valneva

Clinical Study Protocol

DRUG SUBSTANCE(S)	VLA1553
VERSION NO.	Version 4.0
STUDY CODE	VLA1553-303
DATE	12-Jun-2023

AN OPEN-LABEL, SINGLE ARM STUDY TO EVALUATE ANTIBODY PERSISTENCE AND LONG TERM SAFETY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADULTS AGED 18 YEARS AND ABOVE

Phase 3 Study

PROTOCOL NUMBER: **VLA1553-303**

IND NUMBER: **17854**

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

1. PROTOCOL SIGNATURE PAGE

Title of Clinical Trial: **AN OPEN-LABEL, SINGLE ARM STUDY TO EVALUATE ANTIBODY PERSISTENCE AND LONG TERM SAFETY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADULTS AGED 18 YEARS AND ABOVE**

Protocol Number: **VLA1553-303**

IND Number: **17854**

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]

[redacted]

2. STUDY PERSONNEL

[redacted]

Sponsor's Medical / Safety Officer

[redacted]

Study Medical Monitor

[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).

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:

4. 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: performing phone call visits to capture safety information and 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.

5. CLINICAL STUDY SYNOPSIS

INVESTIGATIONAL PRODUCT, DOSAGE AND MODE OF ADMINISTRATION	
Title	**AN OPEN-LABEL, SINGLE ARM STUDY TO EVALUATE ANTIBODY PERSISTENCE AND LONG TERM SAFETY OF A LIVE-ATTENUATED CHIKUNGUNYA VIRUS VACCINE CANDIDATE (VLA1553) IN ADULTS AGED 18 YEARS AND ABOVE**
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 genetic deletion in the nsP3 gene (del5nsP3) encoding the non-structural replicase complex protein nsP3, which leads to attenuation of the virus *in vivo*.

In this open-label Phase 3b, single arm study, persistence of antibodies and long-term safety i.e. SAEs will be evaluated in subjects recruited from the VLA1553-301 study. Up to 375 subjects vaccinated with VLA1553 as part of study VLA1553-301 will participate in VLA1553-303. These subjects will have annual follow-up visits at Months 12, 24, 36, 48 and 60 after immunization within this study.

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 three 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.

Two Phase 3 studies have been completed with the CHIKV vaccine candidate VLA1553. The pivotal Phase 3 study VLA1553-301 was a multi-center, randomized, placebo-controlled trial conducted in the United States (U.S.). More than 4,000 healthy adults, aged 18 years and older, were randomized 3:1 to receive a single vaccination of VLA1553 or a saline control. Recruitment was stratified by age and the first 462 participants were selected as the immunogenicity subset. Vaccinations took place on day 1 and the primary endpoint was evaluated at day 29. The primary endpoint of the pivotal Phase 3 study was met: VLA1553 induced seroresponse (defined as $\mu PRNT_{50}$ ≥150 for baseline negative subjects) in 98.9% of participants (263 of 266 participants from the per protocol subset tested for immunogenicity, 95%CI: 96.7-99.8) after a single vaccination with the lower bound of the confidence interval exceeding the FDA non-acceptance threshold of 70%. Furthermore, seroresponse rates (SRR) over time demonstrated sustained benefits after a single vaccination of VLA1553. Participants were followed through Month 6 in the pivotal trial. The SRR stayed well above the seroprotective level of antibodies in 96.3% of participants until Day 180 (233 of 242 participants in the per protocol subset tested for immunogenicity). The vaccine was also confirmed to be highly immunogenic in older adults (≥65 years), who achieved equally high SRR and neutralizing antibody titers as younger adults (<65 years).
The safety profile was consistent with previous results across all age groups (Day 29 analysis, Lot-to-lot consistency study (Day 29 analysis), preceding Phase 1 clinical study) and comparable with other vaccines.
Also the Phase 3 Lot-to-Lot consistency study VLA1553-302 met the primary endpoint of Lot-to-Lot consistency: In the PP population, the CIs on the Geometric Mean Titer (GMT) ratios of all three lots were in the defined acceptance margins of 0.67 and 1.5.

CLINICAL CONDITION(S)/INDICATION(S)	
Active immunization for the prevention of disease caused by CHIKV	
STUDY PHASE	Phase 3b
PLANNED STUDY PERIOD	
Initiation	Q2 / 2021
Duration	The overall study duration (First Subject In – Last Subject Out) is estimated to be approximately 57 months. Individual subject participation is approximately 54 months from enrollment to study completion unless prematurely discontinued.

Completion	Part A (Visit 1, Year 1): planned Q4 / 2021 Part B (Visit 2, Year 2): planned Q4 / 2022 Part C (Visit 3, Year 3): planned Q4 / 2023 Part D (Visit 4, Year 4): planned Q4 / 2024 Part E (Visit 5, Year 5): planned Q4 / 2025 Individual study parts will be analyzed sequentially. For each study Part A – D, a Clinical Study Report will be written based on the respective statistical analysis integrating previous study periods. For Part E, the final report including all data from preceding Parts will be prepared after completion of the study. Part E final report will be submitted for BLA filing.

STUDY OBJECTIVES

Primary Objective

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

Secondary Objective

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

STUDY DESIGN

Investigator and sites	Multicenter study. The study will be conducted at approximately 13 study sites throughout the U.S.
Study participants	A total of up to 375 male and female subjects in study VLA1553-301 having received VLA1553 will participate in this study.
Study Type	Long-term Immunogenicity and Safety
Vaccine Type	Live-attenuated CHIKV in a lyophilized formulation (VLA1553) Vaccine was administered during the primary study VLA1553-301
Control Type	None
Study Indication Type	Prevention
Blinding Scheme	Open-label, single arm

Study Design

This is a prospective, multicenter, open-label Phase 3b, single arm clinical study evaluating the persistence of antibodies and long-term safety i.e. SAEs in subjects recruited from the VLA1553-301 study. In this precursor study 4,060 male and female subjects aged 18 years and above were randomized in a ratio 3:1 to either the final dose of VLA1553 or placebo. The first enrolled and randomized approximately 500 subjects constituted the initial group of subjects, i.e. the

immunogenicity subset of study VLA1553-301, that will be approached to join VLA1553-303. Thereafter, additional subjects will be approached in a predefined manner (see Section 7.4.1) to achieve a more robust number of participants in study VLA1553-303. Overall, up to 375 subjects vaccinated with VLA1553 as part of study VLA1553-301 will be recruited into the study and stratified into two age strata of subjects aged 18 to 64 years (Stratum A) and subjects of 65 years of age or above (Stratum B). This subject population will be enrolled at approximately 13 pre-selected study sites across the U.S. representative of the whole study population.

