunogenic after two vaccinations supporting Phase 3 entry (Chen et al. 2020; Chang et al. 2014).

With the increase of international travel in the past years and the spread of potential vectors, infections caused by CHIKV are likely to expand on a global scale and may result in overlapping regions of endemicity (Hochedez, Jaureguiberry et al. 2006). Consequently, morbidity due to this virus is a serious threat to global health, and CHIKV has been listed as a priority pathogen by the National Institutes of Allergy and Infectious Disease (NIAID) in the United States (Suhrbier, Jaffar-Bandjee et al. 2012; Simon, Javelle et al. 2015; NIAID October 26, 2016) and raises an urgent demand for efficient prophylaxis, providing a strong justification for the development of a vaccine.

10.3 Findings From Nonclinical and Clinical Studies

10.3.1 Non-clinical Summary

The safety, immunogenicity and protective potency of the CHIKV vaccine candidate VLA1553 have been assessed in numerous non-clinical studies.

The non-clinical development program has focused on the establishment of a small animal as well as non-human primate (NHP) model allowing for the evaluation of the CHIKV candidate vaccine with respect to safety and efficacy, i.e. immunogenicity and protection (Hallengärd et al. 2014). To this end, mouse models have been investigated and were shown to be permissive for infection with a wild-type (wt) CHIKV isolate, LR2006 OPY-1. Thus, infection of mice with wt CHIKV was shown to cause significant viremia, a major sign also in humans (Couderc and Lecuit 2009). In addition, NHPs serve as excellent animal models for understanding CHIKV pathogenesis as they are a natural amplification host for CHIKV and share significant genetic and physiological homology with humans. CHIKV infection in NHPs results in acute fever, rash, viremia and the production of CHIKV-specific neutralizing antibodies, type I interferon and pro-inflammatory cytokines. CHIKV establishes a persistent infection in NHPs, particularly in cynomolgus macaques (Broeckel, Haese et al. 2015).

Valneva's preclinical data package generated with the CHIKV vaccine candidate demonstrates that a single shot of the CHIKV del5nsP3 vaccine/VLA1553:

- is highly immunogenic and induces a strong and long lasting neutralizing antibody response in a mouse and NHP model;
- protects NHPs from a high-dose wt CHIKV challenge;
- causes no clinical manifestations typically associated with wt CHIKV infections in the NHP model;
- shows a delayed and strongly reduced viremia as compared to wt CHIKV infection in a mouse and NHP model;
- shows strongly reduced cytokine production compared to wt CHIKV infection and
- shows a more sporadic, transient and lower dissemination in tissues of VLA1553 immunized NHPs compared to wt CHIKV infected NHPs;
- confirms the stability of the virus del5nsP3 attenuation in humans post-vaccination;
- is able to protect NHPs from CHIKV infection based on passive transfer of human immune sera to NHPs followed by wt CHIKV challenge and
- shows a negligible risk of VLA1553 virus transmission from vaccinated to non-vaccinated humans by mosquitoes;
- demonstrate that all VLA1553-101 post-vaccination samples obtained at Day 14 or later show substantial neutralizing activity against the wt La Reunion CHIKV as well as against the heterologous strain of the West African lineage.

An overview of further non-clinical studies can be found in **Table 2** below.

Table 2. Non-clinical Studies with VLA1553

Study	Species	Summary
Repeat-Dose Toxicity	Rabbits *(New Zealand Whites)*	Upon two high dose vaccinations at a two week interval, all findings were transient and resolved within the 30 days recovery period; No adverse findings.
Persistence of infection and biodistribution	NHPs *(Cynomolgus macaques)*	› VLA1553 replication in blood was 3 logs lower than replication of wt CHIKV; › shedding of VLA1553 in saliva and vaginal fluids was much lower than wt CHIKV; › in cerebrospinal fluid, synovial fluid and urine, no shedding of VLA1553 nor wt CHIKV was detected; › VLA1553 dissemination in tissues, when detected, was more sporadic, transient and lower than observed for wild-type CHIKV; › VLA1553 was not detected in joints and muscles.
Mosquito Transmission Study	Mosquitos *(Aedes albopictus)*	Probability of mosquitoes transmitting VLA1553 virus from a human vaccinated with the vaccine appears to be minisculy low [redacted]
Passive transfer in NHPs using human serum from VLA1553-101	NHPs *(Cynomolgus macaques)*	After vaccination with VLA1553 a [redacted] is proposed as a titer reasonably likely to predict protection.

For further details please refer to the Investigator's Brochure.

10.3.2 Clinical Summary

The first clinical study with Valneva's live-attenuated vaccine candidate was initiated with first-subject-in on 05 March 2018 in the U.S. and was designed as randomized, observer-blinded, multicenter Phase 1 dose-escalation study (VLA1553-101) assessing both safety and immunogenicity. The study has been conducted in healthy male and female (of non-childbearing potential) volunteers aged 18 to 45 years, who are unlikely to have had a natural infection with CHIKV (naïve population). Three escalating dose levels of VLA1553 of $3.2x10^3$, $3.2x10^4$ or $3.2x10^5$ $TCID_{50}$/dose in a liquid formulation (30 subjects in the low and medium and 60 subjects in the high dose group) were investigated after a single immunization in a total of 120 subjects. Dosing was adjusted by injection volume. All subjects returned to the study site at Day 28 and Day 84, Month 6 and at Month 12 for safety and immunogenicity evaluations. Subjects in dose groups L and M were re-vaccinated ("intrinsic human virus challenge") with the highest dose at Month 12 and followed up for safety and immunogenicity until 28 days after re-vaccination, i.e. until Month 13. Subjects in dose group H were re-randomized 1:1 at Month 6 to receive either a re-

vaccination with the highest dose at this Visit 4 or at Month 12. Subjects were followed up for safety and immunogenicity until 28 days after their respective re-vaccination, i.e. until Month 7 and further continued until Month 12 or Month 13, respectively. Additionally, throughout the study, subjects returned to the study site for safety, including the assessment of viremia, shedding and CHIKV-associated events, as well as immunogenicity evaluations on Days 3, 7 and 14 post-vaccinations. Should a subject present with a positive viremia or shedding sample on Day 14 after vaccination, weekly tests are performed until viremia/shedding were resolved.

Taken together, VLA1553 was well-tolerated in the low and medium dose groups and generally safe in all dose groups after a single vaccination. The low and medium doses of VLA1553 have a superior safety profile (including viremia) compared to the high dose group. No AESIs and no SAEs related to vaccination were reported up to Month 12. The local tolerability profile at all dose levels was considered excellent. Notable systemic AEs included short-term fever, headache and muscle pain. Adverse event rates were significantly lower in the low and medium dose groups compared to the high dose group. Following a re-vaccination („intrinsic human virus challenge") with the highest dose at Month 6 (Group H2) or Month 12 (Group L, M and H1) vaccinees were protected from vaccine-induced viremia as indicated by the absence of positive viremia results in plasma, urinary shedding as well as associated adverse events. On Day 14, the GMTs in the Low, Medium and High dose groups were [redacted] respectively; with statistically significant differences between the Low and Medium and Low and High dose groups.

VLA1553 shows an excellent immunogenicity profile in all dose groups after a single vaccination. 100% Seroconversion rates (conservatively defined as the proportion of subjects achieving a CHIKV-specific neutralizing antibody titer of [redacted] was already achieved at Day 14 after the single vaccination in all dose groups and was sustained at 100% until Month 12. 96.3% or more subjects in all dose groups achieved at least a 16-fold increase in antibody titers at Day 28. Mean peak antibody titers at Day 28 ranged from [redacted] from groups L to H, respectively, with maximum titers at Day 28 reaching [redacted] Neutralizing antibodies persisted up to a year (GMTs ranging from [redacted] in Group H2 to [redacted] in Group M). The absence of an anamnestic response following any re-vaccination demonstrates that a single vaccination of VLA1553 is sufficient to induce sustaining high titer neutralizing antibodies at all dose levels one year after priming characterized by the development of sterilizing immunity (≤ 4-fold increase in 94.4 to 100.0% of subjects) in neutralizing antibody titers as compared to titers prior re-vaccination.

For further information on Study VLA1553-101, please refer to the Investigator's Brochure.

In summary, the medium dose was considered equally safe as the low dose but presented with an improved immunogenicity profile in terms of neutralizing antibody kinetics. Based on these results Valneva selected the medium dose of VLA1553 for further clinical development of the vaccine. Based on this solid and comprehensive clinical data package involving data on approximately 120 subjects, wherein 30 have received the final product as a single vaccination, the inclusion of an "intrinsic human virus challenge" with the potential to generate early data on efficacy and the evidence for a single-dose vaccination schedule supports the advancement of this chikungunya virus vaccine candidate (VLA1553) to this pivotal Phase 3 study responding to the urgent medical need for a prophylactic CHIKV vaccine.

10.4 Study Rationale and Justification for Dosage and Study Design

Building on the initial data from the Phase 1 study (study VLA1553-101) and dose selection as discussed in Section 10.3.2, this pivotal Phase 3 study is designed to expand the safety and immunogenicity database of the final dose of VLA1553 administered as a single vaccination. In addition, the rate of subjects with neutralizing antibody titers above a predefined seroprotective threshold will be evaluated. The proposed seroprotective threshold usable as surrogate of protection reasonably likely to predict clinical benefit has been established in non-human primate passive transfer studies using human sera from VLA1553-101.

10.4.1 Selection of Study Population

A total of approximately 4,060 subjects of either gender aged 18 years and above, who meet all inclusion criteria and none of the exclusion criteria and provide a written informed consent, will be invited to participate in the study. Subjects who have a recent CHIKV infection, including a suspected CHIKV infection or subjects who are taking medication or other treatment for unresolved symptoms attributed to a previous CHIKV infection will be excluded. Subjects will not be screened for CHIKV seropositivity at study entry, however a blood sample will be obtained for retrospective investigation of pre-existing antibodies including but not limited to e.g. a panel of alphaviruses (i.e. CHIKV, Mayaro) or dengue and Zika.

10.4.2 Selection of Seroprotective Threshold

Human immune sera derived from individuals of the Phase 1 clinical study (VLA1553-101) with different titers were passively transferred to NHPs to assess protection from viral replication after challenge with a high dose of wt CHIKV LR2006- OPY1. The challenge dose used for this study ([redacted] PFU) corresponds to a dose that is higher than the dose people may encounter when bitten by a mosquito (Dubrulle et al., 2009).

The study revealed, that the animals were fully protected against viremia by using the highest dose of human immune sera (Day 28, [redacted]. In addition, a highly significant decrease of viral RNA based on RT-qPCR was noted, of 3 to 5 logs, compared to the control animals. The duration of viremia was also strongly reduced from more than 10 days in control animals to 2-3 days in animals treated with human VLA1553-101 serum, except for 2 animals treated with the ultra-low serum pool (5 days).

Most importantly, the NHP passive transfer study showed that all human VLA1553-101 serum treated animals with $\mu PRNT_{50}$ titers between [redacted] prior to challenge were fully protected against CHIKV infection-associated fever or modification of blood parameters. The threshold for protection (lowest observed Day 0 titer at which at least 90% of animals are protected from viremia) was [redacted]

When comparing the protection against CHIKV infection provided by the human sera of Day 180 post-immunization with the data of the Day 28 (or Day 14 and 84) sera, our analysis showed there is no significant difference in terms of viremia or protection at the same level of $\mu PRNT_{50}$ titer prior to challenge.

Based on a FDA request to include a conservative safety margin, a neutralizing antibody titer of [redacted] determined by $\mu PRNT_{50}$ is proposed as a titer reasonably likely to predict protection in humans and is therefore an agreed surrogate endpoint to support accelerated approval.

10.5 Evaluation of Anticipated Risks and Benefits of the Investigational Product(s) to Human Subjects

10.5.1 Possible Benefits for Subject

The benefit of participation for the subjects in this study is the possible formation of antibodies against CHIKV after vaccination with the VLA1553 candidate vaccine (among those not in the placebo group). During their participation in this study, the subjects' antibody development will be monitored. Following vaccination with Valneva's VLA1553 vaccine, subjects may acquire immunity to CHIKV as indicated by antibody levels above a pre-defined threshold indicative of protection.

Based on the results of non-clinical studies and epidemiological data it is anticipated that a substantial number of subjects will be protected against CHIKV infection following participation in the study. However, because protection following vaccination has not yet been proven or subjects may have received placebo, subjects planning to travel to CHIKV endemic areas should be counseled to apply personal protective measures against mosquito bites.

An additional benefit for subjects involved in this study is derived from safety monitoring and follow-up as prophylactic measure for an early diagnosis of illness.

10.5.2 Possible Benefits for Society

CHIKV is currently regarded as one of the most-likely re-emerging viruses to spread globally (Simon, Savini et al. 2008), an issue which raises an urgent demand for efficient prophylaxis. However, at present there is no treatment or vaccine available against this CHIKV-induced debilitating disease and its various symptoms (Weaver, Osorio et al. 2012; Ahola, Couderc et al. 2015; Weaver and Lecuit 2015). Thus, morbidity due to this virus is a serious threat to global health and CHIKV has now been listed as a priority pathogen by the National Institutes of Allergy and Infectious Disease (NIAID) in the United States (Ahola, Couderc et al. 2015; Weaver and Lecuit 2015; NIAID October 26, 2016). Due to the increasing frequency and risk of CHIKV, the development of a safe and effective vaccine is a high priority.

Through their participation in this Phase 3 study, the subjects will contribute to the development of a novel vaccine against chikungunya. This Phase 3 study is the pivotal study within a complete clinical development program, which is intended to lead to subsequent licensure of the VLA1553 vaccine, if it is shown to be safe and efficacious.

