30 May 2024
EMA/292025/2024 – Corr.1[1]
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Ixchiq

Common name: Chikungunya vaccine (live)

Procedure No. EMEA/H/C/005797/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

[1] Correction 10 June 2024

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List of abbreviations

ADE	Antibody-dependent Enhancement
AEs	Adverse events
AESI	Adverse event of special interest
AID50	Animal Infectious Dose
ANC	Absolute Neutrophil Count
ASIA Syndrome	Autoimmune/Inflammatory Syndrome Induced by Adjuvants
BSE	Bovine Spongiform Encephalopathy
CHIKV	Chikungunya virus
CHIKF	Chikungunya fever
CPE	Cytopathic effect
CHMP	Committee for Medicinal Products for Human Use
CPP	Critical process parameter
CPV	Continued process verification
DoC	declaration of conformity
EEA	European Economic Area
ECDC	European Centre for Disease Prevention and Control
EMA	European Medicines Agency
ECSA	East Central South African
ERA	Environmental risk assessment
ETF	Emergency Taskforce
EVDAS	EudraVigilance data analysis system
EU	Europe
EU-PAS register	The European Union electronic Register of Post-Authorisation Studies
FBS	Foetal bovine serum
FDA	Food and Drug administration
GD	Gestation day
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GMO	Genetically Modified Organism
GSPR	General Safety and Performance Requirements
HCP	Health care professional
HDPE	High Density Polyethylene
HCP	Host cell protein
HPLC	High-Performance Liquid Chromatography

ICSRs	Individual case safety reports
IOM	Institute of Medicine
IOL	Indian Ocean lineage
IPC	In-process control
ITT population	Intent-to-treat population
LMP	Last menstrual period
LR-CHIKV	Chikungunya virus La Reunion strain
MAAE	Medically attended adverse event
MAH	Marketing Authorisation Holder
MALS	Multi Angle Light Scattering
MCB	Master cell bank
MVS	Master virus seed
NHPs	non-human primates
NIP	National Immunisation Program
NSAIDS	nonsteroidal anti-inflammatory drugs
PAHO	Pan American Health Organisation
PFS	Prefilled syringe
PL	Package leaflet
PP population	Per-protocol population
PPQ	Process performance qualification
PRIME	PRIOrity MEdicines
PRNT	plaque-reduction neutralisation antibody test
PS-HCPs	Pregnancy-Specific Healthcare Providers
PSUR	Periodic Safety Update Reports
PRAC	Pharmacovigilance Risk Assessment Committee
qPCR	quantitative Polymerase Chain Reaction
RMP	Risk management plan
rHA	Recombinant human albumin
RP-HPLC	Reversed phase High Performance Liquid Chromatography
SA	Scientific advice
SAE	Serious adverse event
SE-HPLC	Size-Exclusion High-Performance Liquid Chromatography
SCRI	Self-controlled risk interval analysis
SDS-PAGE	Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis
SmPC	Summary of Product Characteristics
TCID50	Tissue culture infection dose 50
TSE	Transmissible Spongiform Encephalopathies

US	United States
VAERS	Vaccine Adverse Event Reporting System
WCB	Working Cell Bank
WFI	Water for injections
WVS	Working virus seed
WT	Wild-type
VLA1553	Clinical development name of Ixchiq
Yoa	Years of age
µPRNT50	50% plaque reduction in a micro plaque reduction neutralisation test;

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Valneva Austria GmbH submitted on 25 October 2023 an application for marketing authorisation to the European Medicines Agency (EMA) for Ixchiq, through the centralised procedure falling within the Article 3(1) and point 3 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 23 November 2023.

The applicant applied for the following indication: Active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0501/2023 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.4.2. New active Substance status

The applicant requested the active substance 'chikungunya virus (CHIKV) Δ5nsP3 strain (live, attenuated)'contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.5. PRIME

Ixchiq was granted eligibility to PRIME on 15/10/2020 in the following indication: Prophylaxis against Chikungunya disease.

Eligibility to PRIME was granted at the time in view of the following:

- The unmet need in the proposed condition is considered justified in particular due to long-term disabilities and severe complications in patients with comorbidities such as encephalopathy, encephalitis, myocarditis, hepatitis and multiorgan failure; no authorised products exist for prevention or treatment and management remains supportive.
- The non-clinical data presented by the sponsor support that in rodents and NHPs, vaccination results in antibodies with cross-neutralizing properties, which are persistent across time and protect against viremia in challenge experiments. Moreover, serum from vaccinated phase-1 HVs, is reported to protect NHP from viremia after challenge.
- In the clinical phase 1 study discussed by the sponsor, single vaccination raises high neutralizing antibody titres after a month, which increase further and remain high a year later; revaccinations did not produce anamnestic responses.
- The product's potential to sufficiently address the unmet need is therefore considered justified.

Upon granting of eligibility to PRIME, Christophe Focke was appointed by the CHMP as rapporteur.

A kick-off meeting was held on 23/04/2021. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

- Approach to the use of, and threshold for surrogate markers for protection.
- Design of co-vaccination studies, studies in vulnerable populations, and design and conduct of post-authorisation vaccine efficacy studies.
- The overall MAA submission approach and generation of comprehensive evidence to support a benefit risk assessment.

1.6. Scientific advice

The applicant received the following Scientific advice on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
26 March 2020	EMEA/H/SA/4412/1/2020/III	*Dr Hans Ovelgönne, Prof Andrea Laslop*
14 October 2021	EMA/SA/0000063772	*Walter Janssens, Ingrid Schellens*
23 June 2022	EMA/SA/0000087378	*Mair Powell, Ingrid Schellens*

The Scientific advice pertained to the following *quality, non-clinical, and clinical* aspects:

- *Regulatory documentation for the Vero Cell Bank*
- *Release and specifications for Virus Seed Banks*
- *Manufacturing process and strategy*
- *Adventitious agents testing*

- *Release tests and specifications for DS and DP*
- *Comparability assessment following changes in manufacturing*
- *Stability program*
- *Process validation*
- *Non-clinical development programme to support MAA*
- *Environmental risk assessment*
- *Neurovirulence testing*
- *Concurrence that vaccine efficacy trials are not feasible and vaccine efficacy can be based on neutralising antibody titres*
- *Clinical development strategy for licensure of VLA1553*
- *Agreement with the surrogate marker and threshold of protection (⩾50 (measured by µPRNT), as defined in a in non-human primate model study following passive transfer of human antibodies*
- *Follow up discussions to define a new threshold µPRNT50 titre ⩾ 150 considering both sero-epidemiological evidence and NHP passive transfer data*
- *Dose regimen selection for the pivotal phase III studies*
- *Design of the pivotal safety and immunogenicity and lot to lot consistency trials*
- *Evidence to support claims on cross protection to heterologous CHIKV strains*
- *Study protocol VLA1553-304 in moderately immunocompromised patients infected with HIV; co-vaccination study protocol VLA1553-305*
- *Agreement on the plan to evaluate post marketing vaccine effectiveness studies in endemic countries and on the outline of the proposed post-marketing effectiveness study*
- *Total safety database*
- *Paediatric development strategy*
- *Strategy regarding characterisation and validation of Clinical CHIKV neutralization assay*

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Christophe Focke Co-Rapporteur: Jayne Crowe

The appointed CHMP co-rapporteur had no such prominent role in Scientific advice relevant for the indication subject to the present application.