All subjects will be asked to return to the study site for Visit 0 of VLA1553-303, followed by Year 1 (Visit 1), Year 2 (Visit 2), Year 3 (Visit 3), Year 4 (Visit 4) and Year 5 (Visit 5) for immunogenicity sampling. Visit 0 may be performed as a visit combined with Visit 5 of study VLA1553-301, or will be performed as a separate study visit, if participants already had their VLA1553-301 Visit 5 before joining study VLA1553-303. If the individual titer drops to a $\mu PRNT_{50}$ ≤40 at a specific timepoint, the respective subject will have an end of study visit at the next scheduled visit. SAEs will also be assessed up until Year 2 in all subjects. Moreover, any AESIs reported to be ongoing at the point in time the subject rolls over to study VLA1553-303 will be monitored. The clinical study design is displayed in **Figure 1** below.

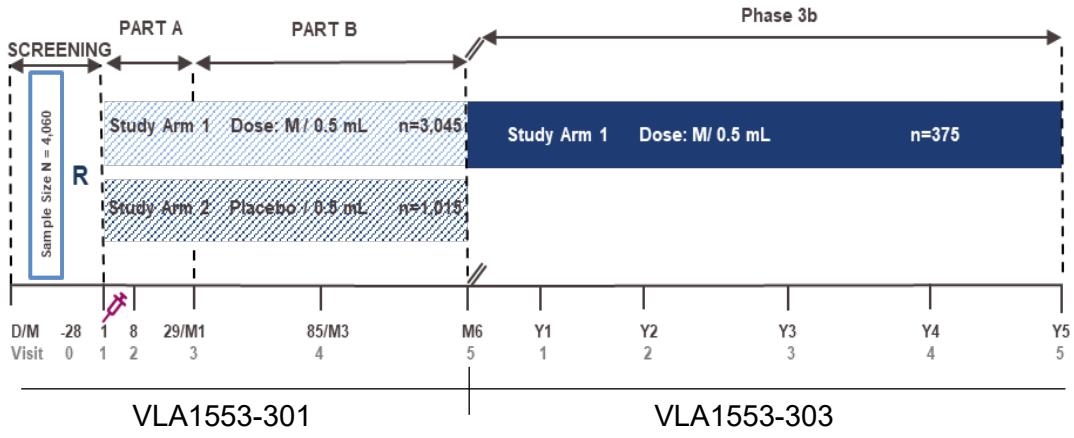


Figure 1 Open-label Phase 3b Study Design.

STUDY ENDPOINTS

Primary Endpoint

- Proportion of subjects with a seroprotective CHIKV antibody level defined as $\mu PRNT_{50}$ ≥150 at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination.

Secondary Endpoints

Safety

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

Immunogenicity

- Immune response as measured by CHIKV-specific neutralizing antibody titers at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as determined by µPRNT assay;
- Proportion of subjects with seroconversion[i] at Year 1, Year 2, Year 3, Year 4 and Year 5 as determined by µPRNT assay;
- Fold increase of CHIKV-specific neutralizing antibody titers determined by µPRNT assay at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as compared to baseline antibody titers at start of study VLA1553-301;
- Proportion of subjects reaching an at least 4-fold, 8-fold, 16-fold or 64-fold increase in CHIKV-specific neutralizing antibody titer compared to baseline as measured by µPRNT assay.

[i] Seroconversion defined as >4-fold increase of µPRNT50 compared to baseline (Day 1); for baseline negative subjects and baseline positive subjects (defined as µPRNT50 >40)

CRITERIA FOR INCLUSION / EXCLUSION

Up to 375 adults of either sex who satisfy the inclusion and exclusion criteria listed below will participate in the study.

Inclusion Criteria

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

1. Subject participated in the VLA1553-301 clinical study;
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 had immunogenicity blood samples taken at baseline (Visit 1) and either Day 29 (Visit 3), Day 85 (Visit 4) or Day 180 (Visit 5) in Study VLA1553-301 and was negative for neutralizing antibodies at baseline.

Exclusion Criteria

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

1. Subject presents with clinical conditions representing a contraindication to blood draws;
2. Subject has donated blood or use of blood products prior 30 days of immunogenicity sampling;
3. Subject has received an active drug with potential immunosuppressive action or investigational drug or device within a period of 30 days prior a study visit
4. Subject has a known or suspected problem with alcohol or drug abuse as determined by the Investigator;
5. Subject has any condition that, in the opinion of the Investigator, may compromise the subject's well-being, might interfere with evaluation of study endpoints, or would limit the subject's ability to complete the study;
6. Subject is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
7. 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.

STATISTICAL ANALYSIS

Sample Size Justification

No formal sample size calculation was performed. Up to 375 subjects of study VLA1553-301 will

participate in this study.

However, with a sample size of 375, two-sided exact (Clopper-Pearson) 95%-confidence intervals for an actual SRR of 60% will have a width of 10.2%. Confidence intervals for an SRR of 80% will have a width of 8.3%.

Statistical Methods

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

All analyses of immunogenicity data will be performed primarily on the Per-Protocol population and secondarily on the whole sample population.

The primary immunogenicity analysis will analyze the observed proportion of subjects with seroresponse (defined as $\mu PRNT_{50}$ ≥150 for baseline negative subjects) over time, accompanied by two-sided exact (Clopper-Pearson) 95% confidence limits.

Secondary immunogenicity analysis will include seroconversion rates by study years, accompanied by 95% confidence intervals. Immunogenicity analyses will also be generated stratified by age stratum.

The kinetics of long-term antibody titers will be evaluated by summary statistics of the GMTs. Annual decline rate of antibody titers will be estimated from a mixed model with repeated measures.