10.5.3 Possible Risks / Inconveniences for the Subject

VLA1553 is a live-attenuated vaccine where the attenuation of the virus leads to a reduced replication capability of the vaccine. Thus, as demonstrated in preclinical studies, viremia was delayed and strongly reduced and no clinical manifestations typically associated with wt CHIKV infections occurred in non-human primates. Viremia was observed in some subjects in the Phase 1 study albeit short-lived and greatly reduced from levels observed after natural infection. Only a single subject shed virus in urine.

Overall the vaccine was well-tolerated in a healthy adult population aged 18 to 45 years following a single vaccination at the low and medium dose level.

Solicited systemic reaction rates in the medium dose group were reported in 40.0% of subjects after the single vaccination. The most frequently reported symptoms of related systemic reactions (occurring at a frequency of ≥1:10) were headache, fever, fatigue and myalgia reported in 23.3%, 20.0%, 16.7% and 13.3% of subjects in the medium dose group, respectively. The majority of reactions were transient and of mild or moderate severity. For 13.3% of subjects, neutropenia, leukopenia and/or lymphocytopenia were reported as clinically relevant laboratory parameter deviations, without associated clinical signs or symptoms.

Few solicited injection site reactions (6.7%) were reported by two subjects in the medium dose group after the single vaccination. All solicited injection site reactions were of mild severity.

Allergic reactions to components of the vaccine or - in the worst case – an anaphylactic shock cannot be excluded, although such reactions have been reported in very rare cases with other vaccinations.

The possibility that vaccination may activate an autoimmune disease (e.g., multiple sclerosis) in predisposed subjects cannot be excluded.

In addition, as with any IMP, there may be unforeseeable risks associated with the use of the VLA1553 vaccine.

The blood draws performed during the study carry the possible risks of pain, hematoma, and in very rare cases an infection at the venipuncture site. The process of vaccination may also trigger syncope.

For further details on known and potential risks of this investigational product, please refer to the Investigator's Brochure.

11. STUDY PURPOSE AND OBJECTIVES

11.1 Study Purpose

To verify the safety and immunogenicity of the final dose of the live-attenuated CHIKV vaccine candidate (VLA1553) in a population aged 18 years and above after a single immunization.

To evaluate the proportion of subjects with neutralizing antibody titers above a threshold indicative of protection as derived from animal passive transfer experiments.

The outcome shall provide the basis for licensure of the vaccine.

11.2 Primary Objective

The primary objective is to evaluate the immunogenicity and safety of the final dose of the live-attenuated CHIKV vaccine candidate (VLA1553) 28 days following vaccination in a population aged 18 years and above after a single immunization.

11.3 Secondary Objective

The secondary objectives are to assess the immunogenicity and safety of the final dose of VLA1553 up to 180 days following vaccination in a population aged 18 years and above after a single immunization.

12. STUDY DESIGN

12.1 Overall Study Design

This is a prospective, randomized, double-blinded, multicenter, pivotal clinical study evaluating the final dose of VLA1553 (1 x10E4 $TCID_{50}$ per 0.5 mL) in comparison to a placebo control. The final dose of VLA1553 or control will be administered as single immunization on Day 1. Overall, approximately 4,060 male and female subjects aged 18 years and above will be enrolled (i.e. ICF signed) in this study.

Subjects will be allocated in a 3:1 ratio to VLA1553 (n= approximately 3,045) or control group (n= approximately 1,015). The approximately 4,060 subjects in this study will be 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 346 subjects in Stratum A and 154 subjects in Stratum B will be included in the immunogenicity evaluation and comprise the immunogenicity subset of in total approximately 500 subjects. The immunogenicity subset will be randomly enrolled at approximately 15 pre-selected study sites across the U.S. representative of the whole study population.

Due to the difficulty to enroll elderly subjects of Stratum B (≥65 yrs.), enrollment was opened to all subjects in December 2020 and Stratum B was filled up with either subjects of Stratum A (18 – 64 yrs.) or Stratum B (≥65 yrs.) changing the composition of the immunogenicity subset, but not its total number. Due to study blinding, the exact figures for study arms 1 and 2 are not available for the time being.

Table 12.1-1. below illustrates the subject distribution scheme.

Table 12.1-1. Subject Distribution

Study Arm	Study Arm	Stratum	Number of subjects (n)	Immunogenicity evaluation (n)	Dose ($TCID_{50}$/dose)	Injection Volume (mL)
1	VLA1553		3,045		1 x10E4	0.5
		A (18 – 64 yrs.)	2,740	292*		
		B (≥ 65 yrs.)	305	83*		
2	Control		1,015		n.a.	0.5
		A (18 – 64 yrs.)	913	98*		
		B (≥ 65 yrs.)	102	28*		
		Total N:	**4,060**	**501***		

Note: * with actually 111 subjects enrolled in Stratum B, we expect approximately 83 subjects receiving VLA1553 and 28 subjects receiving placebo, similarly with 390 Stratum A subjects enrolled into the immunogenicity subset we expect approximately 292 on VLA1533 and 98 on placebo (to be confirmed after unblinding of the study).

All subjects will be asked to return to the study site at Day 8 (Visit 2), Day 29 (Visit 3) and Day 85 (Visit 4) and Month 6 (Day 180, Visit 5) for immunogenicity sampling. However, immunogenicity analysis and evaluations were only planned to be done in the immunogenicity subset. Due to the addition of non-immunogenicity subset subjects in the Immunogenicity elderly population, some immunogenicity samples will also be evaluated and analyzed for these subjects. In addition, a clinical sample for safety laboratory evaluations will be obtained at all study visits from the immunogenicity subset only. Safety data collection will capture solicited AEs until Day 11 and unsolicited AEs up to Day 180 (Month 6, Visit 5) from all subjects. AESIs will be captured from 2 to 21 days post-vaccination. Subjects presenting with acute arthralgia within this time period will be followed-up until resolution and monitored for recurrences until the end of the study. SAEs will also be assessed until the end of the study (Month 6, Visit 5).

The overall study design is displayed in the **Figure 12.1-2** below.

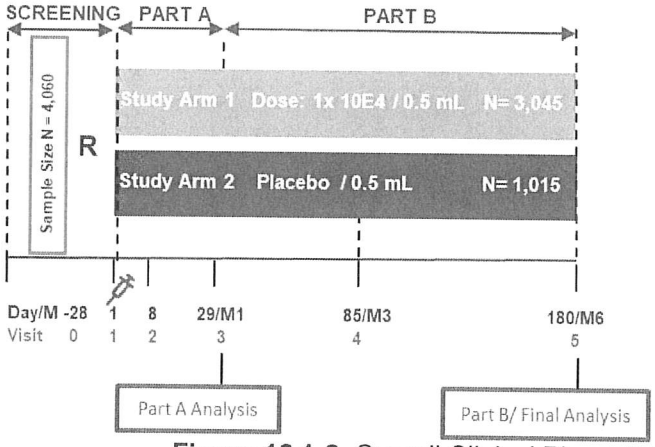


Figure 12.1-2. Overall Clinical Phase 3 Study Design.

12.2 Study Duration

The overall duration of the study is estimated to be approximately 13 months from study initiation (First Subject In) to study completion (Last Subject Out).

The individual subject participation is approximately 7 months from enrollment to study completion unless prematurely discontinued.

12.3 Study Endpoints

12.3.1 Primary Endpoint

- Proportion of subjects with a seroprotective CHIKV antibody level defined as [redacted] for baseline negative subjects 28 days post-vaccination.

12.3.2 Secondary Endpoints

Immunogenicity

The following secondary immunogenicity endpoints will be evaluated:

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

Safety

The following secondary safety endpoints will be determined:

- Frequency and severity of unsolicited AEs within 28 days post-vaccination;
- Frequency and severity of solicited injection site and systemic reactions within ten days post vaccination;
- Frequency, severity and relatedness of any adverse event (AE) during the entire study period;
- Frequency and relatedness of any serious adverse event (SAE) during the entire study period;

- Frequency and relatedness of any adverse event of special interest (AESI) within 2 to 21 days post-vaccination.

12.4 Randomization and Blinding

12.4.1 Randomization

The approximately 4,060 subjects will be randomized by age group into the two study arms at a ratio of 3:1 at Visit 1 as described in Section 12.1.

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

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

Randomization will be performed via the IXRS. At Day 1 (Visit 1, day of vaccination) eligible subjects will be assigned to VLA1553 or Placebo. Each subject will receive a unique randomization number when he/she is assigned to the study arm. Subjects will be allocated to study arms according to the randomization code.

The first 500 subjects allocated to the immunogenicity subset will be randomized to the study arms via the IXRS across approximately 15 pre-selected study sites across the U.S. while ensuring 3:1 randomization per site.

The IMP will be prepared by unblinded study staff in accordance with the information in the IXRS.

An overview of persons who will be (un)blinded is provided below:

Unblinded:

- Designated study staff who are concerned with IMP handling (i.e. perform preparation of the study vaccine or placebo, maintain the drug accountability logs detailing the dates and quantities of IMP administered to each subject, etc.). The unblinded study staff will not be involved in any other study procedures/assessments;
- CRA's responsible for monitoring of IMP handling and for verifying drug accountability during the study and performing overall drug accountability;
- DSMB voting members;
- Bio-statistician preparing unblinded analysis for the DSMB.

Blinded:

- Investigators and other study staff involved in general study conduct and safety assessments;
- Study participant;
- Bio-statistician (except the one involved in DSMB);
- CRA's responsible for monitoring study data;
- All laboratory personnel at central laboratories for safety and immunogenicity laboratory assessments;
- All other Sponsor and CRO staff including medical monitor and laboratory personnel at the Sponsor's labs for additional testing procedures.

In addition, IMP administration (i.e. vaccination of subjects) can be performed by either unblinded or blinded study staff.

12.4.2 Random selection of subjects to fill up the elMM population

Missing subjects of Stratum B in the immunogenicity subset will be filled up with randomly selected subjects of Stratum B from the safety analysis population. Additional immunogenicity analyses will be performed using the newly defined IMM elderly population. This analysis will be included in the Part A CSR if all elderly patients are enrolled in a timely manner to allow the processing of immunogenicity samples within the timeframe of the Part A unblinding and CSR creation, else it will be performed at a later date and filed with the Part B analysis.

To this aim, the following algorithm will be applied. All non-immunogenicity subjects in Stratum B (aged >65) from the 15 sites initially contributing to the immunogenicity subset will be counted, with the number of such subjects noted as N. If there are more than 43 such subjects then these will be considered to make up the IMM elderly population. 43 of these N subjects will then be selected to be included in the new IMM elderly group by a random sampling method. All N subjects will be sorted by subject number and then allocated a random number using the rand(uniform) procedure in SAS. A seed number of 15532021 will be used. The subjects will then be sorted by the randomly allocated value. The 43 subjects will be selected sequentially from this list, while maintaining the

approximate 3:1 ratio of treatment arms. Hence the first 11 placebo, and the first 32 active subjects will be added to the eIMM population.

If N is less than 43, then all N subjects will be included in the IMM elderly population. In addition, the additional [43-N] subjects will be selected from the other sites via random sampling using the method above while maintaining as close to the 3:1 ratio as possible for treatment arms.

12.4.3 Blinding Process

In order to ensure the blinding of study participants and site staff performing the safety assessments with respect to the vaccine dose, preparation of IMP must be done by unblinded staff members in a separate room applying the 4-eyes principle, unobserved by blinded staff members and the subject.

The syringes for VLA1553 as well as placebo are provided to the sites already in a masked manner. Content in the syringe is masked by a yellow tint, transparent adhesive label wrapped around the syringe. The unblinded study staff will not discuss randomizations with study clinicians. Identification of the syringe is guaranteed by placing a tear-off label containing Kit number, Subject number, date of injection and operator onto the label.

For further details please refer to the IMP Manual.

12.4.4 Unblinding

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

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

12.5 Study Termination - Study Stopping Rules

The study may be paused or prematurely terminated, after consultation with the DSMB, if vaccine-related SAEs or other significant vaccine-related side effects occur. In addition, the Sponsor may stop the entire study for any reason at any time.

If the Sponsor or Investigator decides to terminate the study before it is completed, they will notify each other in writing, stating the reasons for early termination. In terminating the

study, the Sponsor and the Investigator will ensure that adequate consideration is given to the protection of the subjects' interests. The Sponsor or Investigator will notify the relevant regulatory authorities or IRB in writing in accordance with local requirements. Documentation will be submitted for filing in the Investigator File and the Trial Master File.

13. SUBJECT SELECTION, WITHDRAWAL AND DISCONTINUATION

Approximately 4,060 adults of either gender, who satisfy the inclusion and exclusion criteria listed below will be invited to participate in the study.

13.1 Inclusion Criteria

Subjects who meet **ALL** of the following criteria are eligible for this study:

1. Subject is 18[iv] years of age or above on the Day of screening (Visit 0);
2. Subject has an understanding of the study and its procedures, agrees to its provisions, and voluntarily gives written informed consent prior to any study-related procedures;
3. Subject is generally healthy[v] as determined by the Investigator's clinical judgement based on medical history, physical examination and screening laboratory tests;
4. If subject is of childbearing potential:
    a) Subject has practiced an adequate method of contraception (see below) during the 30 days before screening (Visit 0);
    b) Subject has a negative serum or urine pregnancy test at screening (Visit 0) or Visit 1, respectively.
    c) Subject agrees to employ adequate birth control measures for the first three months post-vaccination (i.e. until Day 85, Visit 4). This includes one of the following measures:
        - Hormonal contraceptives (e.g. implants, birth control pills, patches);
        - Intrauterine hormone-release systems or intrauterine device;
        - Barrier type of birth control measure (e.g. diaphragms, cervical caps);
        - Vasectomy in the male sex partner ≥ 3 months prior to first vaccination;
        - Sexual abstinence;
        - Same sex relationships.