The application was received by the EMA on	25 October 2023
Accelerated Assessment procedure was agreed-upon by CHMP on	14 September 2023
The procedure started on	23 November 2023
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	23 January 2024

The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	02 February 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	30 January 2024
In accordance with Article 6(3) of Regulation (EC) No 726/2004, the CHMP Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	08 February 2024
ETF discussions took place on	16 February 2024
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	20 February 2024
The applicant submitted the responses to the CHMP consolidated List of Questions on	21 March 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	12 April 2024
The CHMP agreed on a list of outstanding issues in writing and to be sent to the applicant on	23 April 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues on	02 May 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	16 May 2024
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Ixchiq on	30 May 2024
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	30 May 2024

During the assessment of the application for the marketing authorisation of Ixchiq, the following non-EU authorities were allowed to participate as part of the OPEN framework and contribute to the scientific discussions of the ETF, BWP and CHMP: WHO and ANVISA. These authorities did not participate in the overall benefit/risk determination, which was decided by the CHMP.

The (Co-) Rapporteurs assessment reports have been discussed and supported by the Emergency Task Force (ETF) in the context of its public health preparedness activities.

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Chikungunya (CHIK) disease (also called CHIK fever) is a mosquito-borne viral disease caused by infection with Chikungunya virus (CHIKV), which is an alphavirus transmitted to humans by the bites of infected female mosquitoes (*Aedes aegypti* and *Ae. albopictus*). Human-to-human transmission (vertical and blood-borne transmission) has been described. Infected travellers can import CHIKV into new areas and local transmission can follow when the vector *Ae. aegypti* and/or *Ae. albopictus* are present ((https://www.ecdc.europa.eu/en/chikungunya/facts/factsheet; https://www.cdc.gov/chikungunya/index.html).).

All ages groups are at risk for CHIKV infection irrespective of their sex. Although CHIK is self-limiting and non-lethal, in most individuals CHIK may lead to significant, long-term disability. Patients at extremes of the age spectrum are at higher risk for severe disease and risk factors for more severe CHIK include intrapartum exposure for neonates, older age (>65 YoA) and co-morbidities. Newborns infected during delivery and older people with underlying medical conditions may become severely ill and are at increased risk of death.

2.1.2. Epidemiology

The first identified outbreak of CHIK occurred in 1952-1953 in East Africa (estimated incidence at 23%) and afterwards sporadic outbreaks of CHIK occurred in Africa (mainly in rural tropical regions) and in Asia, with major activity in the 1960s-1980s followed by a decrease in activity until 2004. Since 2004, CHIKV is responsible of major emerging and re-emerging outbreaks of disease in the Indian Ocean islands, Southeast Asia, and the Americas (Zeller et al.; 2016). It is estimated that during the sudden and large outbreaks caused by CHIKV, one third to three quarters of the population is affected in the areas where the virus is circulating. Approximately 50-97% of infected individuals will become symptomatic with fever and arthralgia (Silva JVJ et al.; 2018).

In 2004, a large epidemic started in Kenya and rapidly spread to several islands in the Indian Ocean (including the French oversea department of La Réunion), to India, and to Southeast Asia. Over 300,000 persons were estimated to be affected during the 2004 to 2006 epidemics in Indian Ocean islands, with over 95% of cases contributed by La Réunion where the estimated overall attack rate was 35%. In 2005-2006 in India, there were 1.3 million cases estimated in 13 states (Pialoux et al.2007). This epidemic was caused by an East/Central/South African (ECSA) CHIKV strain, which evolved into a new lineage termed Indian Ocean Lineage (IOL) ((Weaver et al. 2015). Concomitantly, the disease re-emerged in several countries in Central and West Africa (Zeller et al. 2016).

In 2013, a second major outbreak occurred when a strain from the Asian lineage emerged in the Caribbean Sea (Saint Martin Island) rapidly spreading to neighbouring islands and Central, South, and North America. More than 1.2 million autochthonous cases were reported to Pan American Health Organization in the Americas for the period 2013–2014 (Zeller et al.; 2016).

In Europe, small outbreaks originating from imported cases have been reported since 2007. Italy reported the first CHIKV outbreak in 2007 (n= 330 cases). France was the second country in Europe to report an outbreak with autochthonous transmission events detected in 2010 (n= 2 cases) and 2014 (n=12 cases). The last outbreaks in continental Europe were in 2017 in France (n=17 cases) and in

Italy (n=489 cases). No autochthonous cases were detected in continental Europe between 2019-2024 (Source: ECDC situation update April 2024).

A higher number of cases have been reported in some areas which are part of French overseas collectivities. In Guadeloupe, 143,422 cases were reported between 2013 and 2017; In French Guiana, 86,216 cases were reported between 2014 and 2018; and in French Polynesia, 69,059 cases were reported in 2014 and 2015. In all these areas, no cases were reported between 2019 and 2022.

According to the ECDC, in 2023 and as of 31 of December, approximately 500 000 CHIKV cases and over 400 deaths had been reported worldwide. A total of 26 countries reported CHIKV cases from the Americas (16), Africa (5) and Asia (5). The majority of countries reporting high CHIKV burden are from the Americas, in South and Central America. Countries reporting the highest number of cases are Brazil (256 927), Paraguay (140 905), Argentina (1 746), and Bolivia (1 455).) In Asia, majority of cases were from India (93 465), Philippines (2 561), Thailand (1 422) and in Africa from Burkina Faso (545), Senegal (337). CHIK associated deaths were reported from Paraguay (297) and Brazil (106).

In 2024 and as of 31 March 2024, approximately 160 000 CHIK cases and 50 deaths were reported worldwide from a total of 17 countries (Americas (11), Asia (5) and Africa (1)). No autochthonous cases have been reported in Europe in 2024 so far.

Further geographical expansion of CHIKV beyond the tropics and neotropics is to be expected due to viral adaptation, climate change and globalization. Currently an estimated 1.3 billion people are at risk of chikungunya fever with already >100 countries reporting circulation and >10 million cumulative cases globally. Climate change models generally anticipate an expansion of the global distribution of *Ae. albopictus* and *Ae. aegypti* and thereby increasing the risk of chikungunya transmission including to parts of China, sub-Saharan Africa, Europe and the Americas (Bartholomeeusen K. et al. 2023).

In view of the autochthonous outbreaks of CHIKV infections in continental Europe, the widespread presence of competent vectors (*Aedes albopictus*) in the Mediterranean basin, and the return of travellers from endemic areas, in EU, CHIK is included in the list of communicable diseases threatening public health that have emerged or re-emerged to be covered by epidemiological surveillance (Commission implementing decision (EU) 2018/945- 22nd June 2019). Systematic surveillance is necessary to prevent the spread of CHIKV in the EU.

2.1.3. Aetiology and pathogenesis

Aetiology

CHIKV is an enveloped positive-sense single-stranded RNA virus of the family Togaviridae, genus Alphavirus. CHIKV viral particles are spherical and measure ~70 nm in diameter, the ~12 kb genomic RNA (gRNA) of CHIKV is packaged by a viral capsid core and enveloped by a host cell-derived membrane with the viral envelope proteins that make up the glycoprotein shell (Bartholomeeusen K. et al.; 2023).