All subjects entered into the study, who had received a single vaccination in VLA1553-301, will be included in the safety analysis.

The number and percentage of subjects with any SAEs and any related SAEs up until Year 2 will be presented for each age stratum, overall and by system organ class/preferred term. AESI follow-up will be provided as narratives.

Data Analysis

The following data analyses will be performed:

- Part A includes immunogenicity and safety data after all subjects have completed Visit 1 (Year 1);
- Part B includes immunogenicity and safety data after all subjects have completed Visit 2 (Year 2);
- Part C includes immunogenicity data after all subjects have completed Visit 3 (Year 3);
- Part D includes immunogenicity data after all subjects have completed Visit 4 (Year 4);
- Part E includes immunogenicity data after all subjects have completed Visit 5 (Year 5).

Individual study parts will be analyzed sequentially.

Final Report

For each study Part A – D, a Clinical Study Report will be written based on the respective statistical analysis integrating previous study periods. For Part E, the final report including all data from preceding Parts will be prepared after completion of the study. Part E final report will be submitted for BLA filing.

Contents

6. LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
C	capsid protein
CHIKV	chikungunya virus
CI	Confidence interval
CRA	Clinical research associate
CRO	Contract Research Organization
CSR	Clinical Study Report
DSMB	Data and Safety Monitoring Board
ECSA	East/ Central/ South African
eCRF	electronic Case Report Form
e.g.	for example
FDA	Food and Drug Administration
GMT	Geometric mean titer
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
i.e.	that is
i.m.	intramuscularly
IMP	Investigational medicinal product
IRB	Intitutional Review Board
kDa	kilo Dalton
MedDRA	Medical Dictionary for Regulatory Activities
µL	microliter
mL	Milliliter(s)
n.a.	not applicable
NT	Neutralizing Titer
NT50	Antibody titer at 50% virus neutralization determined by µNT assay
pH	Potential of hydrogen
PP	Per-protocol population
µPRNT	Micro Plaque Reduction Neutralization Test
SAE	Serious Adverse Event
SRR	Seroresponse Rate

7. INTRODUCTION

7.1 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 large genetic 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.

7.2 Clinical Condition/Indication

7.2.1 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.

7.2.2 Prospects for Vaccine Development

A number of CHIKV vaccine candidates are 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 intramuscularly (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, then Emergent BioSolutions, now Bavarian Nordic, developed a Virus-like particle (VLP) vaccine for prophylaxis of CHIKV. First-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.

7.3 Findings from Nonclinical and Clinical Studies

7.3.1 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 dose 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;
- 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.

An overview of further non-clinical studies can be found in **Table 1** below.

Table 1. 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 (threshold titer of 3.875 log10 CCID50/mL).
Passive transfer in NHPs using human serum from VLA1553-101	NHPs *(Cynomolgus macaques)*	After vaccination with VLA1553 a neutralizing antibody titer of ≥50 determined by $\mu PRNT_{50}$ is proposed as a titer reasonably likely to predict protection.

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

7.3.2 Clinical Summary

In a Phase 1 clinical trial (study code: VLA1553-101), three dose levels of VLA1553 were administered i.m. in the deltoid muscle as a single dose immunization. As primary outcome safety and as secondary outcomes immunogenicity and antibody persistence were investigated. 120 healthy volunteers aged 18 to 45 years were randomly assigned 1:1:2 to one of three escalating dose groups (Group L, low 3.2x10E3/0.1mL; Group M, medium 3.2x10E4 /1mL or Group H, high dose of 3.2x10E5 TCID50/1mL) and received a single dose immunization on Day 0. Half of individuals in all Groups H were re-vaccinated with the highest dose on either Month 6 or 12 and followed up for 28 days post re-vaccination.

The vaccine was generally safe in all and well tolerated in the low and medium dose group. VLA1553 showed an excellent immunogenicity profile in all dose groups after a single vaccination. 100% seroconversion (defined as the proportion of participants achieving a CHIKV-specific neutralizing antibody titer of NT50 ≥20) was achieved at Day 14 after a single vaccination in all dose groups and sustained at 100% until Month 12. The absence of an anamnestic response following 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. Even at the Month 12 re-vaccination („intrinsic human viral challenge“) vaccinees were protected from vaccine induced viremia and associated clinical symptoms as early indication of VLA1553‘s efficacy at all dose levels. Based on a superior safety along an improved immunogenicity profile in terms of antibody kinetics the medium dose (3.2x10E4 TCID50/1 mL) was selected for further development (Wressnigg et al., 2020).

A pivotal Phase 3 trial (study code: VLA1553-301) was performed across 43 U.S. sites (study code: VLA1553-301). The final dose of VLA1553 (target 1x 10E4 TCID50/ 0.5 mL) or placebo as control was administered i.m. into the deltoid muscle as a single dose immunization on Day 1 to 4,115 adults, aged 18 years and above. The trial met its primary endpoint inducing seroresponse (defined as $\mu PRNT_{50}$ ≥150 for baseline negative subjects) in 98.9% of participants 28 days after receiving a single dose (263 of 266 participants from the per protocol subset tested for immunogenicity, 95%CI: 96.7-99.8). The seroresponse rate (SRR) of 98.9% exceeded the 70% threshold (for non-acceptance) agreed with the US FDA. The SRR remained high, with 241/246 (98.0%) and 233/242 (96.3%) seroprotected participants in the VLA1553 arm on Day 85 and Day 180, respectively. The seroprotective titer was agreed with FDA to serve as a surrogate of protection and this corresponds now to the term seroresponse rate.