13.2 Exclusion Criteria

Subjects who meet **ANY** of the following criteria are **NOT** eligible for this study:

[iv] From the 18th birthday or above.
[v] Subjects are considered **generally healthy** if (1) any chronic illness/condition, e.g. hypertension, type 2 diabetes mellitus, or hyperlipidemia is stable and well-controlled on therapy for the past 6 months, and (2) they do not have a disease that is identified as an exclusion criterion.

1. Subject has had a CHIKV infection in the past, including suspected CHIKV infection; is taking medication or other treatment for unresolved symptoms attributed to a previous CHIKV infection; or has participated in a clinical study involving an investigational CHIKV vaccine;
2. Subject has an acute or recent infection (and who is not symptom-free in the week prior to the Screening Visit (Visit 0);
3. Subject tests positive for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV);
4. Subject has received another live virus vaccine within 28 days or inactivated vaccine within 14 days prior to vaccination in this study or plans to receive a vaccine within 28 days or 14 days after vaccination, respectively;
5. Subject has abnormal findings in any required study investigations (including medical history, physical examination, and clinical laboratory) considered clinically relevant by the Investigator which pose a risk for participation in the study based on his/her judgement;
6. Subject has a medical history of or currently has acute or progressive, unstable or uncontrolled clinical conditions (e.g. cardiovascular, respiratory, neurologic, psychiatric, or rheumatologic conditions) that poses a risk for participation in the study, based on Investigators clinical judgement. Examples include individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment, or presence of high risk comorbidities (e.g. significant cardiopulmonary disease);
7. Subject has a history of immune-mediated or clinically relevant arthritis/arthralgia;
8. Subject has a history of malignancy in the past 5 years other than squamous cell or basal cell skin cancer. If there has been surgical excision or treatment more than 5 years ago that is considered to have achieved a cure, the subject may be enrolled. A history of hematologic malignancy is a permanent exclusion. Subjects with a history of skin cancer must not be vaccinated at the previous tumor site;
9. Subject has a known or suspected defect of the immune system, such as subjects with congenital or acquired immunodeficiency, including infection with 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 ≥ 0.05 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.

10. Subject has a history of any vaccine related contraindicating event (e.g., anaphylaxis, allergy to components of the candidate vaccine, other known contraindications);
11. Subject presents with clinical conditions representing a contraindication to intramuscular vaccination and blood draws;
12. Subject is pregnant (positive serum or urine pregnancy test at screening or Visit 1, respectively), has plans to become pregnant during the first three months post-vaccination or lactating at the time of enrollment;
13. Subject has donated blood, blood fractions or plasma within 30 days or received blood-derived products (e.g. plasma) within 90 days prior to vaccination in this study or plans to donate blood or use blood products until Day 180 of the study;
14. Subject has a rash, dermatological condition or tattoos that would, in the opinion of the Investigator, interfere with injection site reaction rating;
15. Subject has a known or suspected problem with alcohol or drug abuse as determined by the Investigator;
16. Subject has any condition that, in the opinion of the Investigator, may compromise the subjects well-being, might interfere with evaluation of study endpoints, or would limit the subject's ability to complete the study;
17. Subject is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
18. Subject has participated in another clinical study involving an investigational medicinal product (IMP) or device within 30 days prior to study enrollment or is scheduled to participate in another clinical study involving an IMP, or device during the course of this study;
19. Subject is a member of the team conducting the study or in a dependent relationship with one of the study team members. Dependent relationships include close relatives (i.e., children, partner/spouse, siblings, parents) as well as employees of the Investigator or site personnel conducting the study.

13.3 Delay Criteria

Vaccination will be delayed if:

1. Subject has an acute febrile infection within 72 hours prior to the scheduled vaccination or oral temperature greater than or equal 38.0 °C/ 100.4 °F on the day of vaccination. Subject may be rescheduled within the screening visit window provided that the illness has resolved (72 hours without fever);

2. Subject has received antipyretics within 4 hours prior to the scheduled time of vaccination. Subject may be rescheduled within the screening visit window.
3. Subject has received any live or inactivated vaccine within 28 days or 14 days prior to vaccination, respectively.

In addition, for a rescheduled vaccination all inclusion and none of the exclusion criteria must be met; in case not all of these criteria are met, the subject will be excluded from the study.

The rescheduled visit should be within the specified time window for the vaccination visit. In case the time window for the rescheduled visit cannot be met, the subject might be invited for a re-screening.

13.4 Pregnancy Testing and Birth Control

The risk of maternal-to-fetal transmission of chikungunya vaccine virus during pregnancy cannot be excluded. Therefore, only women of childbearing potential presenting with a negative pregnancy test and practicing the use of adequate birth control before conduct and during the first three months of the study are eligible for inclusion into the study.

A woman is considered of childbearing potential if fertile, following menarche and until becoming post-menopausal unless permanently sterile.

Women of childbearing potential must have practiced an adequate contraceptive method during the 30 days before Visit 0 (Screening Visit) and the first three months until Visit 4 (Month 3) and present with a negative **serum** pregnancy test at Visit 0 (Screening Visit), a negative **urine** pregnancy test prior to vaccination. In addition, a **urine** pregnancy test will need to be performed at the Early Termination Visit (if applicable).

Women of childbearing potential are required to practice an acceptable method of birth control for the first three months post-vaccination. An acceptable method of birth control is defined as those, which result in a low failure rate (i.e. less than 1% per year) when used consistently and correctly.

This includes one of the following measures:

- Hormonal contraceptives (e.g. implants, birth control pills, patches);
- Intrauterine hormone-releasing system or intrauterine device;
- Barrier type of birth control measure (e.g. diaphragms, cervical caps);
- Vasectomy in the male sex partner ≥ 3 months prior to first vaccination;
- Sexual abstinence;
- Same-sex relationships.

Women without childbearing potential are not required to perform any birth control measure. A woman is considered of non-childbearing potential, if she is:

- Surgically sterilized for ≥ 3 months prior to Visit 1 (permanent sterilization methods include hysterectomy, bilateral salpingectomy or bilateral oophorectomy, or transcervical sterilization (Essure and Adiana procedures), or tubal ligation;
- Postmenopausal for ≥ 1 year prior to study start as historically confirmed by a gynecologist.

If a subject becomes pregnant during the study, she must immediately inform the Investigator and the subject is asked to attend all remaining visits according to schedule.

13.5 Subject Withdrawal or Discontinuation

Any subject has the right to withdraw from the study at any time for any reason, without the need to justify. The Investigator and Sponsor also have the right to prematurely terminate a subject's further participation in the study, e. g. in the case of non-compliance or if – in the judgment of the Investigator and/or Sponsor – continued participation would pose an unacceptable risk for the subject.

The primary reason for withdrawal / discontinuation of a subject from treatment and/or from the study should be documented in the electronic Case Report Form (eCRF) (e.g. withdrawal of consent, Investigator/Sponsor recommended withdrawal, lost to follow up, death).

The primary reasons for discontinuation will be reported on the Discontinuation eCRF, including:

- Withdrawal of consent (not due to AE);
- Withdrawal due to AE;
- Lost to follow-up (defined as 3 documented unsuccessful attempts to contact the subject[vi]);
- Investigator decision (e.g. non-compliance with protocol);
- Study terminated by Sponsor;
- Death;
- Or other (reason to be specified by the Investigator, e.g. technical problems).

[vi] **Note** that a subject who is lost to follow-up but later returns to the study site for a follow-up can still complete the study provided he/she has received the vaccination according to the protocol.

Regardless of the reason, all data available for the subject up to the time of completion/discontinuation should be recorded on the appropriate CRF.

Data collected on withdrawn subjects will be used in the analysis and included in the clinical study report.

Subjects who do not complete the entire study due to withdrawal or discontinuation for any reason will not be replaced.

14. INVESTIGATIONAL MEDICINAL PRODUCT

14.1 Description of VLA1553

The CHIKV vaccine (VLA1553) is a live-attenuated vaccine comprising a large deletion of 60 amino acids in the nsP3 gene encoding the non-structural replicase complex protein nsP3, which leads to attenuation of the virus *in vivo*.

VLA1553 is present in a freeze dried presentation and must be reconstituted with a solvent consisting of sterile water for injection in a prefilled syringe before use.

One dose (0.5 mL) of the VLA1553 vaccine contains between 1.6 x10E3 and 2.5 x10E4 $TCID_{50}$ per dose. The active ingredient is suspended in a formulation buffer of pH 7.3, before freeze drying.

Table 14.1-1
Composition of the VLA1553 lyophilized Drug Product

Active substance	
Live-attenuated CHIKV	**Target $TCID_{50}$/dose**
	1x 10E4 $TCID_{50}$
Excipients and buffer components	**Freeze Dried Formulation/ dose**
di-Potassium Hydrogen Phosphate	0.313 mg
Potassium di-Hydrogen Phosphate	0.098 mg
Trisodium citrate dihydrate	3.68 mg
Sucrose	25 mg
Magnesium Chloride hexahydrate	0.51 mg
D-Sorbitol	2.5 mg
L-Methionine	0.75 mg
Recombinant Human Albumin	0.01% (equals 0.05 mg)
pH	7.3

14.2 Description of Placebo

VLA1553 Placebo consists of a PBS buffer based on Dulbecco's PBS media formulation without Calcium and Magnesium. The concentration of the PBS is 1x and is produced with raw material classified as free from animal origin. The filling volume is 0.6 mL, ensuring an extractable volume of 0.5 mL. The glass vials are 2R Type I Plus® glass vials closed with 13 mm injection Flurotec® secured by aluminum crimp caps.

Table 14.2-1 Composition of Placebo	
Ingredient	**Concentration per dose (0.5 mL)**
Potassium Chloride (KCl)	100 µg
Potassium Dihydrogen phosphate (KH_2PO_4)	100 µg
Sodium Chloride (NaCl)	4000 µg
Di Sodium Hydrogen Phosphate Heptahydrate (Na_2HPO_4 x$7H_2O$)	1080 µg

14.3 IMP Packaging

14.3.1 VLA1553 Packaging

VLA1553 will be provided in kit containing one single-use 2R vial with the lyophilized powder of VLA1553 vaccine, one solvent consisting of 0.5 mL sterile water for injection in a prefilled syringe and a sterile needle set (i.e. 2x 25G x 1½, 0.50x40 mm) for reconstitution and administration.

14.3.2 Placebo Packaging

Placebo will be provided in a kit containing one single-use 2R Placebo vial and one prefilled syringe with 0.5 mL sterile water for injection and a sterile needle set (i.e. 2x 25G x 1½, 0.50x40 mm) for preparation and administration.

The Placebo preparation will mimic as far as technically possible the preparation of the VLA1553, due to clinical study blinding reasons. Hence for Placebo administration the 1.5 mL pre-filled syringe in the Placebo kit will be used.

The solvent is sterile water for injection and supplied in a 1.5 mL pre-filled syringe, filled with 0.5 mL and closed with a temper-evident closure system (Vetter V-OVS®) to safeguard the product integrity.

14.4 IMP Labeling

The IMP will be labeled according to the valid regulatory requirements for clinical trials. The expiry date of VLA1553 and Placebo differ on the outer kit box and vials, respectively.

14.5 IMP Storage

14.5.1 VLA1553 Storage

VLA1553 has an expected retest date of 24 months from the date of manufacture, considering the specified storage conditions of +2-8°C (35.6 °F to +46.4 °F). A stability program is currently ongoing and if any impact on the stability profile are observed, the study sites will be informed accordingly. The vaccine must not be used after the retest date indicated on the package.

VLA1553 must be stored at +2-8°C (35.6 °F to +46.4 °F) in a refrigerator in a room not accessible to unauthorized persons. Storage at room temperature or higher should be avoided because of potential impairment to immunogenicity and tolerability. Temperature monitoring systems will be used.

14.5.2 Placebo Storage

The Placebo has an expected retest date of 36 months from the date of manufacture, considering the specified storage conditions of +2-8°C (35.6 °F to +46.4 °F). A confirmatory stability program is currently ongoing and if any impact on the stability profile are observed, the study sites will be informed accordingly. The Placebo must not be used after the retest date indicated on the package.

The Placebo must be stored at +2-8°C (35.6 °F to +46.4 °F) in a refrigerator in a room not accessible to unauthorized persons. Storage at room temperature or higher should be avoided. Temperature monitoring systems will be used.

14.5.3 Dispensing and Accountability of IMP

Current drug accountability logs have to be maintained, detailing the dates and quantities of IMP administered to each subject. Records will be maintained that include subject identification code (SIC), dispensation date, and amount dispensed. This documentation will be available to the designated unblinded CRA to verify drug accountability during the study and to perform overall drug accountability.

Used and unused IMP will be accounted for and returned to the CMO/ Sponsor. In addition, used IMP can be destroyed on-site upon Sponsor approval.

A study specific IMP manual with further details on IMP handling will be provided.

15. STUDY PROCEDURES

15.1 Informed Consent and Enrollment

Any healthy volunteer who provides informed consent (i.e., signs and dates the informed consent form) is considered enrolled in the study.

The Investigator will inform the subject about the procedures, risks and benefits of the study. Fully informed, written consent must be obtained from each subject prior to any assessment being performed. It is important that the subject is allowed sufficient time to decide on his / her participation in the study.