The Alphavirus genus also includes other pathogenic mosquito-transmitted viruses, which are classified according to their pathogenic characteristics into arthritogenic and encephalitic alphaviruses:

- Arthritogenic alphaviruses - causing arthralgic diseases- include the different genotypes of CHIKV, O'nyong'nyong virus (ONNV), Ross River virus (RRV), Barmah Forest virus (BFV), Mayaro virus (MAYV), Sindbis virus (SINV) and Semliki Forest virus (SFV).
- Encephalitic alphaviruses - causing neuroinvasive diseases - include Venezuelan equine encephalitis virus (VEEV), western equine encephalitis virus (WEEV) and eastern equine encephalitis virus (EEEV).

There are three distinct lineages for CHIKV identified by phylogenetic analysis, which correspond to their respective geographical origin: West African, East Central South African (ECSA) and Asian lineage. The ECSA lineage is divided further into two clades, ECSA1 (entirely consisting of ancestral CHIKV sequences) and ECSA2 (contains sequences from the Central African Republic, Cameroon, Gabon and the Republic of Congo). Following the outbreak that started in Kenya in 2004 and that spread to the Indian Ocean Islands, a fourth phylogenetic lineage has emerged, which is termed Indian Ocean lineage (IOL). The IOL lineage subsequently dispersed to Asia and India and it caused autochthonous transmission in Italy and France (Bartholomeeusen K. et al.; 2023).

Transmission

CHIKV is transmitted to humans by the bites of infected female mosquitoes, mainly by *Aedes aegypti* but more recently also by *Aedes albopictus* mosquitoes. The IOL strain that evolved from the ECSA CHIKV strain during the 2004-2006 epidemic, harbours a mutation in the E1 glycoprotein (E1-A226V) regarded as contributing to the observed enhanced transmission through *Ae. albopictus* mosquitos. *Ae. albopictus* mosquitos are better adapted to surviving cold winters and their habitat is extending to new temperate regions, which may result in disease transmission in new areas (https://www.ecdc.europa.eu/en/disease-vectors/facts/mosquito-factsheets/aedes-albopictus).

Two distinct CHIKV transmission cycles exist, the enzootic sylvatic cycle and the urban cycle. The enzootic sylvatic transmission cycle occurs between *Aedes mosquitoes* and non-human primates (although other yet undetermined animal species might also be involved). Periodic outbreaks of CHIK are thought to be caused by occasional introduction of the virus into urban areas and driven by human-mosquito-human transmission cycle (Bartholomeeusen K. et al.; 2023).

Aedes mosquitoes also transmit other arboviruses (e.g. Dengue virus, Zika virus, Yellow Fever virus). This complicates diagnosis of CHIK based on symptoms as some clinical signs are shared with diseases caused by other arboviral infections circulating in the same regions. Notably, CHIK is frequently misdiagnosed as dengue.

In addition to vector-borne transmission, other transmission routes of CHIKV have been documented: blood-borne transmission among laboratory personnel and healthcare providers and mother-to-child transmission, mainly intrapartum when the mother is viraemic around the time of delivery. Rare in utero transmission has been documented, mostly during the second trimester. There have been no reports to date of infants acquiring CHIKV infection through breastfeeding (CDC).

Pathogenesis

At first, CHIKV infection through bites of infected mosquitoes will result in a dermal infection phase of skin-resident cells (dermal macrophages, fibroblasts, mesenchymal stromal cells, and Langerhans cells). Further CHIKV replication occurs in peripheral organs, including the lymph nodes, spleen and, in severe cases, the liver, brain and other organs (Bartholomeeusen K. et al.; 2023).

The main mechanism of CHIKV cellular entry is via receptor binding and clathrin-mediated endocytosis and involves the E1 and E2 glycoproteins. MXRA8 – a cell adhesion molecule expressed on epithelial, myeloid, and mesenchymal cells - was identified as a receptor in human cells for CHIKV and related arthritogenic alphaviruses. In addition to MXRA8, the presence of additional receptors or attachment factors involved in CHIKV cell entry is considered likely given the broad reported cellular and tissue tropism of CHIKV (Kril et al.; Annu Rev Virol. 2021).

Upon release into the host cell cytoplasm, the genomic RNA of CHIKV can immediately be translated as it harbours a 5'-Cap and a 3'-polyadenylated tail. The genomic RNA of CHIKV contains two open-reading frames. The first codes for the four non-structural proteins (nsP1–4) that form the replicase complex catalysing the production of new viral RNA (including genomic and sub-genomic RNAs). The

second open-reading frame codes for the structural proteins that are translated from sub-genomic RNA as a single polyprotein (C–E3–E2–6K/TF–E1), which is further processed (Bartholomeeusen K. et al.; 2023).

Noteworthy, VLA1553 derives from the La Reunion strain (LR-CHIKV clone LR2006-OPY1) of ECSA genotype that was attenuated by reverse genetics to delete 61 amino acids in the C-terminal part of the nsP3 viral replicase complex protein (Δ5nsP3), which results in a reduced replication capability of the virus in vivo. The nsP3 protein has roles in viral replication (within the viral replication complex/spherules) and in host adaptation, including hypothesized roles in downmodulation of innate immune responses (Kril et al.; 2021).

Initiated by CHIKV detection by different pathogen recognition receptors, CHIKV infection results in a strong antiviral type I IFN response and production of pro-inflammatory cytokines and chemokines, such as TNF-alpha and CCL2. Onset of symptoms is coincident with rising viral loads and IFN-alpha responses. The clearance of viraemia requires specific antibodies. Anti-CHIKV neutralizing IgM are detected as early as 4 days after the onset of symptoms, while specific IgG are detected at later time-points. CHIKV-specific CD4 T cells responses are also generated, these are involved in IgG class switching and efficient production of IgG antibodies, but also in promoting arthritic inflammation (Bartholomeeusen K. et al.;2023)

It is considered that natural infection with CHIKV will induce life-long protective immunity against re-infection or disease caused by re-infection. This would theoretically be driven by antibody responses, as supported by different sero epidemiological studies that have shown long-term persistence of CHIKV-specific neutralizing antibodies and by different passive-transfer studies in animal models.

However, an immune correlate of protection for CHIK or CHIKV infection has yet not been established.

2.1.4. Clinical presentation and diagnosis

Clinical presentation

The World Health Organization classifies CHIK into four major categories (acute CHIK, atypical CHIK, severe CHIK, chronic CHIK and proposed standardised case definitions (chik-outbreak-toolbox---25092019.pdf (who.int)).

Brief description of each category is provided below:

Acute CHIK

Symptoms will appear between 3 and 7 days after the patient has been bitten by an infected female *Aedes* mosquito.

Acute CHIK is divided in a viraemic phase (range 5-10 days) and in a post-viraemic phase (range 6-21 days). The most common symptoms are rapid onset of high-grade fever in the viraemic phase, polyarthralgia and often polyarthritis. Polyarthralgia is often incapacitating, usually symmetrical and involves primarily peripheral joints. In addition, other common symptoms of acute CHIK include headache, myalgia, joint swelling, rash (usually maculopapular), fatigue, diarrhoea, or oedema (Suhrbier, 2019). Although this spectrum of clinical manifestations corresponds to the one experienced by the majority of subjects with acute CHIK, for some patients the spectrum of manifestations in the acute phase is more complex than initially appreciated.