VLA1553 was generally well tolerated among the 3,082 participants evaluated for safety in study VLA1553-301. An independent Data Safety Monitoring Board (DSMB) continuously monitored the study and identified no safety concerns. The safety profile was consistent with previous results across all age groups (Lot to lot consistency study, VLA1553-302, described below and preceding Phase 1 clinical study) and comparable with other vaccines. The majority of solicited AEs were mild or moderate and resolved within 3 days; 2.1% of participants reported severe solicited systemic AEs (most commonly fever), thereof 1.9% were assessed as treatment-related by the investigators. Approximately 50% of participants experienced solicited systemic AEs; headache, fatigue and myalgia were most common (seen in more than 20% of participants) after vaccination with VLA1553. Approximately 15% of participants experienced solicited injection site AEs after having received VLA1553. Two SAEs considered related to VLA1553 by the assessing investigators were reported during the entire study period; one case of myalgia and one case of syndrome of inappropriate antidiuretic hormone secretion (sponsor assessment: hypovolemic hyponatremia); both participants completely recovered. In total 11 participants reported events meeting the criteria of adverse event of special interest, 10 participants received VLA1553. Most of the events were mild or moderate, 5 participants had severe fever. The majority of events were self-limited after 2 to 4 days.

The above described immunogenicity and safety profile was mirrored in the pivotal lot-to-lot consistency study (study code: VLA1553-302), where 409 healthy adults were block randomized 1:1:1 to receive one of three manufacturing lots of VLA1553. The primary endpoint was met as the Confidence Intervals (CIs) of the GMT ratios of all lots were within the defined acceptance margins of 0.67 and 1.5. The antibody titer, determined by µPRNT, peaked at Day 29 with a GMT of 2,643.2; titers subsequently decreased to 846.1 at Day 85 and 708.8 at Day 180. The SRR was 97.8% (348/356) on Day 29 and remained high, with 321/330 (97.3%) and 316/329 (96.0%) seroprotected participants on Day 85 and Day 180, respectively. No significant difference between the lots was observed. Moreover, seroconversion rate, defined as a µPRNT50 titer of ≥20 in participants seronegative for CHIKV at baseline (Day 1), was achieved in 97.5% (353/362) on Day 29 and remained stable throughout the study, with 327/335 (97.6%) participants maintaining seroconversion on Day 180.

With regards to safety, after administration of VLA1553 there were no significant differences in any AE occurrences between lots. The majority of AEs at Day 180 were solicited AEs (61%); overall 236 participants (57.8%) reported solicited AEs assessed to be related to VLA1553 by the Investigator. Furthermore, there were no significant differences in solicited AE occurrences between lots. Overall, AEs were mostly mild or moderate; 16 participants of 408 (3.9%) experienced 22 severe AEs (8 unsolicited events, 14 solicited events) at Day 180. Five SAEs were reported until Day 180 (met seriousness criterion of hospitalization or medically-important event); all cases were not related (two cases of acute appendicitis, one case of acute cholecystitis and two cases of miscarriage). Finally, in total one participant reported events meeting the criteria of adverse event of special interest: mild arthralgia (6 days) and severe fever (3 days), the events were self-limited.

7.4 VLA1553-303 Study Rationale and Justification of Study Design

To elucidate the long-term safety i.e. SAEs and persistence of antibodies in subjects, the VLA1553-303 study builds on the data from VLA1553-301 and is designed to evaluate the persistence of antibodies and long-term safety i.e. SAEs in up to 375 subjects who participated in the VLA1553-301 study. All subjects will be asked to return to the study site at Month 6 (Visit 0), Year 1 (Visit 1), Year 2 (Visit 2), Year 3 (Visit 3), Year 4 (Visit 4) and Year 5 (Visit 5) for immunogenicity sampling. SAEs will also be assessed until Year 2 of the study.

7.4.1 Selection of Study Population and justification of study population size

In the precursor study VLA1553-301, approximately 4,060 male and female subjects aged 18 years and above were randomized in a ratio 3:1 to either the final dose of VLA1553 or

placebo. The first enrolled and randomized approximately 500 subjects constituted the initial group of subjects (i.e. the immunogenicity subset of study VLA1553-301 recruited at approximately 13 pre-selected sites across the U.S.) that will be approached to join VLA1553-303. Thereafter, additional subjects who were recruited by the same approximately 13 sites will be approached in a predefined manner to achieve a more robust number of participants in study VLA1553-303: At first participants aged ≥65 years of age will be asked to join study VLA1553-303 by way of ascending subject IDs to ensure adequate representation of this age group among the study participants, followed by subjects aged 18 to 64 years of age who will also be invited to participate in study VLA1553-303 in ascending subject ID order on a study site level. For those additional subjects, who are not part of the VLA1553-301 immunogenicity subset, immunogenicity samples were collected but not tested during the VLA1553-301. Hence no immunogenicity results from the precursor study are available at the time they will be approached. No selection bias to favor convenient subjects is possible for the described approach. Those 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. The subjects will be stratified into two age strata of subjects aged 18 to 64 years (Stratum A) and subjects of 65 years of age or above (Stratum B). No formal sample size calculation was performed. However, up to 375 subjects who received VLA-1553 in the study VLA1553-301 will be recruited to this study. With a sample size of 375, two-sided exact (Clopper-Pearson) 95%-confidence intervals for an actual SRR of 60% will have a width of 10.2%. Confidence intervals for an SRR of 80% will have a width of 8.3%.

7.4.2 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 (7,000 to 10,000 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, titer range 82-155 $\mu PRNT_{50}$). 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 10 and 155 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 52.

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 the above summarized experiment, a neutralizing antibody titer of ≥50 determined by $\mu PRNT_{50}$ was proposed as a titer reasonably likely to predict protection. Finally, a seroprotective titer ($\mu PRNT_{50}$ antibody titer ≥150, i.e., seroresponse) was agreed with the US FDA to serve as a surrogate of protection in the Phase 3 studies.

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

7.5.1 Possible Benefits for Subject

Following vaccination with Valneva's VLA1553 vaccine, subjects may have acquired immunity to CHIKV as indicated by antibody levels above a pre-defined threshold indicative of protection. During their participation in this study, the subjects' antibody persistence will be monitored.

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, subjects planning to travel to CHIKV endemic areas should be advised 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.

7.5.2 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 VLA1553-303 phase 3 study, the subjects will contribute to the development of a novel vaccine against chikungunya. This VLA1553-303 study is the long-term follow-up of the pivotal study VLA1553-301 within a complete clinical development program. The pivotal study VLA1553-301 is intended to lead to subsequent licensure of the VLA1553 vaccine, if it is shown to be safe and efficacious.