15.2 Subject Identification Code

At Visit 0, a 13-character subject identification code will be assigned to each subject. The first four digits are the product identifier (e.g. 1553 for this product) provided by the Sponsor. The fifth digit is the study identifier (i.e. 1553-1 for this Phase 3 study), the sixth and seventh digits are site identification number (i.e. 1553-1-01). The last three digits are assigned in ascending order as the subjects are enrolled (i.e., signing the informed consent form, e.g. 1553-1-01-001).

15.3 Investigational Treatment

15.3.1 Description of Treatment

All subjects will receive a single intramuscular vaccination in the deltoid region of the arm of VLA1553 vaccine according to the vaccination schedule as described in Table 24.2-1 of Study Procedures. Subjects will be assigned in a 3:1 ratio to one of the two Study Arms and will receive either the final dose level of between 1 x 10E4 $TCID_{50}$ per dose or placebo. All subjects will be followed up for approximately 6 months following the single study vaccination (see also Section 12.1).

15.3.2 Vaccine Preparation and Administration

VLA1553 is available as a suspension after reconstitution of targeted 1 x 10E4 $TCID_{50}$ per 0.5 mL dose in a prefilled syringe.

IMP preparation (to be done in a separate room by unblinded study staff (four eyes principle), unobserved by the subject and blinded study staff) and administration (by unblinded or blinded study staff) will be done according to the following procedure:

1. CAUTION: Preservatives, antiseptics, detergents, and other anti-viral substances may inactivate the vaccine. Use only provided sterile syringes that are free of preservatives, antiseptics, detergents, and other anti-viral substances for reconstitution and injection of VLA1553.
2. Before reconstitution, the lyophilized VLA1553 is a white to pale yellow compact crystalline plug. VLA1553 when reconstituted, is a clear pale to slightly yellow liquid solution.
3. The solvent is sterile water for injection and supplied in a 1.5 mL pre-filled syringe, filled with 0.5 mL and closed with a temper evident closure system (Vetter V-OVS®) to safeguard the product integrity.
4. VLA1553 must be reconstituted by adding the entire content of the pre-filled syringe of the solvent into the vial containing the lyophilized powder.
5. Twist the white temper evident closure system on the syringe bottom and remove the part of the closure system. For reconstitution, immediately attach the first provided needle on the luer lock of the syringe by twisting the needle clockwise until it locks.
6. Puncture the stopper of the vial with the needle on the syringe and add the entire amount of the solvent of the syringe into the vial containing the powder cake. Gently agitate the vial to dissolve completely and wait for at least one minute for complete reconstitution of the vaccine. Avoid strong shaking or vortexing. Inspect the liquid solution by visual control for any particulate matter and discoloration prior to administration.
7. Withdraw the entire amount from the vial of the reconstituted vaccine into the same syringe with the same attached needle.
8. To administer the vaccine, the second provided needle must be used. Change the needle and inject the reconstituted vaccine intramuscularly (i.m.) into the deltoid muscle as soon as possible within an allowable time window of maximal 2 hours.
9. The used vials and syringes should be kept within the empty kits for drug accountability purposes. Used needles should be disposed in accordance with local requirements.
10. For further information please refer to the IMP Manual provided in the Investigator File.

Under no circumstances should the VLA1553 vaccine be administered intravascularly, as this could lead to hypersensitivity reactions such as shock.

Anaphylaxis or other possible severe acute, post-vaccination adverse reactions to vaccines, including VLA1553 vaccine, are very rare, but can occur. Therefore, appropriate emergency equipment and medication as well as adequately trained personnel must be on site whenever a vaccination is performed.

A study specific IMP manual with further details on IMP handling will be provided.

15.3.3 Placebo Preparation and Administration

The Placebo preparation will mimic as far as technically possible the preparation of the VLA1553, due to clinical study blinding reasons. Hence for Placebo administration, the 1.5 mL pre-filled syringe in the Placebo kit will be used, after discarding its content.

The pre-filled syringe contains sterile water for injection, filled with 0.5 mL and closed with a temper evident closure system (Vetter V-OVS®) to safeguard the product integrity.

Twist the white temper evident closure system on the syringe bottom and remove the part of the closure system. Immediately attach the reconstitution needle on the luer lock of the syringe by twisting the needle clockwise until it locks. **Empty the pre-filled syringe** (into normal waste/ sewer) by pressing slowly the plunger rod, until all sterile water inside the syringe is removed.

Remove the flip off seal from the vial and puncture the stopper of the Placebo vial with the needle and withdraw the entire volume of the Placebo vial into the syringe. Inspect the liquid solution by visual control for any particulate matter and discoloration prior to administration.

To administer the Placebo, a new provided needle must be used. Change the needle and inject the Placebo intramuscularly (i.m.) into the deltoid muscle as soon as possible within an allowable time window of maximal 2 hours.

The used vials and syringes should be kept within the empty kits for drug accountability purposes. Used needles should be disposed in accordance with local requirements.

A study specific IMP manual with further details on IMP handling will be provided.

15.3.4 Post- Vaccination Observation

Following vaccination, the subject will be observed for at least 30 minutes at the study site in order to provide appropriate emergency treatment should this be necessary. In addition, vital signs including pulse rate and blood pressure while seated and at rest will be measured prior to discharge (for further information see Section 17.5). Any injection site and systemic reactions will be recorded.

Prior to leaving the study site, the subject will be instructed on the use of the respective electronic Subject Questionnaire for documentation of AEs (for further information see Section 17.3), a digital thermometer for measuring oral body temperature and a measuring device for assessing injection site reactions (at the vaccination visit only).

15.4 Screening and Study Visits

The overall study design is illustrated in Figure 24.1-1 (in Section 24.1). For a tabular outline of study procedures and assessments required at each visit, please see Table 15.4-1, Table 15.4-2 as well as the Schedule of Study Procedures and Assessments in Section 24.2.

15.4.1 Part A

Table 15.4-1
Study VLA1553-301 Study Visit Schedule – Part A

VISIT	TIME	ACTION
Visit 0 Screening	**Day -28 to 0 (prior to Visit 1)**	1. Informed consent [a] 2. Inclusion and exclusion criteria (Section 13.1 and 13.2) 3. Demographics [b] 4. Medical history incl. vaccination history [c] (Section 17.4.1) 5. Prior and concomitant Medications (Section 17.4.2) 6. Physical examination, Hand Stiffness Test and Vital Signs (Section 17.6, and 17.5) 7. Blood draw and urine sample for: a) Serum Pregnancy test [d] b) Safety Sample [e]
Visit 1	**Day 1**	1. Inclusion and exclusion criteria (review) (Section 13.1 and 13.2) 2. Medical History incl. vaccination history (update) (Section 17.4.1) 3. Concomitant Medication (Section 17.4.2) 4. Symptom-driven physical examination, Hand Stiffness Test and Vital Signs (Section 17.6 and 17.5) 5. Blood draw and urine sample for: a) Urine Pregnancy test b) Baseline Sample [f] c) Immunogenicity [g] d) Viremia [h] e) Clinical Sample (for Immunogenicity subset only) [i] 6. Randomization (Section 12.4) 7. **VACCINATION** (Section 15.3.2) 8. Post-vaccination observation (Section 15.3.4) 9. AE documentation (Section 17.2) 10. Instruct on Subject eDiary [j] (Section 17.3)
Visit 2	**Day 8 after Visit 1 (+/- 1d)**	1. Review Subject eDiary (Section 17.3) 2. AE documentation (Section 17.2) 3. Concomitant medication (Section 17.4.2) 4. Symptom-driven physical examination and Hand Stiffness Test (Section 17.6) 5. Blood draw and urine sample for: a) Immunogenicity [g] b) Viremia [h] c) Clinical Sample (for Immunogenicity subset only) [i]

Table 15.4-1
Study VLA1553-301 Study Visit Schedule – Part A

VISIT	TIME	ACTION
		6. Instruct on eMemory Aid (Section 17.3)
Visit 3	**Day 29 Month 1 (+/- 4d)**	1. Review eDiary and eMemory Aid (Section 17.3) 2. AE documentation (Section 17.2) 3. Concomitant medication (Section 17.4.2) 4. Symptom-driven physical examination and Hand Stiffness Test (Section 17.6) 5. Blood draw and urine sample for: a) Urine Pregnancy test b) Immunogenicity [g] c) Viremia [h] d) Clinical Sample (for Immunogenicity subset only) [i]

[a] Occurs at enrollment (before Screening).
[b] Demographics data include year of birth, height, weight, BMI, gender, race and ethnicity.
[c] Prior vaccination against relevant traveler diseases should be documented in the Medical History, i.e. YF and JEV.
[d] A **serum** pregnancy test will be performed for all female subjects of childbearing potential at the screening visit.
[e] Baseline safety laboratory sample obtained from ALL subjects. Safety laboratory assessments according to Section 17.7. Positive HIV test obtained by ELISA will have to be confirmed by a second method (e.g. Western blot or PCR).
[f] A baseline sample will be drawn from ALL subjects for potential retrospective investigation of pre-existing antibodies including but not limited to other alphaviruses (i.e. Mayaro) or Dengue and ZIKA.
[g] Immunogenicity sample to be obtained from ALL subjects for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis. For further information on the preparation, storage and shipment of blood samples please refer to the instructions in the respective Laboratory Manual provided in the Investigator File.
[h] Viremia plasma sample obtained from ALL subjects for clinically indicated **retrospective** investigation of viremia by RT-qPCR.
[i] Clinical sample obtained from subjects in the **Immunogenicity Subset** ONLY for standard safety laboratory assessments according to Section 17.7.
[j] Distribute thermometer and measuring device: Instruct subject how and when to complete the eDiary. Subject will also be instructed to immediately inform the site in case of any severe solicited AEs or other severe symptoms.

15.4.2 Part B

Table 15.4-2
Study VLA1553-301 Study Visit Schedule – Part B

VISIT	TIME	ACTION
Visit 4	**Day 85 Month 3 (+/- 7d)**	1. Review eMemory Aid (Section 17.3) 2. AE documentation (Section 17.2) 3. Concomitant medication (Section 17.4.2) 4. Symptom-driven physical examination and Hand Stiffness Test (Section 17.6) 5. Blood draw and urine sample for: a) Urine Pregnancy test [a] b) Immunogenicity [b] c) Clinical Sample (for Immunogenicity subset only) [c]
Visit 5	**Day 180 Month 6 (+/- 14d)**	1. Review eMemory Aid (Section 17.3) 2. AE documentation (Section 17.2) 3. Concomitant medication (Section 17.4.2) 4. Symptom-driven physical examination and Hand Stiffness Test (Section 17.6) 5. Blood draw and urine sample for: a) Urine Pregnancy test [a]

Table 15.4-2
Study VLA1553-301 Study Visit Schedule – Part B

		b) Immunogenicity [b] c) Clinical Sample (for Immunogenicity subset only) [c]

[a] A **urine** pregnancy test will be performed for all female subjects of childbearing potential.
[b] Immunogenicity sample to be obtained from ALL subjects for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis. For further information on the preparation, storage and shipment of blood samples please refer to the instructions in the respective Laboratory Manual provided in the Investigator File.
[c] Clinical sample obtained from subjects in the **Immunogenicity Subset** ONLY for standard safety laboratory assessments according to Section 17.7.

15.4.3 Unscheduled Visit

An unscheduled visit can be held at any time during the study if deemed necessary by the Investigator (e.g. follow-up on unexpected AEs or SAEs) or the DSMB. Assessments performed at an unscheduled visit will be at the Investigator's or DSMB's discretion. Unscheduled visits and any procedures/assessments performed during such a visit (e.g. physical examination, laboratory test) should be documented in the source data and the eCRF.

15.4.4 Early Termination Visit

Subjects who terminate participation or who are withdrawn from the study prematurely will undergo investigations as outlined below during an Early Termination Visit, if possible. Every effort should be made to have discontinued subjects complete the study Early Termination (ET) Visit (see Table 15.4-3).

Table 15.4-3
Study VLA1553-101 Study Visit Schedule – Early Termination

VISIT	TIME	ACTION
ET	unscheduled	1. Review Subject eDiary or eMemory Aid, as applicable (Section 17.3) 2. AE/AESI/SAE documentation (Section 17.2) 3. Concomitant medication (Section 17.4.2) 4. Symptom-driven physical examination and Hand Stiffness Test (Section 17.6) 5. Blood draw and urine sample for: a) Urine Pregnancy test b) Immunogenicity [h] c) Viremia [i] d) Clinical Sample (for Immunogenicity subset only) [j] 6. Documentation of reason for early termination

[f] A **urine** pregnancy test will be performed for all female subjects of childbearing potential.
[h] Immunogenicity sample for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis. For further information on the preparation, storage and shipment of blood samples please refer to the instructions in the respective Laboratory Manual provided in the Investigator File.
[i] Only if the ET visit occurs prior to Day 29, a viremia plasma sample should be obtained from the subject for clinically indicated **retrospective** investigation of viremia by RT-qPCR.
[j] Clinical sample obtained from subjects in the **Immunogenicity Subset** ONLY for standard safety laboratory assessments according to Section 17.7.

In case an ET Visit is not possible, a follow-up safety phone call should be made as soon as possible after termination to capture at least concomitant medications and solicited and unsolicited AEs since the last study visit.

The reason for early termination should be clarified in as much detail as possible. If an AE was the reason for early study termination details on that specific AE(s) should be captured (see Section 13.5). The reason for discontinuation will be recorded on the eCRF, and data collected up to the time of discontinuation will be used in the analysis and included in the clinical study report.

In the event of subject discontinuation due to an AE, clinical and/or laboratory investigations that are beyond the scope of the required study observations/assessments may be performed as part of the evaluation of the event. These investigations will take place under the direction of the Investigator in consultation with the Sponsor, and the details of the outcome may be reported to the appropriate regulatory authorities by the Sponsor.