Atypical CHIK

Clinical manifestations of atypical and severe acute CHIK are typically observed in older adults (>65 years); people with medical conditions such as high blood pressure, diabetes, or heart disease;

children (<1 year) and newborns (infected intrapartum). Atypical acute CHIK affect different systems and organs. Examples of atypical manifestations include encephalitis, meningoencephalitis, Guillain–Barre syndrome, myocarditis, nephritis, dyspnoea, respiratory failure.

Severe CHIK

In the case of severe acute CHIK, which requires hospitalisation, the most prevalent manifestations are cardiac or multiple organ failure ((Suhrbier, 2019).

Chronic CHIK

Acute CHIK is typically self-limiting and more than 50% of patients reports resolution after 1 month. However, a significant proportion of patients will progress to chronic CHIK following the acute stage, with estimates ranging from ~14% to ~87% and an average prevalence of approximately 48% among infected patients that has been estimated (Silva LA et al.; 2017). In some classifications of the CHIK stages, a so-called "post-acute" stage of CHIK (from day 21 to the 3rd month after the onset of symptoms) is included between the acute and chronic stages (Simon et al.; 2015, Zaid et al. 2018).

Chronic CHIK is characterised predominantly by persistence of arthritic conditions for more than 3 months. Risk factors that have been associated to progression to chronic CHIK include patient age (>45 years), preexisting chronic inflammatory arthropathy, CHIKV genotype, increased severity of symptoms during the acute phase (arthralgias, body aches and weakness) and increased viral loads during the acute stage (Silva LA et al.; 2017; Bartholomeeusen K. et al.; 2023). Several clinical and non-clinical evidence support immunopathological mechanisms for chronic CHIK and it is hypothesized that chronic CHIK might be mediated by persistent virus, but replicative virus has not been detected.

CHIK in neonates

A meta-analysis was recently performed to evaluate the risk for mother-to-child transmission; antepartum foetal deaths; symptomatic neonatal disease; neonatal deaths from maternal CHIKV-infections during gestation. The authors concluded that perinatal infections do occur and can be related to neonatal death and long-term disabilities, with high rates during intrapartum period (Contopoulos-Ioannidis et al. 2018). It is hypothesized that intrapartum transmission results from placental openings from contractions during labour and a study performed in La Réunion reports that 21% of the infected neonates had persisting disabilities (Gérardin et al. 2008).

Diagnosis

Diagnosis of CHIK based on clinical presentation is complicated by the fact that CHIK shares clinical signs with diseases caused by other arboviral infections, such as dengue. Laboratory confirmation of CHIK disease is needed in order to guide appropriate treatment and for epidemiological surveillance to identify outbreaks.

CHIKV can be detected in blood samples collected during the first week of illness, since viraemia typically clears 14 days post-infection. During the acute phase of the disease, CHIKV can be detected by culture and/or by nucleic acid amplification methods detecting viral RNA (e.g. RT-qPCR assays). During the first 8 days of CHIKV infection, use of nucleic acid amplification techniques is considered the preferred diagnostic method.

There are also indirect serological diagnostic methods that are typically used after the first week of infection to test for antibodies to the virus. Specific IgM responses are typically detected within 5–7 days after the onset of symptoms and IgG responses can be detected approximately 7–10 days after onset of illness, often after viraemia has been cleared.

WHO recommends use of both serological and virological testing methods for patient specimens collected during the first week after the onset of symptoms (Bartholomeeusen K. et al.; 2023).

2.1.5. Management

Prophylaxis by vaccination

In the EU, no vaccine is approved for the prevention of disease caused by CHIKV infection.

VLA1553 was granted approval from the U.S. Food and Drug Administration (FDA) in November 2023 for active immunization for the prevention of disease caused by CHIKV in individuals 18 years of age or older who are at increased risk of exposure to CHIKV.

Some additional vaccine candidates are in advanced clinical development (Bartholomeeusen K. et al.; 2023).

Vector control to prevent exposure

Prevention and control of outbreaks of CHIK depend on the implementation of integrated vector management strategies to reduce mosquito densities and personal protection to prevent mosquito bites and prevent mosquitos from biting infectious people. There should be a surveillance control system in place. Examples of vector control measurements are eliminating larval habitats, for example by removing or covering up water holding containers, larvicidal treatment or chemical control measures. Personal protection (especially during daytime) includes the use of mosquito repellent, impregnated mosquito bed nets, use of long sleeved shirts and long pants among others (Caribbean Public Health Agency (CARPHA) – CHIKUNGUNYA – Information for Vector Control Personnel).

Therapeutics

There are no approved therapeutics for CHIK. Supportive symptomatic treatments are applied, which differ according to the disease phase. During the acute phase, treatments include hydration or pain relief. During chronic phases of the disease, treatments include pain relief and corticosteroid therapy and/or administration of antirheumatic drugs to act on rheumatological symptoms (Bartholomeeusen K. et al.; 2023).

2.2. About the product

Ixchiq (VLA1553) is a single-dose monovalent live-attenuated vaccine derived by reverse genetics from the CHIKV La Reunion strain LR2006-OPY1. Attenuation was achieved by deleting 61 amino acids in the C-terminal part of the non-structural replicase protein 3 (nsP3). As compared to the parent strain, this genetic modification reduces replication capability of the modified virus in vivo.

The vaccine is propagated on Vero cells and purified by centrifugation, ultrafiltration, chromatography, and sucrose gradient centrifugation. Ixchiq contains no adjuvant.

The applicant seeks approval for active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older.

The proposed posology is 0.5 mL after reconstitution of ≥3.0 log10 TCID50 Chikungunya virus CHIKV Δ5nsP3 strain administered as a solution for injection through intramuscular administration.

Mechanism of action

Ixchiq contains live-attenuated CHIKV of the ECSA/IOL genotype. The exact mechanism of protection against CHIKV infection and/or disease has not been determined. Ixchiq elicits neutralizing antibodies against CHIKV.

2.3. Type of Application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the therapeutic innovation of the candidate vaccine, in the context of an identified unmet medical need for the prevention of chikungunya disease in adults.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as two components (powder and solvent for solution for injection): 1) a single-dose vial with the lyophilized powder of Chikungunya virus (CHIKV) Δ5nsP3 strain (live, attenuated (not less than 3.0 $\log_{10}$ Tissue Culture Infectious Dose ($TCID_{50}$)) and 2) a solvent consisting of water for injections in a prefilled syringe (PFS).

Other ingredients (powder) are: sucrose, D-sorbitol, L-methionine, trisodium citrate di-hydrate, magnesium chloride, di-potassium-hydrogen phosphate, potassium-di-hydrogen-phosphate, and recombinant human albumin (rHA).

The powder's primary packaging is a vial (type I glass) with a rubber stopper (bromobutyl) and an aluminium flip-off cap with polypropylene closure.

The solvent's primary packaging is a prefilled syringe (type I glass) with a rubber stopper (Flurotec) and a tip cap (bromobutyl) packaged without needles.