7.5.3 Possible Risks / Inconveniences for the Subject

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.

For further details on known and potential risks of the investigational product (administered in predecessor study VLA1553-301), please refer to the Investigator's Brochure.

7.6 COVID-19 Management

The COVID-19 pandemic may impact the conduct of this clinical study. Therefore, the study Sponsor will continuously monitor and evaluate the development of the COVID-19 pandemic in the area of the study sites.

To protect the subject's safety, welfare, and rights, the study Sponsor will continuously evaluate specific circumstances, such as the ability to conduct appropriate safety monitoring.

Depending on the local situation, subjects may not be able to come to the site for protocol-specified visits. Therefore, existing processes will be modified or new processes will be implemented. Such measures may include; performing phone call visits to capture safety information and employing mobile teams to collect serum samples. In all cases, the subject and/or the legal representative(s) will be informed. Changes in study visit schedules, missed visits or subject discontinuations due to COVID-19 will be recorded. In addition, the reason for the implementation of any contingency measures will be documented.

Moreover, the Sponsor will communicate any measures or modifications of the study protocol to the relevant CA and IRB in line with local requirements.

The COVID-19 measures are based on the FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency (as of 02-July-2020) and will be adjusted, if needed. WHO declared an end to COVID-19 as a public health emergency in May 2023.

8. STUDY PURPOSE AND OBJECTIVES

8.1 Study Purpose

To evaluate antibody persistence and long-term safety after single immunization of a live-attenuated chikungunya virus vaccine candidate (VLA1553) in adults aged 18 years and above.

8.2 Primary Objective

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

8.3 Secondary Objective

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

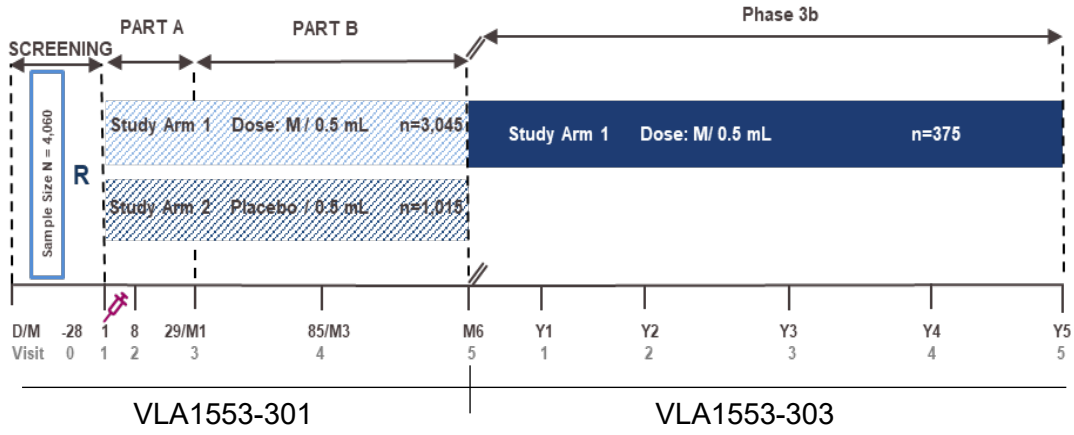
9. STUDY DESIGN

9.1 Overall Study Design

This is a prospective, multicenter, open-label Phase 3b, single arm clinical study evaluating the persistence of antibodies and long-term safety i.e. SAEs in up to 375 subjects in study VLA1553-301. In this precursor study VLA1553-301, 4,060 male and female subjects aged 18 years and above were randomized in a ratio 3:1 to either the final dose of VLA1553 or placebo. Up to 375 subjects vaccinated with VLA1553 in study VLA1553-301 will be recruited into the study and stratified into two age strata of subjects aged 18 to 64 years (Stratum A) and subjects of 65 years of age or above (Stratum B).

All subjects will be asked to return to the study site at Month 6 VLA1553-301 (Visit 0 of VLA1553-303), followed by Year 1 (Visit 1), Year 2 (Visit 2), Year 3 (Visit 3), Year 4 (Visit 4) and Year 5 (Visit 5) for immunogenicity sampling. If the individual titer drops to a $\mu PRNT_{50}$ ≤40 at a specific timepoint, the respective subject will have an end of study visit at the next scheduled visit. SAEs will also be assessed up until Year 2 in all subjects. Moreover, any AESIs reported to be ongoing at the point in time the subject rolls over to study VLA1553-303 will be monitored. The clinical study design is displayed in **Figure 2**.

Figure 2 Open-label Phase 3b Study Design



9.2 Study Duration

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

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

9.3 Study Endpoints

9.3.1 Primary Endpoint

Proportion of subjects with a seroprotective CHIKV antibody level defined as $\mu PRNT_{50}$ ≥150 at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination.

9.3.2 Secondary Endpoints

Safety

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

Immunogenicity

- Immune response as measured by CHIKV-specific neutralizing antibody titers at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as determined by µPRNT assay;
- Proportion of subjects with seroconversion[ii] at Year 1, Year 2, Year 3, Year 4 and Year 5 as determined by µPRNT assay;
- Fold increase of CHIKV-specific neutralizing antibody titers determined by µPRNT assay at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination as compared to antibody titers at start of study VLA1553-301;
- Proportion of subjects reaching an at least 4-fold, 8-fold, 16-fold or 64-fold increase in CHIKV-specific neutralizing antibody titer compared to baseline as measured by µPRNT assay.

[ii] Seroconversion defined as >4-fold increase of µPRNT50 compared to baseline (Day 1); for baseline negative subjects and baseline positive subjects (defined as µPRNT50 >40).

9.4 Study Termination - Study Stopping Rules

The study may be paused or prematurely terminated, after consultation with the DSMB. 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.

10. SUBJECT SELECTION, WITHDRAWAL AND DISCONTINUATION

Approximately 375 adults of either gender who satisfy the inclusion or exclusion criteria listed below will participate in the study.

10.1 Inclusion Criteria

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

1. Subject participated in the VLA1553-301 clinical study;

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 had immunogenicity blood samples taken at baseline (Visit 1) and either Day 29 (Visit 3), Day 85 (Visit 4) or Day 180 (Visit 5) in Study VLA1553-301 and was negative for neutralizing antibodies at baseline.