15.5 Procedures for Monitoring Subject Compliance

All study procedures are to be performed under the supervision of the Investigator at the study site, and thus, no separate procedures will be used to monitor subject compliance.

16. ASSESSMENT OF IMMUNOGENICITY

All serum samples for neutralization titer determination will be handled according to the procedures supplied to each investigative site for the preparation, storage and shipment of samples (refer to Laboratory Manual). Each clinical study site will be responsible for the separation of serum from whole blood samples and the safe and controlled storage of serum samples prior to shipment to the central laboratory.

At the end of the study, results of immunogenicity assessments will be provided to the Investigator.

Immunogenicity samples will be collected from ALL subjects at the indicated time points and may be used for additional testing procedures (see Section 16.2). However, immunogenicity assessment measuring neutralizing antibodies will be performed on samples collected at Day 1, 8, 29, 85 and at Month 6 after a single immunization in the immunogenicity subset only.

16.1 Determination of vaccine-induced neutralizing antibody response

Immunogenicity of VLA1553 will be evaluated using a micro Plaque Reduction Neutralization Test (µPRNT), which is based on the same principle as a plaque reduction neutralization assay (PRNT), but allows testing with higher throughput. This assay differs from the µNT assay used for the assessment of neutralizing antibodies during Phase 1 clinical testing. Chikungunya virus neutralizing antibodies will be measured in the µPRNT using a serially passaged, live-attenuated CHIKV vaccine (CHIKV 181/25, TSI-GSD-218 or 181/clone 25) developed by the Walter Reed Army Institute of Research (WRAIR, US).

The CHIKV µPRNT evaluates the levels of antibodies that neutralize CHIKV infection of Vero cells in human serum samples.

Seven (7) two-fold serial dilutions of the de-complemented serum samples are prepared in round-bottom 96-well plates. CHIKV 181/25 at a target working dilution (to obtain 100 PFU/well) is added sequentially to the serum dilutions and incubated at 37°C and 5% CO_2 for 60 minutes. Serum-virus complexes are then transferred onto plates, previously seeded overnight with Vero cells, and incubated at 37°C and 5% CO_2 for 60 minutes. Then, the serum-virus complexes are removed and a virus maintenance medium containing 0.5% Methyl Cellulose is added to the wells, followed by an incubation of 17 ± 1 hours at 37°C

and 5% CO_2. The following day, cells are fixed with 4% Paraformaldehyde for 15 minutes and permeabilized with 0.2% Triton X-100. Following cell permeabilization, indirect immunostaining is performed. Primary antibody diluted in blocking agent is added to the plates and incubated at 37°C for 60 minutes. Afterwards, the secondary antibody diluted in blocking agent is added to the plates and incubated at 37°C for 30 minutes. Substrate is added to the plates and incubated for 10 minutes in the dark. Plates are rinsed with sterile water and left to be dried completely. Images from each well are acquired by an automated microscope system (ScanLab reader). The number of resulting PFUs in the wells is inversely proportional to the level of functional antibodies present in the serum, which is directly proportional to the immunological response of the subject.

The cut-off of CHIKV-specific neutralization antibody titer to be used in the analysis of seroconversion has been defined during µPRNT assay validation as CHIKV-specific neutralizing antibody titer [redacted] for baseline negative subjects. Seroconversion for baseline positive subjects is defined as a [redacted]-fold increase over baseline.

16.2 Additional Testing Procedures

Serum samples obtained in this study may, in addition to its use for assessment of CHIKV-specific neutralizing antibody titers, also be used for further development of the vaccine, including but not limited to the following assays:

- Passive immunization assays to evaluate the potency of immune sera to protect animals from infection after wild-type challenge;
- Development of additional neutralization assays (e.g. µNT, PRNT, virus replicon particle neutralization assays) for the assessment of cross-neutralization of heterologous CHIKV strains or other related (alpha)viruses;
- Detection of anti-CHIKV antibodies by enzyme linked immunosorbent assay (ELISA);
- Detection of viremia by viral culture;
- CHIKV sequencing;
- Clinical diagnostic work-up.

Such development may occur at laboratories other than the central analytical laboratory used for this study.

17. ASSESSMENT OF SAFETY

17.1 Definitions

17.1.1 Adverse Events

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

17.1.2 Serious Adverse Event

A **serious** adverse event (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 that were planned prior to vaccination to treat a pre-existing condition that did not change in severity, neither the condition leading to the hospitalization or prolonged hospitalization, nor the medical procedure itself need to be reported as an SAE. In this case, the underlying diagnosis or condition should be reported in the medical history section of the eCRF and the corresponding medical procedure should be documented as a comment to the underlying diagnosis or condition in the eCRF's medical history section.

The Sponsor will classify the SAEs as either expected or unexpected:

- **Expected:** An AE that is listed in the current Investigator's Brochure (IB);
- **Unexpected:** An AE that is not listed in the current IB, or it differs because of greater severity or greater specificity.

For the purpose of this study, AEs graded as potentially life-threatening (Grade 4) (see Section 17.2.3 for solicited AEs) will be reported as SAEs.

17.1.3 Adverse Events of Special Interest

An AESI (serious or non-serious) is an event of scientific and medical concern specific to the Sponsor's product.

17.1.4 Medically-Attended Adverse Event

All AEs where subjects are seeking medical care (i.e. doctor's office, emergency service, hospital, but not including use of self-medication).

17.1.5 Preexisting Diseases

Preexisting diseases that are present before entry in to the study, that are described in the medical history, and that manifest with the same severity, frequency, or duration after vaccine exposure, will not be recorded as AEs. Furthermore, routine health checks required due to pre-existing diseases will not be recorded. However, when there is an increase in the severity of a preexisting disease, the event must be described on the AE CRF page.

17.1.6 Untoward Medical Occurrences Not Considered Adverse Events

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

17.2 Collection, Documentation and Assessment of Adverse Events

17.2.1 Unsolicited Adverse Events

Subjects will be provided with an eMemory Aid to collect unsolicited AEs occurring until the end of study (Visit 5, see Section 17.3). Additionally, the Investigator will enquire about AEs during study visits. Clinically relevant laboratory parameter changes constitute unsolicited

AEs, too, unless they are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection). In addition, symptoms noted during the symptom-driven physical exams (unless already covered by an AE) constitute AEs.

All unsolicited AEs need to be documented in the respective AE section of the eCRF during applicable study visit (Visits 1 to 5 or unscheduled visit(s), if applicable), regardless of their source (AEs noted in the eMemory Aid (see Section 17.3), open question to subject, laboratory parameters, symptom-driven physical examination). Any symptom is regarded as a separate AE. However, if the Investigator considers several symptoms to be in the context of one underlying diagnosis, the Investigator may merge these symptoms into one single appropriate AE. The AE term entered into the eCRF should contain all symptoms summarized to one event (e.g. "Influenza with flu-like-symptoms, fever and headache").

The Investigator will follow-up on each AE until it is resolved or until the medical condition of the subject is stable. All relevant follow-up information will be reported to the Sponsor until the end of the study for each subject. SAEs ongoing at the time of Visit 6 will be followed until resolution or achievement of stable clinical conditions, latest until the overall end of the study.

Beyond study end, SAEs that are fatal, life-threatening or suspected to be related to study treatment will continue to be reported until 6 months after the last study visit of the respective subject (i.e. Visit 5).

The following information will be documented for each AE: severity, causality, outcome, seriousness, medically-attended, action taken to treat AE, start and stop dates.

17.2.1.1 Severity

The investigator will assess the severity of AEs using his/her clinical expertise and judgment based on the most appropriate description below:

Mild (Grade 1):	Awareness of signs or symptoms, but easily tolerated, does not interfere with daily activities.
Moderate (Grade 2):	Discomfort enough to interfere with usual activity and with or without requiring medical intervention.
Severe (Grade 3):	Incapable of work or usual activity and requiring medical intervention.

17.2.1.2 Causality

Causality is a determination of whether there is a reasonable possibility that the vaccine administration is etiologically related to/associated with the AE.

For AEs, the Investigator will assess the causal relationship between the IMP and the AE using his/her clinical expertise and judgement according to the following most appropriate algorithm for the circumstances of the AE:

Probable: Reaction that follows a reasonable temporal sequence from administration of the IMP, or that follows a known or expected response pattern to the suspected treatment; and that could not reasonably be explained by known characteristics of the subject's clinical state.

Possible: Reaction that follows a reasonable temporal sequence from administration of the IMP, or that follows a known or expected response pattern to the suspected treatment; but that could readily have been produced by a number of other factors.

Unlikely: Reports not following a reasonable temporal sequence from administration of the IMP; an event, which may have been produced by the subject's clinical state or by other environmental factors. A more likely alternative etiology exists.

Not related (unrelated): Events for which sufficient information exists to conclude that the etiology is unrelated to the IMP.

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

17.2.2 Assessment and Outcome of Adverse Events

Each AE from vaccination until study completion/termination will be described in the eCRF (i.e., 1 AE per form) using the medical diagnosis (preferred), symptom, or sign, in standard medical terminology in order to avoid the use of vague, ambiguous, or colloquial expressions (see definition in Section 17.1.1). AEs will be evaluated by the Investigator for:

- Seriousness as defined in Section 17.1.2
- Severity as defined in Section 17.2.1.1
- Causal relationship to vaccine exposure as defined in Section 17.2.1.2

For each AE, the outcome will also be documented as either:

- recovering/resolving
- recovered/resolved
- recovered/resolved with sequelae
- not recovered/not resolved
- fatal
- unknown

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

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

17.2.3 Solicited Adverse Events

17.2.3.1 Injection Site Reaction – Measurement and Evaluation

Subjects will be provided with a measuring device to measure the size of any measurable injection site reaction that may develop after vaccination. The subject will be instructed on how to measure any such reactions over a period of 10 consecutive days after vaccination along the longest diameter of the reaction area and record this measurement in the Subject eDiary. Injection site reactions include injection site pain, tenderness, erythema/redness, induration and swelling.

Injection site reactions will be rated based on the FDA Guidance on Toxicity Grading Scales as described in Table 17.2-1. Any grade 4 injection site reaction should be reported as an SAE (see Section 17.1.2).

Table 17.2-1
Grading of Injection Site Reactions – Vaccine Specific Criteria

Vaccine-specific Criteria	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[c]
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema / Redness [a]	2.5 – 5.0 cm 0.98 – 1.96 inch	5.1 – 10.0 cm 1.97 – 3.94 inch	> 10.0 cm > 3.94 inch	Necrosis or exfoliative dermatitis
Induration / Swelling [b]	2.5 – 5.0 cm (0.98–1.96 inch) and does not interfere with activity	5.1– 10.0 cm (1.97 – 3.94 inch) or interferes with activity	> 10.0 cm (> 3.94 inch) or prevents daily activity	Necrosis

[a] In addition to grading the measured local reactions at the greatest single diameter.
[b] Induration / swelling should be evaluated and graded using the functional scale as well as the actual measurement.
[c] Any Grade 4 injection site reaction will be reported as SAE, Grade 3 severity should be documented.

17.2.3.2 Systemic Reactions – Measurement and Evaluation

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

Systemic reactions will be rated based on the FDA Guidance on Toxicity Grading Scales as described in Table 17.2-2. Any grade 4 systemic reaction should be reported as an SAE (see Section 17.1.2).

Table 17.2-2
Grading of Systemic Reactions – Vaccine Specific Criteria

Vaccine-specific Criteria	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)[d]
Fever[a]	38.0 – 38.4 °C 100.4–101.1 °F	38.5 – 38.9 °C 101.2–102.0 °F	39.0 – 40.0 °C 102.1–104 °F	> 40.0 °C >104 °F
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia[b]	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Rash[c]	Macules/papules covering <10% body surface area (BSA) with or without symptoms (e.g., pruritus, burning, tightness)	Macules/papules covering 10- 30% BSA with or without symptom (e.g., pruritus, burning, tightness); limiting instrumental activity of daily living	Macules/papules covering >30% BSA with or without associated symptoms; limiting self care activity of daily living	-

[a] Oral temperature; no recent hot or cold beverages or smoking.
[b] Symptom not described in FDA Toxicity Grading Scale.
[c] Grading based on Common Terminology Criteria for Adverse Events (CTCAE), NIH, v4.03, 2010.
[d] Any Grade 4 systemic reaction will be reported as SAE, Grade 3 severity should be documented.

17.2.3.3 Body Temperature Measurement

From vaccination (Day 1) until Day 11 after vaccination (i.e., the first 10 post-vaccination days including the day of vaccination), the subject should measure his/her body temperature orally once every evening, assessments by the subject should occur at the same time each day, starting approximately 8 hours after vaccination. To optimize the comparability of the documented body temperatures, all subjects will be provided with a digital oral thermometer and be instructed in its use. The subjects may keep the thermometer after study termination.

If fever (oral body temperature ≥ 38.0 °C/100.4 °F) occurs, body temperature should be measured at least every 4 to 8 hours until it returns to normal (< 38.0 °C/100.4 °F). All body temperature measurements including the date and time should be recorded in the Subject eDiary (see Section 17.3). In case of fever, the subject should record all fever measurements in the diary including the first value that shows a return to normal body temperature.

Body temperature measurements from vaccination (Day 1) until Day 11 after vaccination (i.e., the first 10 post-vaccination days including the day of vaccination) will be transcribed automatically or by the Investigator into the eCRF. If more than one body temperature value is recorded in the diary for a given day, the highest daily temperature reading will be recorded in the eCRF.

Body temperature measurements will be analyzed according to the FDA Guidance on Toxicity Grading Scales for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (Food and Drug Administration) for data analysis as described in Table 17.2-2 above.