At the time of administration, the lyophilized vaccine is reconstituted with the entire contents of the solvent in the PFS so that upon withdrawal of the reconstituted finished product a 0.5 mL dose of vaccine can be administered.

2.4.2. Active substance

2.4.2.1. General information

The active substance [common name: Chikungunya vaccine (live)] of the Chikungunya vaccine is a live-attenuated chikungunya virus CHIKV (Δ5nsP3), propagated on Vero cells and purified by centrifugation, ultrafiltration, chromatography, and sucrose gradient centrifugation. The Chikungunya vaccine contains no adjuvant.

Chikungunya virus (CHIKV) is an Alphavirus belonging to the *Togaviridae* family. The virus is enveloped and has a spherical shape with a diameter of approximately 70 nm. The genome of CHIKV is a single-stranded positive sense RNA of approximately 12 kb; it contains two open reading frames that encode four non-structural replicase proteins (nsP1-4) and a structural polyprotein consisting of the capsid protein (C) and the envelope proteins (E3-E2-6K-E1).

The Chikungunya vaccine candidate is a genetically stable live-attenuated CHIKV mutant which is based on the La Reunion strain (LR-CHIKV clone LR2006-OPY1) of East Central South Africa of the Indian Ocean lineage, which generates cross neutralizing antibodies against Chikungunya virus of the West African and Asian lineages. It is a genetically modified virus strain with a 61 amino acid deletion in the non-structural protein 3 (Δ5nsP3). The 61 amino acid deletion was generated by substitution of the CHIKV-specific sequence with an amino acid linker (AYRGAAAG) which was designed to avoid possible structural constraints caused by the deletion. The structure of the virus genome is presented below.

Figure 1. Structure of the Δ5nsP3 vaccine virus genome

2.4.2.2. Description of manufacturing process and process controls

Manufacturers

The active substance is manufactured by Valneva Scotland Limited (UK). All sites involved in manufacturing and controls of the active substance operate in accordance with EU Good Manufacturing Practice (GMP).

Description of the manufacturing process

Manufacture of the active substance consists of the upscaling of Vero cells, infection of Vero cells with virus, virus production, viral harvest, harvest concentration and purification, active substance formulation with formulation buffer and active substance filtration and filling. The batch size is defined.

Upstream Manufacture - Cell Culture, Viral Amplification and Viral Harvest
Vero cells from the Working Cell Bank (WCB) are thawed and cultured in flasks, followed by serial expansion in culture flasks and roller bottles. Viral inoculation is performed based on the cell count. The required number of Working Virus Seed (WVS) vials are thawed and cells are infected. Post infection the roller bottles are observed until the presence of significant cytopathic effect (CPE). Following confirmation of cytopathic effect, harvest is taken, sampled for testing, filtered and stored.

Downstream Manufacture - Pooled Harvest Concentration and Diafiltration

The filtered harvest material is concentrated and diafiltered into diafiltration buffer. The harvest concentrate is conditioned by addition of sucrose solution. The conditioned harvest concentrate is stored.

Downstream Manufacture - Viral Clarification and Purification

The concentrated virus is clarified by a protamine sulphate treatment. Following DNA precipitation by protamine sulphate, host cell protein is removed by adsorption. The clarified harvest concentrate is then purified by sucrose gradient centrifugation. Following centrifugation, fractions containing the virus are selected and pooled. The resulting sucrose gradient pool is further processed.

Downstream Manufacture - Active Substance Formulation

The active substance formulation buffer is prepared and filtered. The sucrose gradient pool is diluted to active substance using formulation buffer. The formulated active substance is mixed, filtered and dispensed in bottles. The bottles are individually heat sealed in bags and stored.

The active substance manufacturing process has been adequately described.

Control of materials

All materials have been described in detail. For non-compendial raw materials, adequate specifications are in place to control their quality. Apart from foetal bovine serum (FBS) and protamine sulphate, no other raw materials of human or animal origin are used in the active substance manufacturing process.

Foetal bovine serum has been used during establishment of the cell bank system and virus bank system and is also used during active substance manufacturing. FBS is covered by Transmissible Spongiform Encephalopathies (TSE) Certificates of Suitability. The FBS used was/is compliant to the effective version of EMA/410/01 (European Commission: Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products), and thus does not pose any risk for transmission of TSE/ Bovine Spongiform Encephalopathy (BSE). In addition, FBS is sterile filtered, heat inactivated and gamma-irradiated, and tested for adventitious viruses. Trypsin was only used for cell dissociation during cell expansion phases in the manufacture of the current MCB and WCB. All other cell banks will be produced using a recombinant enzyme derived from microbial fermentation for cell dissociation during cell expansion phases.

Protamine sulphate (fish origin: salmon or herring) consists of sulphates of basic peptides. The manufacturing process of protamine sulphate includes several steps with harsh conditions (heat treatment at 90°C, extraction with HCl, concentrated ethanol precipitation) which eliminates and /or inactivates any potential contaminating viruses.

Cell bank system: The 2-tiered Vero cell bank system consists of a master cell bank (MCB) and working cell banks (WCB) and has been documented in detail and is in line with ICH Q5D, ICH Q5A (R1) and Ph.Eur.5.2.3. Extensive testing has been performed which confirms that the MCB and WCB have been properly qualified. Information on storage and stability testing of cell banks is provided. Testing of cell banks for viral and non-viral adventitious agents and screening for retroviruses has been described. As the active substance is a live virus and as the active substance process does not contain any virus-inactivating or -removing steps, the applicant has committed to test a WCB for the following viruses: Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis E virus, CMV, EBV, HHV-1, HHV-2, varicella virus, HHV-6, HHV-7, HHV-8, human papilloma virus, human polyoma virus and parvovirus B19 and to communicate the outcome of these tests upon availability.

Tumorigenicity studies have been performed on the Vero cells (end of production cells). Characterisation testing confirmed correct identity of the cell bank system. The applicant also described the manufacturing of future WCBs. The proposed testing programme to qualify future WCBs is considered adequate.

Virus seed system: The process uses a 2-tiered virus seed system (master virus seed (MVS) and working virus seed (WVS)). The recombinant virus was generated by reverse genetics: the virus genome was cloned in a plasmid and after *in vitro* RNA transcription the viral RNA was transfected in Vero cells to generate recombinant virus. The virus genome has a deletion in the nsP3 gene which causes the attenuated phenotype. Generation of the recombinant virus and production and testing of the virus seed system has been described in detail. Testing included tests for identity, virus titre, sterility, mycoplasma, mycobacterium, retroviruses, *in vitro* and *in vivo* adventitious virus assay, porcine and bovine viruses. Also genetic stability was adequately demonstrated via sequencing of the pre-MVS, MVS, WVS and various active substance lots. The proposed tests are considered adequate.

With regards to future WVSs, the applicant proposes not to include a qualification protocol in Module 3 but to submit a variation when a new WVS needs to be qualified. This approach is acceptable and it is

expected that future WSV testing will be compliant with Ph.Eur.2.6.16, including an *in vitro* adventitious agents test with an incubation period of 28 days.

Control of critical steps and intermediates

The applicant provided an overview of critical process parameters (CPPs) and in-process controls.