10.2 Exclusion Criteria

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

1. Subject presents with clinical conditions representing a contraindication to blood draws;

2. Subject has donated blood or use of blood products prior 30 days of immunogenicity sampling;

3. Subject has received an active drug with potential immunosuppressive action or investigational drug or device within a period of 30 days prior a study visit;

4. Subject has a known or suspected problem with alcohol or drug abuse as determined by the Investigator;

5. Subject has any condition that, in the opinion of the Investigator, may compromise the subject’s well-being, might interfere with evaluation of study endpoints, or would limit the subject’s ability to complete the study;

6. Subject is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);

7. 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.

10.3 Pregnancy during study

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

10.4 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 in special circumstances, e. g. in the case of non-compliance (meeting exclusion criteria) 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 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 SAE);
- Withdrawal due to SAE;
- Lost to follow-up (defined as 3 documented unsuccessful attempts to contact the subject [iii]);
- 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).

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

[iii] 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.

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.

11. INVESTIGATIONAL MEDICINAL PRODUCT

11.1 Description of VLA1553

The CHIKV vaccine (VLA1553) is a live-attenuated vaccine comprising a large genetic deletion 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 form 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 2 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

Subjects participating in the VLA1553-303 study were vaccinated with VLA1553 at Day 1 in the VLA1553-301 study.

12. STUDY PROCEDURES

12.1 Informed Consent and Enrollment

Any healthy volunteer who provides informed consent i.e., signs and dates the informed consent form) and is part of the VLA1553-301 study can be considered for enrollment 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.

12.2 Subject Identification Code

In the VLA1553-301 study, a 13-character subject identification code was assigned to each subject. This number will be the same identification code used to identify subjects in the VLA1553-303 study. For the VLA1553-301 study the first four digits are the IMP identifier (i.e. 1553) provided by the Sponsor). The fifth digit is the study identifier (i.e. 1553-1), 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).

12.3 Study Visits

The overall study design is illustrated in **Figure 3** (in Section 21.1). For a tabular outline of study procedures and assessments required at each visit, please see **Table 3** as well as the Schedule of Study Procedures and Assessments in Section 21.2.

Visit 0 may be performed as a visit combined with Visit 5 of study VLA1553-301, or will be performed as a separate study visit, if participants already had their VLA1553-301 Visit 5 before joining study VLA1553-303.

For subjects which are enrolled at a later time point Visit 0 and Visit 1 may be performed on the same day. V1 has to be scheduled within the allowed visit window.

Table 3 Study VLA1553-303: Study Visit Schedule		
VISIT	**TIME**	**ACTION**
0	**On or after month 6 of VLA1553-301**	1. Informed consent form 2. Exclusion /Inclusion appraisal 3. No blood draw specifically for VLA1553-303, Immunogenicity sample available from study VLA1553-301[a] 4. SAE assessment [b] 5. Concomitant medications [c]
1	**Year 1**	1. Inclusion/Exclusion criteria status check 2. Concomitant medications 3. Immunogenicity sample [20.0 mL] [a] 4. SAE assessment [b]
2	**Year 2**	1. Inclusion/Exclusion criteria status check 2. Concomitant medications 3. Immunogenicity sample [20.0 mL] [a] 4. SAE assessment [b]
3	**Year 3**	1. Inclusion/Exclusion criteria status check 2. Concomitant medications 3. Immunogenicity sample [20.0 mL] [a]
4	**Year 4**	1. Inclusion/Exclusion criteria status check 2. Concomitant medications 3. Immunogenicity sample [20.0 mL] [a]
5	**Year 5**	1. Inclusion/Exclusion criteria status check 2. Concomitant medications 3. Immunogenicity sample [20.0 mL] [a]

[a] Blood draw Immunogenicity Sample [20.0 mL] for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis upon clinical indication or on demand if requested later by regulatory authorities (backup sample).

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

[c] The strategy outlined above for capturing of SAEs if there is a gap between the end of the VLA1553-301 and the subjects start in VLA1553-303 also applies to the capture of concomitant medications.

12.3.1 Unscheduled Visit

An unscheduled visit can be held at any time during the study if deemed necessary by the Investigator. During the unscheduled visit the investigator should query what concomitant medications have been taken by the subject and if there are any updates on ongoing SAEs

or if any new SAEs have occurred since their last visit. Immunogenicity samples will be collected at all unscheduled visits throughout the study unless the subjects have had a sample taken at their previous regular VLA1553-303 study visit (see **Table 4**).

Table 4 Study VLA1553-303 Study Visit Schedule – unscheduled

VISIT	TIME	ACTION
unscheduled	**unscheduled**	1. Concomitant medications 2. Immunogenicity Sample [20.0 mL] [a] 3. SAE Assessment [b]

[a] Blood draw Immunogenicity Sample [20.0 mL] for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis upon clinical indication or on demand if requested later by regulatory authorities (backup sample).

[b] SAE Assessment will be performed from Visit 0 until Year 2.

12.3.2 End of study visit

Subjects who terminate participation or who are withdrawn from the study prematurely will undergo investigations as outlined below during an end of study visit, if possible. Every effort should be made to have discontinued subjects complete the study End of Study (EoS) Visit (see **Table 5**).

Table 5 Study VLA1553-303 Study Visit Schedule –End of study visit

VISIT	TIME	ACTION
EoS	**unscheduled**	1. Inclusion/Exclusion criteria status check 2. Concomitant medications 3. Immunogenicity Sample [20.0 mL] [a] 4. SAE Assessment [b]

[a] Blood draw Immunogenicity Sample [20.0 mL] (only applicable during on-site visits) for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis upon clinical indication or on demand if requested later by regulatory authorities (backup sample).

[b] SAE Assessment will be performed from Visit 0 until Year 2.

In case an End of Study visit is not possible, a follow-up safety phone call should be made as soon as possible after termination to capture at least relevant concomitant medications and SAEs since the last study visit.