Outside of the first 10 post-vaccination days, daily body temperature measurement is **not required**. However, subjects experiencing an AE with symptoms of fever are recommended to measure their body temperature in order to document fever as an AE in the eMemory Aid. If fever (i.e., oral body temperature ≥38.0°C/100.4 °F) occurs, it is recommended that the subject measures his/her body temperature every 4 to 8 hours until fever resolves (i.e., oral body temperature <38.0°C/100.4 °F) in order to document onset and resolution, as defined by the date of first oral body temperature measurement of ≥38.0°C/100.4 °F (date of onset) and the date of first oral body temperature measurement of <38.0°C/100.4 °F (date of resolution).

17.3 Study eQuestionnaires

Subjects will be provided with two types of electronic questionnaires throughout the course of this study:

- **Subject eDiary** will be distributed to all subjects for the collection of safety information from the day of vaccination until the first 10 post-vaccination days (i.e. including the day of vaccination)
- **eMemory Aid** will be distributed to all subjects for the collection of safety information outside the first 10 post-vaccination days until the next Visit.

<u>The following information will be collected in the Subject eDiary (10 days post-vaccination):</u>

- Measurement of oral body temperature (For further information see Section 17.2.3.3);

- Solicited injection site reactions[vii] (injection site pain, tenderness, redness, induration and swelling), for further information see Section 17.2.3.1;
- Solicited systemic reactions[vii] (fever, fatigue, headache, nausea/vomiting, muscle pain, joint pain, rash);
- Other AEs;
- Any new concomitant medication or changes in medication taken after vaccination;
- Information on travel to geographical regions where CHIKV is endemic.

The following information will be collected in the eMemory Aid (outside 10 day post-vaccination period):

- AEs;
- Any new concomitant medication or changes in medication taken after vaccination;
- Ongoing solicited reactions;
- Information on travel to geographical regions where CHIKV is endemic.

Assessments by the subject should occur at the same time each day, starting approximately 8 hours after vaccination. The subject will be properly instructed on the reporting requirements and how to complete and use the subject questionnaires, thermometer and measuring device (for assessment of measurable injection site reactions).

Questionnaires are always to be assessed at the next visit by the Investigator together with the subject, prior to these data being entered into the electronic case report form (eCRF). The Investigator will review and discuss the questionnaires with the subject, ask about AEs occurring since the last visit and both the subject and Investigator have to sign the questionnaires to ensure completeness and reliability of self-reporting. The entries in the respective questionnaires shall be evaluated and graded for severity and relatedness to the vaccination by the Investigator.

The eQuestionnaires will serve as source documentation. Entries in the eQuestionnaires will be transcribed into the appropriate eCRFs. Any entry on the eCRF that does not correspond with an entry in the questionnaire will be explained by the Investigator on the relevant questionnaire page.

[vii] Solicited injection site and systemic reactions in the Subject eDiary will be assessed by the subject for absence, presence and duration.

17.4 Medical, Medication, and Non-Drug Therapy History

17.4.1 Medical history

At screening, the subject's medical history will be described for the following body systems including severity (mild, moderate, or severe as defined in Section 17.2.1.1) or surgery and start and end dates, if known: eyes, ears, nose, and throat; respiratory; cardiovascular; gastrointestinal; musculoskeletal; neurological; endocrine; metabolic; hematopoietic/lymphatic; dermatological; and genitourinary.

In addition, the medical history covering the last 3 years prior to screening will include the following:

- Information on prior vaccination against relevant traveler diseases (e.g. yellow fever, Japanese encephalitis virus vaccine)
- Previous chikungunya virus infection;
- Other vector-borne diseases in the past;
- Previous trauma to joints;
- Information on planned hospitalizations (including elective surgery) during the study for medical conditions existing prior to or at study entry. Such planned hospitalizations will not need to be reported as SAEs.

17.4.2 Concomitant Medications and Non-Drug Therapies

All medications (including vaccinations) received from 2 weeks prior to study enrollment until completion/termination should be collected from all subjects, and recorded on the appropriate eCRF. In addition to product name (generic name), the dose, indication, route of administration and frequency as well as the start and end date of treatment will be documented.

In addition, medications to treat SAEs will be reported to the Sponsor on SAE Report Forms as described in Section 3. In context of this study, information on non-drug therapies will only be collected in relation to SAEs.

The following medications are **not permitted** if administered within the specified study periods (unless such treatment has to be administered in an emergency situation):

- Any blood products or immunoglobulins during the course of the study;
- Immunosuppressive therapies (e.g. systemic or high dose inhaled [>800 µg/day of beclomethasone dipropionate or equivalent] corticosteroids, radiation treatment or other immunosuppressive or cytotoxic drugs) during the course of the study;

- Prophylactic administration of antipyretics within 4 hours prior to and during the first 72 hours after vaccination;
- Subjects are requested to refrain from donation of blood, blood fractions and plasma within 30 days prior to vaccination and for the entire study duration

For documentary purposes, any of the treatments listed above (including emergency treatment) given within these time periods requires special documentation and is to be documented as a protocol deviation.

The following medications and procedures will **delay** vaccination:

- antipyretics received within 4 hours prior to vaccination.
- any live or inactivated vaccine received within 28 days or 14 days prior to vaccination, respectively.

Usage of any other medications or non-drug therapies is not restricted.

Additionally, medications that are not permitted prior to study enrollment, resulting in exclusion from the study, are reflected in the exclusion criteria in Section 13.2.

17.5 Vital Signs

Vital signs will include body temperature (°C / °F) measured orally, pulse rate (beats/min), and systolic and diastolic blood pressure (mmHg) while seated and at rest.

Vital signs will be measured at screening (Visit 0) and at the vaccination visit (Visit 1) and are to be recorded before the vaccination is given. In addition, after an observation period of 30 minutes at the study site following vaccination pulse rate as well as blood pressure while seated and at rest will again be assessed.

Vital sign values are to be recorded on the appropriate eCRF. For each vital sign value, the Investigator will determine whether the value is considered an AE (see definition in Section 17.1.1). If assessed as an AE, the medical diagnosis (preferably), symptom, or sign, will be recorded on the AE CRF. Additional tests and other evaluations required to establish the significance or etiology of an abnormal result, or to monitor the course of an AE should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the Investigator.

17.6 Physical Examinations

At screening (Visit 0), 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.

A symptom-driven physical examination will be performed at all study Visits except the Screening Visit (Visit 0), i.e. only in case a symptom is reported by the subject, a system-based assessment will be performed for a detailed check of the affected body system(s). A symptom-driven examination should also be performed in case the subject has complaints within the observation time after vaccination (as described in 24.2, Visit 0 Screening)

A hand stiffness examination will be performed at all study Visits irrespective of any clinical signs or symptoms. The subject will be asked to bend simultaneously the four fingers of both hands, i.e. index, middle, ring, and little finger and the range of motion will be measured with a measuring device. Specifically, the distance between the fingers and the ball of the thumb should be measured in cm and recorded. Special attention should be paid to measuring consistently to the same part of the ball of the thumb.

Abnormal conditions detected at screening or prior to vaccination at Visit 1 will be recorded as medical history. At all other study visits, if a new abnormal or worsened abnormal pre-existing condition is detected, the condition will be recorded as an AE.

17.7 Clinical Laboratory Parameters

Blood and urine samples will be obtained for assessment of clinical laboratory parameters as outlined in the Schedule of Study Procedures (see Section 24.2) or in the Screening and Study Visits Section (see Section 15.4). Parameters will be analyzed by central laboratories according to the applicable laboratory SOP.

17.7.1 Safety Sample

A baseline safety laboratory blood [18.0 mL] and urine sample will be obtained at Visit 0 (Screening Visit) from all subjects for standard clinical chemistry, hematology, coagulation panel and urinalysis as well as for HIV / HBsAg / HCV testing.

Clinical chemistry (approx. 3.5 mL)	Creatinine, sodium, potassium, calcium, aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin and C-reactive protein (CRP).
Hematology panel (approx. 5.0 mL)	Hemoglobin, hematocrit, erythrocyte count, WBC count, differential WBC count (basophils, eosinophils, lymphocytes, monocytes, neutrophils), platelets, ESR.
Coagulation panel (approx. 4.5 mL)	Small blood coagulation (prothrombin time, aPTT and

	fibrinogen).
Urinalysis	Standard urine dipstick for determining pH-value, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
HBsAg/ HCV / HIV test (approx. 5.0 mL)	A positive HIV test obtained by ELISA will have to be confirmed by a second method [e.g. Western blotting or PCR], at Visit 0 only.

17.7.2 Baseline Sample

A baseline sample [10.0 mL] will be drawn from all subjects at Visit 1 (Day 1) for potential retrospective investigation of pre-existing antibodies including but not limited to other alphaviruses (i.e. Mayaro), dengue or Zika. Such assessments may occur at laboratories other than the central laboratory used for analysis of safety samples.

17.7.3 Clinical Sample

A clinical blood [13.0 mL] and urine sample will be obtained from subjects allocated to the immunogenicity subset ONLY for standard clinical chemistry, hematology, coagulation panel and urinalysis (see Section 17.7.1).

17.7.4 Assessment of Laboratory Values

Laboratory values will be evaluated according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (Food and Drug Administration). For the individual toxicity criteria refer to Section 24.3.

Laboratory assessments for which no severity grading is described in Section 24.3 are graded as described in Section 17.2.1.1 upon investigator's judgment.

The Investigator's assessment of each abnormal laboratory value, including its clinical significance, is to be recorded in the eCRF:

- Abnormal laboratory assessments that are considered clinically relevant, in the opinion of the Investigator, need to be documented as unsolicited AEs and assessed further for severity according to the toxicity grading scale provided in Section 24.3, causality and other assessments done for unsolicited AE (see Section 17.2.1).

- Abnormal laboratory assessments that are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection) do NOT additionally need to be documented as unsolicited AE, but a respective comment should be added to the underlying AE.

Additional tests and other evaluations required to establish the significance or etiology of an abnormal laboratory result, or to monitor the course of an AE should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the Investigator.

17.8 Viremia

Subjects will return to the study site to collect plasma samples from all subjects on the vaccination Day 1 and on Days 8 and 29 (Visit 1-3), and if applicable at the ET Visit. Viremia plasma sample will be obtained from all subjects for a clinically indicated retrospective investigation of viremia by quantitative RT-PCR (RT-qPCR). The retrospective analysis will be conducted if deemed necessary by the Data Safety Monitoring Board upon clinical indication.

Viremia will be analysed in plasma samples. Blood [8.0 mL] will be collected by venous puncture. Samples will be shipped on dry ice at -70°C, viral RNA will be isolated and viral CHIKV RNA will be specifically amplified by RT-qPCR.

17.9 Adverse Events of Special Interest (AESI)

In addition to nonspecific transient muscle pain and joint pain that may occur after vaccination with any vaccine, subjects will be carefully monitored for signs and symptoms suggesting an acute stage CHIKV-associated event.

If a subject develops symptoms suggestive of CHIKV infection, he/she will be asked to contact the site immediately for clinical evaluation at an unscheduled visit.

Therefore, the following cluster of symptoms with or without remissions or exacerbations will receive particular consideration:

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

AND

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

OR

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

AND

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

AND

4. Duration of event ≥ 3 days.

Any suspected clinical case of CHIKV-associated event shall be referred to a clinical expert, be evaluated according to standard diagnostic procedures and treated according to current medical standard until resolved or stabilized. For more detailed information please refer to: French guidelines for the management of chikungunya - acute and persistent presentations (Simon, Javelle et al. 2015).

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

A blood sample for laboratory investigation will be taken upon presentation of the subject. Testing may include but is not limited to rheumatoid factor (RF), anticitrullinated protein antibody (ACPA), Ferritin and CRP. Retrospective investigation of a pre-vaccination sample may be considered for a thorough causality assessment.

Suspected CHIKV-associated events do not constitute a reason for withdrawal from the study.

17.10 Adverse Event Reporting Procedures

17.10.1 Serious Adverse Event

Any SAE should be reported to the Safety Desk by fax or email within 24 hours after the Investigator has become aware of the event. Under certain circumstances the initial notification could be done by phone, but nevertheless a written SAE Report Form has to be submitted to the Safety Desk within 24 hours (see Section 3).

Correct SAE reporting will have to cite a diagnosis or a symptom. Any diagnosis and any symptom is regarded as separate SAE. However, if the Investigator considers several symptoms to be in the context of one underlying diagnosis, he/she may specify the diagnosis as the reportable SAE and describe the attendant symptoms in one single appropriate SAE report.

Medical or diagnostic procedures due to an underlying disease or symptom are not considered an AE but a consequent measure following an AE. A correct SAE report will therefore have to specify the disease or symptom as the reportable AE and the medical or diagnostic procedure as action taken.

In addition, expedited and periodic reporting to Competent Authorities and IRBs will be performed in accordance with local requirements. Further reporting details can be found in the study-specific SAE procedure which is in accordance with respective US/EU requirements, International Conference on harmonization (ICH) GCP, national laws and site-specific requirements. SAEs that are considered as probably or possibly related and additionally are unexpected need to be reported according to the requirements for suspected unexpected serious adverse reactions (SUSARs).

SAE reports will be reviewed by a study site´s physician, the Safety Desk, the Study Medical Monitor, the Sponsor and the independent DSMB.

Beyond study end, SAEs that are fatal, life-threatening or suspected to be related to study treatment will continue to be reported until 6 months after the last study visit of the respective subject (i.e. Visit 5).

17.10.2 Adverse Events of Special Interest (AESI)

Any AESI should be reported to the Safety Desk by fax or email within 48 hours after the Investigator has become aware of the event. Under certain circumstances the initial notification could be done by phone, but nevertheless a written AESI Report Form has to be submitted to the Safety Desk within 48 hours (see Section 4).