Process parameters were identified and ranked according to their critically based on their risk and impact to the process. Depending on the criticality, development work was performed which included small scale and at-scale studies. These characterisation studies were used to define and establish the expected ranges for the parameters. The rationale for deciding the critically of parameters is presented and is considered sufficient.

In-process controls (IPCs) results on the harvest for bioburden, mycoplasma and adventitious viruses comply with acceptance criteria. Overall, the control strategy is considered acceptable.

Process validation

In accordance with the process validation protocol, the process performance qualification (PPQ) was successfully completed by producing six full scale batches of active substance at Valneva Scotland Ltd. All process performance validation tests and test results for critical quality attributes met the acceptance criteria. All CPPs were within the required specification. For commercial batches, the control of the manufacturing process will be monitored and updated as appropriate. Continued process verification (CPV) is conducted during the entire life cycle of the product. The active substance process has been adequately validated and yields active substance of consistent quality.

Impurity clearance was evaluated by analysing specific process-related impurities in active substance and active substance intermediates. Impurities were consistently reduced to sufficiently low levels.

The applicant provided justifications for the proposed maximum duration of several process steps and also clarified the difference between processing times and hold times. In addition, the applicant clarified that the resins used in the multi-modal chromatography are for single use only and that no regeneration is performed.

A transport validation study has been performed demonstrating that the active substance maintained at the proposed storage temperature for the duration of the transport.

Deviations have been appropriately assessed and shown to have no impact on the product or on the PPQ outcome.

Manufacturing process development

The active substance manufacturing process was developed at Valneva Austria GmbH and transferred to another site for production of toxicology studies material and Clinical Phase 1 material. The active substance process was subsequently transferred from this site to Valneva Scotland Ltd for production of Clinical Phase 3 material and commercial lots. The upstream process and downstream process remained the same, only minor process optimizations were introduced. These are considered minor with no impact on product quality, safety or efficacy. Process adaptions by volumetric scale up with slightly larger volumes for centrifugation and subsequent dilution to active substance were performed for clinical phase 3 based on the obtained high virus titre. The downstream process with removal of process related impurities and multi-mode chromatography showed the same purification capability between clinical phase 1 and clinical 3 process.

Developmental studies were performed to investigate the impact of different process parameters. The optimal process parameter settings were defined based on the outcome of these studies.

A comparability analysis was performed which confirmed that Phase 1 material and Phase 3 material were comparable.

Characterisation and impurities

The following methods were applied to monitor particle content, integrity, purity and product-related impurities: Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC), SE-HPLC-Multi Angle Light Scattering (MALS), Dynamic Light Scattering, Electron Microscopy, Nanoparticle Tracking Analysis, Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) Silver stain. Most virus is present as monomer; only a minor portion are multimers. Based on the results obtained the multimers are mostly dimers and trimers; there are no or only very little large aggregates. The size of the virus particles is consistent to the expected external diameter of 70nm for a CHIKV virion.

Genetic stability was demonstrated by sequencing analysis of different virus passages (including the pre-MSV, MSV, WSV and several active substance lots). All sequences were identical thereby confirming that the virus is genetically stable up to production level. Only for three positions genetic heterogeneities (mixed positions) were observed which gradually increased with higher passage number in Vero cells. One of these 3 mixed positions results in a silent mutation (no impact at protein level) whereas the other two mixed positions lead to amino acid changes (one in the non-structural polyprotein and one in the structural polyprotein). The relative proportions of the observed genetic heterogeneities are consistent between active substance batches and do not appear to impact the immune response in animals and humans.

Impurities are consistently removed during virus purification to sufficiently low levels. Nevertheless, active substance release testing includes tests for residual process impurities. Overall, impurity levels are very low in the active substance and are routinely monitored and controlled via the active substance specifications.

2.4.2.3. Specification

The active substance specifications include tests for appearance/solubility, pH, osmolality, identity (quantitative Polymerase Chain Reaction (qPCR)), infectious virus concentration ($TCID_{50}$), residual host cell protein (Enzyme Linked Immunosorbent Assay (ELISA)), residual host cell DNA (PCR)), other residual process components/impurities and other standard safety acceptance tests.

Specifications are set based on current knowledge of the process and capabilities of methods, current limited number of active substance batches and justified by the safety and immunogenicity profile of clinical trials. For the Infectious Virus Concentration, the lower limit of specification represents the minimal target viral concentration to enable production of finished product lots at the desired validated batch size. The proposed active substance specification limits have been justified and are deemed acceptable.

Analytical procedures

Osmolality, pH, endotoxins, bioburden, mycoplasma and mycobacteria testing are compendial tests performed in accordance with the respective Ph. Eur. monographs.

The applicant indicated that alternative methods to quantitate endotoxins are being considered to replace the current test that uses horseshoe crab derived material. The feasibility of such methods will however depend on the method qualification.

Identity: Identity is determined by a quantitative polymerase chain reaction (q-PCR).

Infectious Virus Concentration by TCID50: TCID50 is the measure to indicate the level of replication competent infectious virus from culture supernatant through the detection of cytopathic effect.

Residual Vero Host Cell Protein (ELISA): A commercially available ELISA kit is used to quantify Vero Cell Host Cell Protein (HCP) impurities in the active substance.

Residual Host Cell DNA by qPCR: Residual hcDNA is determined by quantitative PCR (q-PCR).

Validation of analytical procedures

The methods for pH, Osmolality, Endotoxin, Bioburden, the *in vitro* and *in vivo* assays for extraneous agents in viral vaccines for human use and the tests for Mycoplasma and Mycobacteria are compendial methods which have been implemented according to the respective monographs of Ph. Eur. Compendial methods are verified for their intended purpose according to pharmacopeial requirements.

The non-compendial methods were adequately validated.

Batch analysis data

Batch data were provided for the phase 3 active substance material and PPQ active substance lots, as well as for other active substance lots manufactured. The results confirm that the phase 3 material is representative of the commercial product.

Reference standards

The applicant has described the reference materials that are used during active substance release testing. A protocol for the preparation and qualification of CHIKV RNA Reference Standard (CHIKV_Δ5nsP3_Standard) is included in the dossier.

Container closure system

The applicant has adequately described the active substance container closure system: bottles with a screw cap. Container materials are compliant with USP. Both the bottles and screw caps are sterilised. An extractables study was performed which did not reveal any compounds or elements that could pose a safety concern. The container closure system is deemed suitable for active substance storage.

2.4.2.4. Stability

Stability of the active substance was demonstrated by supportive data generated from Clinical Phase 1 batches and primary stability data generated from the Clinical Phase 3 batch and 3 PPQ batches, as well as further supportive GMP batches manufactured according to the commercial scale process. A stability program for active substance has been initiated. Samples are stored under long term and accelerated conditions. Samples have also been stored under forced degradation storage conditions. Furthermore, a freeze-thaw stability study has been performed. The parameters tested include appearance, pH, infectious virus concentration, endotoxin and bioburden.

Stability data are available for a number of representative clinical active substance batches for the duration of the proposed storage period. All stability results comply with the specifications and no decrease in viral titre is observed at long term. Also, at accelerated storage conditions the stability results comply with the specifications and no decrease in viral titre is observed for Clinical Phase 3 active substance and PPQ batches. For samples stored under forced degradation conditions the viral titre decreases over time, which is more pronounced under forced degradation conditions. All batches show a stable profile under freeze thaw conditions. A commitment is made to complete and report all registration stability studies.