The reason for End of Study should be clarified in as much detail as possible. If an SAE was the reason for End of Study details on that specific SAE should be captured (see Section 10.4). 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 SAE, clinical and/or laboratory investigations that are beyond the scope of the required study observations/assessments will 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.

12.4 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.

13. 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 each study part, results of the respective 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 13.2). However, immunogenicity assessment measuring neutralizing antibodies will be performed on samples collected after Year 1, 2, 3, 4 and 5.

13.1 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 was measured by 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.

[redacted]

[redacted]

The cut-off of CHIKV-specific neutralization antibody titer to be used in the analysis of seroconversion has been defined as CHIKV-specific neutralizing antibody titer >40. Seroconversion is defined as >4-fold increase of $\mu PRNT_{50}$ compared to baseline (Day 1); for baseline negative subjects and baseline positive subjects (defined as $\mu PRNT_{50}$ >40).

13.2 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.

14. ASSESSMENT OF SAFETY

14.1.1 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.

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

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

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

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

14.1.2 Ongoing AESIs

An AESI (serious or non-serious) is an event of scientific and medical concern specific to the Sponsor's product. Any AESI ongoing from study VLA1553-301 will be followed-up during VLA1553-303.

14.1.3 Preexisting Diseases

The preexisting disease information compiled from the VLA1553-301 study may be used to supplement clinical study writing for VLA1553-303.

14.2 Collection, Documentation and Assessment of Serious Adverse Events

The Investigator will follow-up on each SAE 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 the last visit will be followed until resolution or achievement of stable clinical conditions, latest until the overall end of the study.

14.2.1.1 Causality

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

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

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.

SAEs with a causality reported as probable or possible will be considered related to the IMP. SAEs with missing causality assessment will be regarded as related unless further specified.

14.2.2 Assessment and Outcome of Serious Adverse Events

Each SAE reported will be described in the eCRF (i.e., 1 SAE 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. SAEs will be evaluated by the Investigator for:

- Causal relationship to vaccine exposure as defined in Section 14.2.1.1

For each SAE, 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 SAE changes before the event resolves, the SAE will not be reported a second time. Instead the original SAE report will be revised. For purposes of data capture the highest severity rating during the course of a single SAE will be the severity rating entered on the 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.*

14.3 Medical, Medication, and Non-Drug Therapy History

14.3.1 Medical history

At screening in the VLA1553-301 study (from which the VLA1553-303 population of subjects derives), the subject's medical history was described for the following body systems including severity (mild, moderate, or severe) 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. Disease information compiled from the VLA1553-301 study may be used to supplement clinical study report writing for VLA1553-303.

14.3.2 Concomitant Medications and Non-Drug Therapies

All medications with known impact on the immune system and those needed during SAE management received from study start 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. In context of this study, information on non-drug therapies will only be collected in relation to SAEs.

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

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

If any of the above situations occur, a subject will be assessed for exclusion from the Per-Protocol population but not from the whole sample population.

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.

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

The strategy outlined above for capture of SAEs if there is a gap between the end of the VLA1553-301 and the subjects start in VLA1553-303 also applies to the capture of concomitant medications.

14.3.3 Serious Adverse Event reporting

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, if applicable.

14.4 Safety Monitoring

14.4.1 Data Safety Monitoring Board

An independent Data Safety Monitoring Board (DSMB) will be utilized for this study. The DSMB will consist of four voting members who will be selected based upon their expertise with and understanding of infectious diseases, vaccines, clinical research and/or skills and knowledge in clinical medicine. The DSMB will review SAE listings quarterly in addition to accruing safety information on an ad-hoc basis throughout the study until Year 2, as applicable. A meeting of the DSMB may be called at the discretion of the Sponsor, e.g. to address any safety concerns arising during the conduct of the study.

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 increases in frequency of SAEs and individual study discontinuations within the study.
- 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 these changes mandate updates to the protocol and suggest a protocol amendment;
- Can propose an unplanned safety analysis of clinical trial data;
- Can suggest unscheduled visits for specific evaluations for individual cases reviewed.

14.4.2 Sponsor

Until the last subject reached Year 2, listings of available safety data will be closely reviewed by the Sponsor to identify any potential safety concerns.

14.4.3 Investigator

To ensure information exchange on safety across sites, Investigators will be provided with safety information pertaining to all SAEs reported in the eCRF.

15. STATISTICS

15.1 Sample Size and Power Calculations

No formal sample size calculation was performed. Up to 375 subjects of study VLA1553-301 will participate in this study.

However, with a sample size of 375, two-sided exact (Clopper-Pearson) 95%-confidence intervals for an actual SRR of 60% will have a width of 10.2%. Confidence intervals for an SRR of 80% will have a width of 8.3%.

15.2 Handling of Missing, Unused, and Spurious Data

All statistical analysis will generally be based on observed values, missing values will be imputed: For subjects who discontinued because their µPRNT50 titer was ≤40 it will be imputed as 10. For the PP population and the analysis of the annual change rate no explicit imputation will be performed for missing data or results excluded from the analysis, however, the mixed model with repeated measures model will yield unbiased results under the assumption that data are missing at random.

15.3 Methods of Analysis

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

15.3.1 Immunogenicity Analysis

The primary immunogenicity analysis will analyze the observed proportion of subjects with seroresponse (defined as $\mu PRNT_{50}$ ≥150 for baseline negative subjects) over time, accompanied by two-sided exact (Clopper-Pearson) 95% confidence limits.

Secondary immunogenicity analysis will include seroconversion rates by study years, accompanied by 95% confidence intervals. Immunogenicity analyses will also be generated stratified by age stratum.

The kinetics of long-term antibody titers will be evaluated by summary statistics of the GMTs. Annual decline rate of antibody titers will be estimated from a mixed model with repeated measures. Any $\mu PRNT_{50}$ value ≤40 will be imputed as 10, for any $\mu PRNT_{50}$ value >40 the actually measured value will be applied.

15.3.2 Safety Analysis

All subjects entered into the study, who had received a single vaccination in VLA1553-301, will be included in the safety analysis.

The number and percentage of subjects with any SAEs up to Year 2 will be presented for each age stratum overall and by system organ class/preferred term. AESI follow-up will be provided as narratives.