Subjects will be carefully monitored for development of AESIs. A cluster of symptoms (see 17.9) associated with CHIKV infection, are defined as AESI. In case an AESI is identified, the Investigator will complete the AESI Report Form with all available information including medical records. This information will be provided to the DSMB. The DSMB will perform a thorough review of each case and advise whether additional clinical work-up is required.

The DSMB will conduct a final adjudication of all AESIs and will assess whether cases were new in onset and whether there is any relationship to administration of the study vaccine. Narratives with detailed case descriptions will be provided for all AESIs.

17.10.3 Pregnancy

The risk of maternal to fetal transmission of Chikungunya virus during pregnancy cannot be excluded. Thus, women must not become pregnant during the first three months post-vaccination.

Reporting requirements start with administration of the vaccination until study completion (or ET Visit). All pregnancies that occur during the clinical study period will be followed-up for three months after delivery or termination of the pregnancy. Any effect on either mother or fetus should be determined. A pregnancy which led to a congenital anomaly/birth defect must be followed-up by the Investigator longer or until resolution or stabilization. Duration of prolonged follow-up will be decided on an individual basis and in accordance with the Sponsor. The Investigator will prepare a narrative on the course of the pregnancy and the outcome.

The Investigator should report pregnancies within 24 hours of being notified using the Pregnancy Report Form. Reporting procedures are similar to SAE reporting procedures (contacts and processing), although a pregnancy is not considered an SAE (see Section 5).

If a seriousness criterion applies in addition to the pregnancy (e.g. hospitalization, congenital anomaly/birth defect) the pregnancy qualifies as an SAE. In such case a Pregnancy Report Form **and** an SAE Report Form have to be filled out.

17.11 Safety Monitoring

17.11.1 Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) will be utilized for this study. The DSMB will consist of five 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 meet to review accumulating safety data on a regular basis until all subjects have received the vaccination at Day 1 and until all subjects have completed Visit 2 or 3. In addition, the DSMB will periodically review accruing safety information throughout the study, 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 ad hoc review cases of SAEs, AESIs and severe (Grade 3) solicited AEs. The DSMB will periodically review listings and summary tabulations of SAEs, Deaths, Solicited AEs, Unsolicited AEs.

A DSMB charter including a detailed description will be prepared.

Responsibilities of the DSMB

- Provides independent monitoring of safety issues, which means it reviews and evaluates all SAE reports and study discontinuations 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;

- Reviews data produced from the study upon the Sponsor's request to determine whether the conditions on which study design is based have remained the same or have changed. If changed, the DSMB will suggest whether or not the changes mandate changes to the protocol and suggest a protocol amendment;
- Upon the Sponsor's request, meets for discussion and gives recommendations to the Sponsor as to whether the study should progress unchanged, or the study requires changes, or the study should be terminated prematurely;
- Can propose an unplanned safety analysis of clinical trial data;
- Can suggest unscheduled visits for specific evaluations for individual cases reviewed.

17.11.2 Sponsor

Until the last subject reached Day 29, listings of available blinded safety data will be closely reviewed by the Sponsor to identify any potential safety concerns.

17.11.3 Investigator

To ensure information exchange on safety across sites, Investigators will be provided with safety information pertaining to all severe (Grade 3) AEs and SAEs reported in the eCRF.

18. STATISTICS

18.1 Sample Size and Power Calculations

The sample size for this study is selected in order to provide a comprehensive safety profile with regards to rare AEs and SAEs. A number of approximately 3,000 VLA1553 vaccinated subjects will allow for the detection of at least one vaccine-related rare event (incidence rate 1/1000) with a probability of 94% in this study.

The sample size of the immunogenicity sub-set will allow for sufficient statistical power when applying a one-sided exact binomial test with a significance level of 2.5% against a non-acceptance threshold of 70% on the proportion of subjects with a seroprotective level (defined as [redacted] for baseline negative subjects) at Day 29. A seroprotection rate of 80% is assumed, and 200 VLA1553-vaccinated subjects would thus be necessary for a statistical power of 90%. With an expected drop-out/major protocol deviations rate of approximately 10%, 225 subjects vaccinated with VLA1553 need to be allocated to the immunogenicity subset. In order to account for placebo subjects, to achieve a meaningful number of subjects in both age strata, and to enroll sufficient numbers of subjects for a long-term follow-up in a potential subsequent trial, a total of 500 subjects will be enrolled into the immunogenicity sub-set.

18.2 Datasets and Analysis Cohorts

18.2.1 Safety Population

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

18.2.2 Immunogenicity Population

The Immunogenicity (IMM) population is defined to include all randomized and vaccinated subjects of the IMM subset who were CHIKV seronegative at baseline (defined as [redacted] and have at least one evaluable post-baseline titer measurement after vaccination. Subjects will be analyzed according to the study arm they had been allocated to, rather than by the actual treatment they received.

This population will be used for sensitivity analyses of the immunogenicity endpoints.

18.2.3 Immunogenicity Elderly Population

As the composition of the IMM population differs from that originally planned in the CSP v4.0 due to recruitment problems, an additional statistical analysis will be performed at a later stage to enhance the number of subjects for which immunogenicity data are available. For this analysis, randomly selected subjects of Stratum B, i.e. subject's ≥65 years of age, will be used to arrive at the originally planned numbers of subjects for this age stratum and to perform the specified immunogenicity analyses.

This analysis will be included in the Part A CSR if all elderly subjects are enrolled in a timely manner to allow the processing of immunogenicity samples within the timeframe of the Part A unblinding and CSR creation, else it will be performed at a later date and filed with the Part B analysis.

Therefore, the Immunogenicity elderly (eIMM) population is defined to include all randomized and vaccinated subjects of the IMM subset as well as randomly selected elderly subjects (Stratum B) of the safety analysis population to achieve 154. Subjects will be analyzed according to the study arm they had been allocated to, rather than by the actual treatment they received. This population will be used for sensitivity analyses of the immunogenicity endpoints.

18.3 Per Protocol Population

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

- 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 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 ≥0.05 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 human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV);
- Subject has received the wrong (not according to randomization) or no IMP.

- Subjects who received an excluded concomitant medication which could influence the immune response.
- Subjects missing Visit 3.

These criteria for protocol violations are identified at the time of planning the study. 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 population will be made by the sponsor on a case by case basis in a blinded manner (prior to study unblinding). Sample testing issues may also lead to exclusion from the PP analysis for particular time points.

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

18.4 Handling of Missing, Unused, and Spurious Data

All statistical analysis will generally be based on observed values, missing values will not be imputed. In case of > 5% of missing values for an immunogenicity comparison involving a statistical test, multiple imputation methods will be applied in order to evaluate the possible impact of missing values on these results.

18.5 Methods of Analysis

A statistical analysis plan will be prepared before database closure/snapshot.

18.5.1 Immunogenicity Analysis

All analyses of immunogenicity data will be performed primarily on the PP population and secondarily on the IMM and eIMM populations.

The primary immunogenicity analysis will be a comparison of the observed proportion of subjects with a seroprotective CHIKV antibody level (defined as [redacted] for baseline negative subjects) at Day 29 (i.e. 28 days post-vaccination) against a non-acceptance threshold of 70%. An exact binomial test for the null-hypothesis H0: SPR ≤ 70% against the alternative H1: SPR > 70% with a one-sided significance level of 2.5% will be applied and exact (Clopper-Pearson) two-sided 95% confidence limits will be calculated.

Secondary immunogenicity analysis will include the comparison of the GMTs between the VLA1553 and placebo groups at Day 29 (i.e. 28 days post-vaccination) by ANOVA (study

group, covariate study site and age stratum as factors), two-sided 95% confidence intervals will be calculated for the GMT.

In addition, the seroprotection and seroconversion rates for various study days, and when applying other threshold titers for defining seroconversion or seroprotection thresholds will be compared between the study arms by Fisher's exact test and 95% confidence intervals will be calculated. Immunogenicity analyses will also be generated stratified by age stratum.

Immune response of baseline sero-positive subjects will be analyzed descriptively and listed.

18.5.2 Safety Analysis

All analyses of safety data will be performed on the safety population. Safety analysis will generally be generated overall and stratified by age stratum.

Safety tabulations will generally be provided separately for solicited AEs and unsolicited AEs, and for both types of AEs combined. 95% confidence intervals according to Altman will generally be provided for all AE rates and differences between the study arms will be assessed for significance using Fisher's exact test and will be assessed regarding the clinical relevance by the DSMB during the ongoing study and discussed in the CSR.

The number and percentage of subjects with any AE, any solicited AE, unsolicited AE, any related unsolicited AE, any related severe AE, any SAEs, any related SAEs, any AESI, any related AESI, any medically attended AE, any AE leading to withdrawal from study, and any AE occurring at a frequency of at least 10% and at least 1% in at least one study arm, up to Day 29, and up to Month 6, will be presented for each study arm, overall and by system organ class/preferred term.

The number and percentage of subjects with solicited local and systemic AEs within 10 days after vaccination will be presented. The occurrence of solicited local and systemic AEs will also be tabulated by Subject Diary day. Changes in laboratory values from study entry will be analyzed descriptively and will be part of the unsolicited AE evaluation only in case of clinically relevant deviations. The rates of subjects with laboratory assessments outside the normal range, and with abnormal laboratory parameters falling into the grade 0 vs. 1 through 3 will be calculated by visit and overall. The rate of subjects with urinalysis results according to the test manufacturer's results categories will be calculated.

18.6 Planned Data Analysis of the Study

The following data analyses will be performed:

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

Individual study parts will be analyzed sequentially.

The Part A analysis will be performed once the last subject has completed the study Visit 3, i.e. Day 29. Part B analysis will be performed once the last subject has completed the study Visit 5, i.e. Month 6. Part A will be unblinded (blind will be maintained for study sites) and Part B final report will be submitted for BLA filing.

19. ETHICS AND REGULATORY ASPECTS

19.1 Compliance Statement

This study will be conducted in accordance with this protocol, current ICH/GCP guidelines, Declaration of Helsinki, and with the applicable national and local regulatory requirements.

19.2 Institutional Review Board (IRB) and Regulatory Authorities

Before enrollment of healthy volunteers into this study, the protocol, informed consent form, any promotional material/advertisements, and any other requested information will be reviewed and approved/given favorable opinion by the IRB and applicable regulatory authorities in accordance with local requirements. The study will commence only upon the Sponsor's receipt of approval/favorable opinion from the IRB.

If the protocol and/or any other information given to the subject is/are amended, the revised document(s) will be reviewed and approved/given favorable opinion by the IRB and applicable regulatory authorities in accordance with local requirements, where applicable. The protocol amendment will only be implemented upon the Sponsor's receipt of approval. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving IRB and authority approval. However, in this case, approval must be obtained as soon as possible after implementation.

19.3 Subject Information and Informed Consent

It is the Investigator's responsibility to obtain freely given, written, informed consent from

the subject before the subject is exposed to any study-related procedures, including screening tests for eligibility.

The informed consent form will include a comprehensive explanation of the proposed treatment without any exculpatory statements, in accordance with the elements required by ICH GCP and applicable regulatory requirements. Volunteers will be allowed sufficient time to consider participation in the study after having the nature and risks of the study explained to them. By signing the informed consent form, volunteers agree that all evaluations required by the study will be completed, unless they withdraw voluntarily or are terminated from the study for any reason.

The Investigator will explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, without any prejudice and need for justification. The subjects will be informed that representatives of the Sponsor and health authority inspector may review their source records, and that these persons are bound by confidentiality obligations.

The subject will be given a copy or a second original of the ICF. An original of the signed and dated ICF must be retained in the site's records, and is subject to inspection by representatives of the Sponsor or representatives from regulatory agencies.

The Sponsor will provide to the Investigator in written form any new information that significantly bears on the subjects' risks associated with study vaccine exposure. The informed consent form will be updated, if necessary. This new information and/or revised informed consent form, that has been approved by the applicable IRB and regulatory authorities, where applicable, will be provided by the Investigator to the subjects who consented to participate in the study.

20. QUALITY CONTROL AND QUALITY ASSURANCE

20.1 Source Data and Records

Source data are defined as all information related to clinical findings, observations or other activities in the study, captured in original records or certified copies of original records. The Investigator will permit study-related monitoring, audits, IRB review and regulatory inspections, by providing direct access to source data/records. Source records should be preserved for the maximum period of time required by local regulations.

Source data entries must be made in accordance with local requirements. Signed and dated copies of the laboratory result reports have to be kept within the subject's source data file.

eCRFs will not be used as source data for any other variable.

20.2 Investigator's Responsibility

The Investigator will comply with the protocol (which has been approved/given favorable opinion by the IRB), ICH GCP, and applicable regulatory requirements. The Investigator is ultimately responsible for the conduct of all aspects of the study at the study site and verifies by signature the integrity of all data transmitted to the Sponsor. The term "Investigator" as used in this protocol, and in study documents refers to the Investigator or authorized study personnel whom the Investigator has designated to perform certain duties. Sub-investigators or other authorized study personnel are eligible to sign for the Investigator, except where the Investigator's signature is specifically required.

20.3 Training

The study monitor will ensure that the Investigator and study site personnel understand all requirements of the protocol, the investigational status of the vaccine, and his/her regulatory responsibilities as an Investigator. Training may be provided at an Investigator's meeting, at the study site, and/or by instruction manuals. In addition, the study monitor will be available for consultation with the Investigator and will serve as the liaison between the study site and the Sponsor.