Based on the stability data provided, the claimed storage period is deemed acceptable for the active substance at the proposed storage conditions.

2.4.3. Finished medicinal product

2.4.3.1. Description of the product and Pharmaceutical development

The active substance in the Chikungunya vaccine is a live-attenuated chikungunya virus CHIKV (Δ5nsP3). The Chikungunya vaccine finished product contains one single-use vial with the lyophilized vaccine and one prefilled syringe of solvent containing 0.5 mL sterile water for injections for reconstitution and administration of the reconstituted vaccine.

The pharmaceutical form is powder and solvent for solution for injection. One dose equals 0.5 mL on reconstitution with sterile water for injections. The composition of the lyophilized vaccine and a list of all components, their amount, function, and reference to quality standard is provided.

Pharmaceutical development

Pharmaceutical development of the finished product has been described in detail.

Developmental studies and risk assessment have been performed to define the criticality of the process parameters and quality attributes. Formulation studies have been conducted to identify suitable formulation conditions, not only to stabilize viral infectivity, but also to minimize unspecific adsorption losses and to guarantee filterability during finished product manufacturing.

Initially, a liquid formulation was developed that was used for the preclinical and phase 1 clinical studies. For phase 3 studies and commercial batches, a lyophilised finished product was developed. Information was provided on the different process variants used during product development and on the finished product batches produced thus far. Comparability studies have been performed which showed that the liquid and lyophilised presentation can be considered comparable.

During formulating of the finished product, a target formulation titre is used that is slightly higher than the release specification to account for any potential loss of viral activity by surface adsorption during finished product bulk preparation, sterile filtration, filling and lyophilization, as well as potential loss of viral activity during storage of the finished product Lyophilized. A target titre range of 5.1 log10 ± 0.5 log10 TCID50/mL is proposed for commercial finished product.

Container closure system

The vials used for the Lyophilized finished product are Type I glass vials closed with a bromobutyl stopper and flip-off cap. The specifications and the suitability of the container closure system are described. Materials of vial and stopper comply with respective Ph. Eur. Monographs.

An extractables study has been performed and did not reveal any elements or compounds of concern. Based on the outcome of this study and given the nature of the finished product (lyophilized material) it was concluded that a leachables study was not deemed necessary. This is deemed acceptable.

The container closure system of the water for injections (WFI) solvent is a syringe consisting of a glass barrel, a bromobutyl rubber stopper and a tip cap. Materials used are all compliant with Ph. Eur. Extractables and leachable studies have been performed and demonstrate suitability of the syringes.

The co-packaged prefilled syringe (PFS) containing the WFI is intended for both reconstitution and administration. It is therefore considered an integral medical device. A declaration of conformity (DoC)

has been provided for the syringe device, demonstrating compliance with the relevant General Safety and Performance Requirements (GSPRs) in Annex I of Regulation (EU) 2017/745.

2.4.3.2. Manufacture of the product and process controls

The finished product is released for commercial distribution at Valneva Austria GmbH (Vienna, Austria). All sites involved in manufacturing and controls of the active substance operate in accordance with EU GMP.

Manufacturing of the lyophilized finished product consists of the thawing of active substance, aseptic formulation of finished product by mixing active substance with the Formulation buffer, sterile filtration of finished product bulk, aseptic filling of the finished product bulk into vials followed by lyophilization, visual inspection, labelling and packaging.
Following visual inspection, the lyophilized finished product (packed into storage boxes) can optionally be stored for a specified period of time. Following a hold step the lyophilized finished product is transferred to 5°C ± 3°C, to be thawed before being sampled for release testing. Stability data have been provided. The batch size is defined.

In accordance with the validation protocol, process performance qualification was successfully completed by producing three consecutive full-scale batches of Chikungunya vaccine finished product lyophilized in campaign mode. Process validation studies have been performed for all process steps (including aseptic filling). Furthermore, all in-process controls also met the predefined acceptance criteria for all PPQ batches. Execution of the process performance qualification demonstrated that the Chikungunya vaccine finished product manufacturing process is in a state of control and capable of producing a product that consistently meets pre-determined acceptance criteria and product quality for commercial manufacturing. Also transport validation studies were successfully performed.

The manufacturing process, process controls and raw materials used for production of the sterile WFI solvent has been described. The WFI process has been appropriately validated.

Excipients

Excipients have been properly described. Apart from recombinant human albumin, all other excipients are of pharmacopoeial grade.

Recombinant human albumin (yeast-derived) is not a novel excipient. However, due to its complexity and its biological origin detailed information is provided in section A.3. Product specification

The finished product specifications include tests for appearance/solubility, pH, extractable volume, osmolality, identity (qPCR), Infectious virus concentration ($TCID_{50}$), recombinant human albumin content (SE-HPLC), sucrose content (HPLC), D-sorbitol content (HPLC), L-methionine content (HPLC), residual moisture, bacterial endotoxins, and sterility.

Specifications are set based on current knowledge of the process and capabilities of methods, current limited number of finished product batches and justified by the safety and immunogenicity profile of clinical trials. For the Infectious Virus Concentration, the lower limit of specification has been clinically justified and is deemed acceptable.

Upon request, the acceptance criteria for recombinant human albumin, sorbitol and sucrose in the finished product specification have been tightened to clinically justified levels.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk

assessment it can be concluded that risk for elemental impurities can be considered negligible to non-existing.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed (as requested) considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that risk is negligible or not existing on the possible presence of nitrosamine impurities in the active substance or the related finished product.

Analytical procedures

Analytical procedures used for lyophilized finished product release testing have been established. Also the methods used for release testing of the WFI solvent have been provided.

The applicant is considering the feasibility of transitioning to Ph. Eur. 2.6.32 Test for bacterial endotoxins using recombinant factor C, eliminating the need for horseshoe crab derived material.

Validation of analytical procedures

Lyophilized finished product: The methods for determination of some parameters are compendial methods which have been implemented according to the respective monographs of Ph. Eur. and/or USP. The compendial methods are verified for their intended purpose according to respective pharmacopeial requirements. The non-compendial methods for other parameters were adequately validated with respect to accuracy, precision (repeatability and intermediate precision), limit of detection and quantification, linearity, range, specificity and robustness.

WFI solvent: All methods are compendial methods.

Batch analysis data

Lyophilized finished product batch data were provided for the phase 3 finished product material and the PPQ finished product lots. The results confirm that the phase 3 material is representative of the commercial finished product.

Batch analysis data were also provided for the WFI solvent confirming compliance with specifications.

Reference standards

Reference materials used during the release testing of the finished product have been described. Representative Certificates of Analysis are available.

A protocol for the preparation and qualification of CHIKV RNA Reference Standard (CHIKV_Δ5nsP3_Standard) is included in the dossier.

2.4.3.3. Stability of the product

A 24-month shelf-life is proposed for the lyophilized finished product when stored at the recommended storage condition of 5°C ± 3°C. This shelf life is supported by the results of the primary stability lyophilized finished product lots: the clinical Phase 3 lot and the PPQ Lots.