15.4 Planned Data Analysis of the Study

The following data analyses will be performed:

VLA1553-301: immunogenicity data from V1 and V3-V5 will be used as baseline for this study.

VLA1553-303:

- Part A includes immunogenicity and safety data after all subjects have completed Visit 1 (Year 1);
- Part B includes immunogenicity and safety data after all subjects have completed Visit 2 (Year 2);
- Part C includes immunogenicity data after all subjects have completed Visit 3 (Year 3);
- Part D includes immunogenicity data after all subjects have completed Visit 4 (Year 4);
- Part E includes immunogenicity data after all subjects have completed Visit 5 (Year 5).

Individual study parts will be analyzed sequentially.

16. ETHICS AND REGULATORY ASPECTS

16.1 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.

16.2 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.

16.3 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.

17. QUALITY CONTROL AND QUALITY ASSURANCE

17.1 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.

17.2 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.

17.3 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.

17.4 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.

17.5 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.

17.6 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.

17.7 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.

18. DATA HANDLING AND RECORD KEEPING

18.1 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.

18.2 Electronic Case Report Forms (eCRFs)

18.2.1 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.

18.2.2 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.

18.2.3 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.

18.2.4 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.

18.2.5 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.

During the precursor study VLA1553-301 immunogenicity samples were collected from all participants. For subjects who are participating in this study the VLA1553-301 immunogenicity sample collection forms in EDC will be integrated in the EDC for study VLA1553-303 and samples and results will be used for this study.

For additional enrolled subjects (see Section 7.4.1) the available immunogenicity samples from VLA1553-301 will be analyzed retrospectively.

Integrated data from VLA1553-301 EDC comprise:

- V1, V3-V5 immunogenicity sample collection forms
- Medical History forms
- Demographics forms
- Prior/Concomitant Medications forms
- Adverse Events from the VLA1553-301 Study
- Ongoing Adverse Events of Special Interest from the VLA1553-301 Study

18.3 Coding of Serious Adverse Events, Drugs and Diseases

After data entry, SAEs 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.

18.4 Investigator File

18.4.1 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.

18.4.2 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.

18.4.3 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.

19. 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.

20. LIABILITIES AND INSURANCE

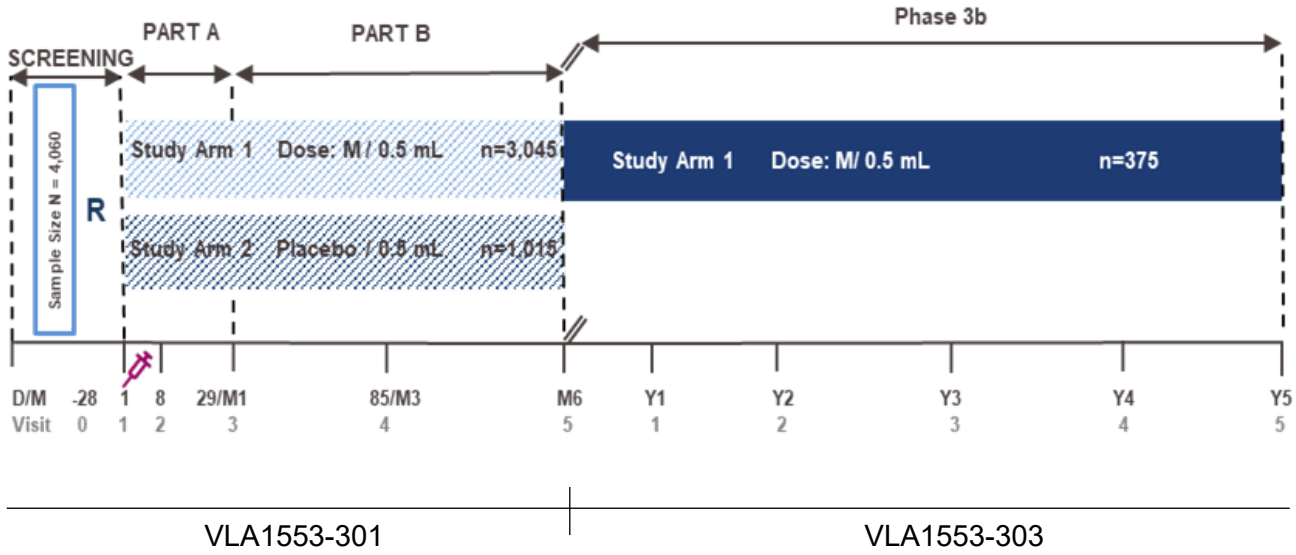
In case of any damage or injury occurring to a subject in association with the participation in the study, insurance has been contracted.

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.

21. SUPPLEMENTS

21.1 Study Flow Chart

Figure 3 Open-label Phase 3b Study Design VLA1553-303



21.2 Schedule of Study Procedures and Assessments

Table 6 Schedule of Study Procedures and Assessments

Part		**A**	**B**	**C**	**D**	**E**	**EoS**
Procedures/Assessments	**Visit 0** On or after *Visit 5 VLA1553-301*	**Visit 1** Year 1 M12 (+/- 84d)	**Visit 2** Year 2 M24 (+/- 42d)	**Visit 3** Year 3 M36 (+/- 42d)	**Visit 4** Year 4 M48 (+/- 42d)	**Visit 5** Year 5 M60 (+/- 42d)	**Visit End of Study**
Informed consent	X						
Inclusion/Exclusion criteria status check	X	X	X	X	X	X	X
Concomitant medications with known impact on the immune system and those needed during SAE management	X[c]	X	X	X	X	X	X
Immunogenicity Sample [a] [20.0 mL]		X	X	X	X	X	X
SAE assessment [b]	X	X	X				X

[a] Blood draw immunogenicity sample [20.0 mL] for CHIKV-specific neutralizing antibody titer evaluation, development of further assays or retrospective safety analysis upon clinical indication or on demand if requested later by regulatory authorities (backup sample).

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

[c] The strategy outlined above for capture of SAEs if there is a gap between the end of the VLA1553-301 and the subjects start in VLA1553-303 also applies to the capture of concomitant medications.

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