20.4 Monitoring

A designated monitor will check electronic system data and source data at regular intervals throughout the study to verify completeness, accuracy and consistency of the data, protocol adherence, and adherence to GCP guidelines. The monitor will work and perform Source Data Verification according to the Clinical Management Plan. The Investigator will cooperate with the monitor to ensure that any discrepancies identified are resolved.

20.5 Audit and Inspection

Upon request, the Investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the Sponsor or to regulatory inspectors. The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for the assessment of safety and efficiency of the investigational product have appropriately been reported to the Sponsor.

20.6 Non-compliance with the Protocol / Protocol Deviations from the Protocol

Any deviations from the protocol will be tracked, actions defined, as feasible, and reviewed in Data Review Meetings for the study part analysis and the final analysis for assessment of their influence on the quality of the study analysis.

20.7 Confidentiality of Subject's Data

The Investigator will exercise all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of subjects' identities. On exported electronic source data or any other documents submitted to the Sponsor, subjects will only be identified by subject number. Documents not for submission to the Sponsor, e.g. subject identification log and original ICF, will be maintained by the Investigator in strict confidence.

21. DATA HANDLING AND RECORD KEEPING

21.1 Information of Investigators

An IB containing all important data relating to the safe use of the IMP will be supplied to the Investigator prior to study start.

The Investigator will be kept informed on new relevant safety data as the study proceeds.

21.2 Electronic Case Report Forms (eCRFs)

21.2.1 Data Recorded Directly on Case Report Forms

An electronic Case Report Form (eCRF) will be used for this study. Data will be recorded directly onto source documents before documentation in the eCRF.

21.2.2 eCRF entries

eCRF entries and corrections will only be performed by study site staff authorized by the Investigator. Each user is informed of the clinical study's web-site internet address and is allocated to a user account with personal password to access the confidential website. The personal password must be kept confidential and must only be used by the person to whom it was assigned. For additional authorized users at the site, a new user account has to be requested to ensure that each entry/change can be allocated to the person who performed the entry/change.

All visit data need to be recorded in the eCRF database as soon as possible after each study visit, no later than 1 business day after data has been collected.

21.2.3 Changes to eCRF data

Corrections may be requested as follows:

- Investigators' responses are checked as they are entered and are rejected if they do not fulfill quality criteria. A message will specify the type of error or syntax error and assist in its correction.
- If required, the CRA can ask for information to be corrected during monitoring.

- Computerized data-check programs and manual checks will identify clinical data discrepancies for resolution. Corresponding queries will be created within the data capturing system and the site will be informed about new issues to be resolved on-line.

All discrepancies will be solved on-line directly by the Investigator or by authorized staff.

Corrections of eCRF data may be performed by authorized staff only. The person performing the changes in the eCRF is required to electronically confirm the changes made.

21.2.4 eCRF Entry Validation

The Investigator will thoroughly review the data on the eCRF, and will finally certify the contents of the eCRF by electronic signature after completion of each subject. If a correction is made to the eCRF data after the Investigator's final approval, the certification must be repeated after the changes have been performed.

21.2.5 Data collection

All visits and assessments are entered into an interactive form. eCRFs will be source document verified following guidelines established before study onset and detailed in the Monitoring Plan. Maintenance of the study database will be performed. Details to eCRF handling are provided in a study specific eCRF manual.

21.3 Coding of Adverse Events, Drugs and Diseases

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 study 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.

21.4 Investigator File

21.4.1 Maintenance

The Investigator will maintain complete and accurate study documentation in a separate file (i.e. Investigator File) provided during the initiation visit. The Investigator is responsible for maintaining complete, up to date and accurate study records to enable the conduct of the study to be fully documented. The records should include the clinical protocol as well as any amendments, study approval letters, all original ICFs, drug dispensing and accountability logs and all relevant correspondence pertaining to the study.

21.4.2 Archiving and Destruction

All study-related documents should be kept by the Investigator for the maximum period of time required by local regulations. No study document should be destroyed without prior written agreement between the Investigator and the Sponsor. Should the Investigator elect to assign the study documents to another party, or move them to another location, the Sponsor must be notified.

21.4.3 Provision of Additional Information

On request, the Investigator will supply the Sponsor with additional data relating to the study or copies of relevant source records, duly anonymized. In case of particular issues or governmental queries, it is also necessary to have access to the complete study records, provided that the subject's confidentiality is protected in accordance with applicable regulations.

22. PUBLICATION POLICY

All results generated in this study will be considered to be strictly confidential. The Investigator may not submit the results for publication or presentation without prior written permission of the Sponsor. Authorship for any publication will be determined in mutual agreement. Within the scope of publication, co-authorship may be offered, at the sole discretion of the Sponsor, on a case-by-case basis taking scientific contribution into consideration. This is according to uniform requirements for manuscripts submitted to biomedical journals proposed by the International Committee of Medical Journal Editors.

23. LIABILITIES AND INSURANCE

In case of any damage or injury occurring to a subject in association with the participation in the study, insurance has been contracted.

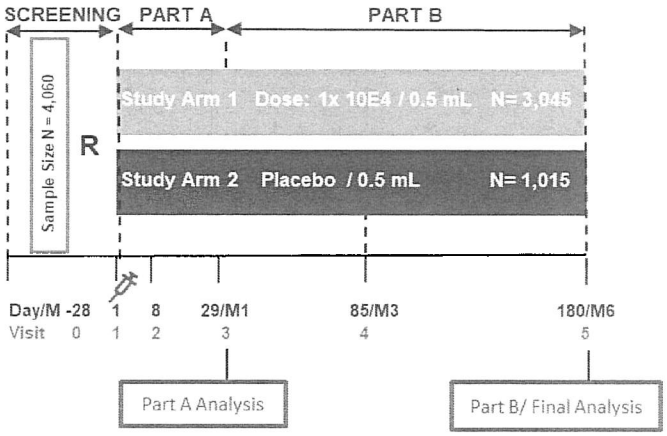
The name, address and the insurance policy number will be given to both the Investigator prior to enrollment. Moreover a copy of the insurance conditions will be filed on site.

The Investigator is responsible for dispensing the investigational product according to this protocol, and for its secure storage and safe handling throughout the study.

24. SUPPLEMENTS

24.1 Study Flow Chart

Figure 24.1-1
Study Design for VLA1553 Clinical Study VLA1553-301



24.2 Schedule of Study Procedures and Assessments – Parts A and B

Table 24.2-1
Schedule of Study Procedures and Assessments

		Part A			Part B		ET
Procedures/Assessments	**Visit 0 Screening** (Day -28 to Day 0)	**Visit 1** [m] Day 1	**Visit 2** Day 8 (+/- 1d)	**Visit 3** Day 29 M1 (+/- 4d)	**Visit 4** Day 85 M3 (+/- 7d)	**Visit 5** Day 180 M6 (+/- 14d)	**Visit ET**
Informed consent [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
Physical examination and Hand Stiffness Test [d]	X	X	X	X	X	X	X
Vital signs [e]	X	X					
Baseline sample [f] [10.0 mL]		X					
Serum/Urine Pregnancy test [g] [3.0 mL]	X	X		X	X	X	X
Immunogenicity [h] [40.0 mL]		X	X	X	X	X	X
Safety Sample [i] [18.0 mL]	X						
Viremia [j] [8.0 mL]		X	X	X			X[k]
Clinical Sample [l] [13.0 mL]		X	X	X	X	X	X
VACCINATION		**X**					
Subject eDiary		I	R				R
eMemory Aid			I	R	R		R
AE/AESI[n]/SAE Assessment		X	X	X	X	X	X

I.....instruct; R.....review

[a] Occurs at enrollment before Screening.
[b] Demographics include year of birth, height, weight, BMI, gender, race and ethnicity.
[c] Symptoms noted at Visit 1 (prior to vaccination) are not considered AEs, but will be recorded as medical history. Prior vaccination against relevant traveler diseases should be documented in the Medical History, e.g. YF and JEV.
[d] At the screening visit, 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. At subsequent visits, a symptom-driven physical examination will be performed, i.e. only in case symptom is reported by the subject, a symptom-based assessment of the affected body system(s) will be performed. A hand stiffness and mobility examination will be performed at all study visits irrespective of prior symptoms.
[e] Vital signs will include systolic and diastolic blood pressure and pulse rate while seated and at rest, and body temperature measured orally before vaccination. In addition, after an observation period of 30 minutes following vaccination pulse rate as well as blood pressure while seated and at rest will again be assessed.
[f] A baseline sample from ALL subjects will be drawn for potential retrospective investigation of pre-existing antibodies including but not limited to e.g. a panel of alphaviruses (i.e. CHIKV, Mayaro) or dengue and Zika [blood (for all tests): 10.0 mL].
[g] A **serum** pregnancy test will be performed for all female subjects of childbearing potential at the screening visit only and a **urine** pregnancy test will be done prior to vaccination at Visit 1 and at indicated visits [blood: 3.0 mL].
[h] Blood draw [40.0 mL] from ALL subjects for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis if deemed necessary by the Data Safety Monitoring Board upon clinical indication or on demand if requested later by regulatory authorities.
[i] Baseline safety laboratory sample obtained from ALL subjects for standard **clinical chemistry** (i.e. creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP), **hematology** (i.e. Hemoglobin, hematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets, erythrocyte sedimentation rate (ESR), **coagulation panel** (i.e. Prothrombin time, aPTT, fibrinogen) and **urinalysis** (i.e. pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) [EDTA blood: 13.0 mL]. A positive HIV test obtained by ELISA will have to be confirmed by a second method (e.g. Western blot or PCR) [blood (for all tests): 5.0 mL].
[j] Viremia plasma sample obtained from ALL subjects for clinically indicated **retrospective** investigation of viremia by RT-qPCR. The retrospective analysis will be conducted if deemed necessary by the Data Safety Monitoring Board upon clinical indication.
[k] Only if the ET visit occurs prior to Day 29, a viremia plasma sample should be obtained from the subject for clinically indicated **retrospective** investigation of viremia by RT-qPCR.
[l] Clinical sample obtained from subjects in the **immunogenicity subset ONLY** for standard **clinical chemistry** (i.e. creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP), **hematology** (i.e. Hemoglobin, hematocrit, erythrocyte count, white blood count, differential white blood cell count, platelets, erythrocyte sedimentation rate (ESR), **coagulation panel** (i.e. Prothrombin time, aPTT, fibrinogen) and **urinalysis** (i.e. pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes) [EDTA blood: 13.0 mL].
[m] All procedures/assessments (apart from Subject eQuestionnaire distribution and AE assessment) occur prior to vaccination (unless stated otherwise).
[n] Assessment of AESIs 2 to 21 days post-vaccination only.

24.3 Toxicity Grading Scale for Abnormal Laboratory Assessments

	Mild (Grade 1)[1]	Moderate (Grade 2)	Severe (Grade 3)	Potentially life threatening (Grade 4)[2,6]
Hematology Parameters				
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	<8.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	<8.5
Hematocrit	Outside normal range[3]			
Erythrocyte count	Outside normal range[3]			
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25,000	>25,000
WBC Decrease - cell/mm^3	2,500 – 3,500[4]	1,500 – 2,499	1,000 – 1,499	<1,000
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	<500
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	<250
Monocytes	Outside normal range[3]			
Eosinophils - cell/mm^3	650 – 1500[4]	1501 - 5000	> 5000	Hyper-eosinophilic
Basophils	Outside normal range[3]			
Platelets Decreased - cell/mm^3	125,000 – 140,000[4]	100,000 – 124,000	25,000 – 99,000	<25,000
ESR	Outside normal range[3]			
Clinical Chemistry Parameters				
Creatinine – mg/dL	1.5 – 1.7[4]	1.8 – 2.0	2.1 – 2.5	>2.5 or requires dialysis
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	<125
Sodium – Hypernatremia mEq/L	144 – 145[4]	146 – 147	148 – 150	>150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2[4]	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6[4]	3.3 – 3.4	3.1 – 3.2	<3.1
Calcium – Hypocalcemia mg/dL	8.0 – 8.4[4]	7.5 – 7.9	7.0 – 7.4	<7.0
Calcium – Hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	>12.0
AST – increase by factor	1.1 – 2.5 x ULN[5]	2.6 – 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN
ALT – increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN
Alkaline phosphatase –	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	>10 x ULN

	Mild (Grade 1)[1]	Moderate (Grade 2)	Severe (Grade 3)	Potentially life threatening (Grade 4)[2,6]
increase by factor				
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	>1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
CRP	Outside normal range[3]			
Coagulation Factors				
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN[5]	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT (aPTT) – increase by factor (activated partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500[4]	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200[4]	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

[1] In case the laboratory's normal ranges and absolute Grade 1 limits overlap, Grade 1 limits will prevail, i.e. the value will be classified as Grade 1 abnormality even if it is within central laboratory normal ranges. Values between the central laboratory normal ranges and absolute Grade 1 limits will be reported as no abnormality (Grade 0).

[2] The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia unsolicited AE if the subject had a new seizure associated with the low sodium value.

[3] As neither the FDA Scale nor the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (December 2004) provide any grading for Hematocrit, Erythrocyte count, Monocytes, Basophils, ESR and CRP, these will only be analyzed as "outside normal range", as determined by central laboratory standards and graded as described in Section 17.2.1.1 upon investigator's judgement.

[4] Central laboratory values should be adjusted to FDA toxicity grading scale. Specifically, if central laboratory reference range is more stringent than FDA toxicity grading scale the central laboratory values should be reported as no abnormality (Grade 0). Similarly, if laboratory values are within the central laboratory normal reference range, but fall into FDA toxicity grading scale, the values should be reported as indicated by the FDA toxicity grading scale.

[5] "ULN" is the upper limit of the normal range

[6] Any grade 4 abnormal laboratory value should be reported as an SAE (see Section 16.1.2).

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