Primary stability studies included long-term data at 5°C ± 3°C (recommended storage conditions), accelerated data and forced degradation data. All lots remained within specifications when stored at the recommended storage condition. At the accelerated condition, a faster decrease of the virus titre was

noted but the finished product lots remained within specifications. During the forced degradation study finished product lots degraded rapidly and only remained within specifications for about 1 week.

To allow more flexibility from a supply chain point of view, stability data are being generated starting the stability study described above for the primary stability data after an initial hold. This study is currently still ongoing. However, available data as well as supportive stability results from the frozen liquid finished product formulation (phase 1 trials) show that the tested hold time has no impact on the vaccine quality and stability and is thus deemed acceptable.

Based on available stability data, the shelf-life (2 years) and storage conditions (2°C - 8°C) as stated in the SmPC are acceptable.

The applicant has characterised possible virus aggregate formation in finished product at (or close to) the end of shelf life. There was no evidence for any aggregate formation in the finished product.

Stability data were also provided for the WFI solvent. The proposed shelf life is considered acceptable.

In-use data were generated on the reconstituted finished product. The SmPC recommends to use the reconstituted vaccine within 2 hours.

2.4.3.4. Adventitious agents

Adequate information has been provided to demonstrate that the active substance and finished product are manufactured in conditions designed to minimise the risk to TSE Agents. All materials used in the process comply with the Guidance on minimising the risk of transmitting spongiform Encephalopathy agents via Human and medicinal products (EMA/410/01). Adventitious virus agents and microbial adventitious agents are adequately controlled through controls and specifications for starting materials, raw materials and appropriate in-process controls and specifications for active substance and finished product.

2.4.3.5. GMO

For the environmental risk assessment relating to GMO products, see ERA section.

2.4.4. Discussion on chemical, and pharmaceutical aspects

Ixchiq is a live attenuated Chikungunya vaccine based on the La Reunion strain (LR-CHIKV clone LR2006-OPY1) of East Central South African genotype and characterized by a 61 amino acid deletion in its nsP3 viral replicase complex protein (Δ5nsP3). The vaccine is presented as two components: 1) a single-dose vial with the lyophilized powder of live attenuated chikungunya virus (Δ5nsP3) and 2) a solvent consisting of 'sterile water for injections' in a prefilled syringe (PFS).

The active substance and finished product manufacturing processes and process controls are described in detail and are deemed sufficient. The active substance process consists of upscaling of the Vero cell substrate, inoculation of the Vero cells with virus, virus production, virus harvest, virus concentration and purification, and active substance formulation. The finished product process consists of active substance thawing, final bulk formulation, sterile filtration, filling and lyophilisation. Quality of process intermediates is adequately controlled by in-process controls. All manufacturing sites for production and QC testing of active substance and finished product have valid GMP certificates.

The production and qualification of the starting materials, i.e. the Vero cell bank system and the Virus seed system, have been described in detail. Information was provided on raw materials used during production of the active substance and during generation of the MCB/WCB and MSV/WSV starting

materials. For non-compendial raw materials, specifications are in place to control their quality. Apart from foetal bovine serum (FBS) and protamine sulphate (fish origin), no other raw materials of human or animal origin are used in the active substance manufacturing process. FBS has been used during establishment of the cell bank system and virus bank system, and is also used during active substance manufacturing. FBS is covered by TSE Certificates of Suitability issued by EDQM. In addition, FBS is sterile filtered, heat inactivated and gamma-irradiated, and tested for adventitious viruses.

An overview of all in-process control tests was provided. Process development studies were performed to define the optimal process parameters for the upstream and downstream active substance process. The history of process development is summarized. Critical process parameters were identified.

Process validation of the upstream and downstream part of the active substance manufacturing process was performed. Results from PPQ runs showed that the active substance process is properly validated and yields active substance of consistent quality. Impurities are cleared to sufficiently low levels during active substance purification.

The applicant has characterised the virus particle size and virus aggregates as well as product-related impurities. Most virus is present as monomer; only a minor portion are multimers. The size of the virus particles is consistent. Impurities are consistently removed during virus purification to sufficiently low levels. Nevertheless, active substance release testing includes tests for residual BSA, HCP, hcDNA and protamine sulphate. Overall, impurity levels are very low in the active substance.

Active substance specifications and corresponding analytical methods have been described. Methods have been adequately validated. active substance batch data were provided for the phase 3 active substance material and the PPQ active substance lots. The results confirm that the phase 3 material is representative of the commercial product. The proposed active substance specification limits have been justified and are deemed acceptable. The applicant has also described the reference materials that are used during active substance release testing.

The active substance container closure system is a PETG bottle with a HDPE screw cap. Container materials are compliant with USP. Both the bottles and screw caps are sterilised by irradiation. An extractables study was performed which did not reveal any compounds or elements that could pose a safety concern. The container closure system is deemed suitable for active substance storage.

The applicant has provided active substance stability data at long term storage conditions as well as at accelerated and stressed conditions. The currently available active substance stability data support the proposed storage period and storage conditions.

The pharmaceutical development of the finished product manufacturing process was described in detail, including the initial frozen liquid formulation for early clinical studies, the development of a lyophilized finished product formulation for storage at 5°C ± 3°C, the upscaling-up of the finished product process and the comparability analysis between liquid frozen formulation and finished product Lyophilized. Formulation studies were performed to define the optimal composition of the vaccine and the optimal quantities of the excipients. A description was also provided for the container closure system and its compatibility. A risk assessment has been executed on extractables of the container closure; based on the outcome of this assessment and the nature of the finished product (lyophilized material), a leachables study was not deemed necessary. This is deemed acceptable.

The finished product manufacturing process was appropriately validated. Supporting validation studies were provided including validation of aseptic filling of the lyophilized vaccine and validation of sterile filtration. In addition, also cleaning validation of reusable units and equipment as well as validation of holding times and shipment was provided.

The applicant provided specifications for all excipients. No excipients derived from Human or Animal origin are used and no novel excipients are included. Detailed information on the Recombinant Human Albumin (produced in yeast) is provided.

Finished product specifications and corresponding analytical methods have been described. Methods have been adequately validated. Finished product batch data were provided for the phase 3 finished product and the PPQ finished product lots. The results confirm that the phase 3 finished product is representative of the commercial product. The proposed finished product specification limits have been adequately justified. The applicant has also described the reference materials that are used during finished product release testing.

Risk assessment have been performed with regard to the possible presence of elemental impurities and nitrosamines in the finished product. Outcome of the analysis showed that the risk was negligible to non-existing in both cases.

The container closure system for the lyophilized finished product consists of a 2-mL Type I glass vial, a bromobutyl rubber stopper and an aluminium cap with polypropylene closure. Vial and stopper materials are compliant with respective Ph. Eur. monographs.

The applicant provided long term, accelerated and stressed stability data for the phase 3 clinical finished product and the PPQ finished product lots. The available finished product stability data support the proposed shelf life of 2 years when stored at 5°C ± 3°C. At higher temperatures, the potency of the vaccine declines rapidly.

Sterile WFI is used as solvent for the lyophilised finished product. The applicant adequately described the manufacturing process, process controls and raw materials for the WFI solvent. The process was properly validated. Specifications were provided as well as analytical methods and method validations. Specification limits were justified. Batch data were shown which demonstrated compliance with the specifications. The container closure of the WFI is a glass syringe with rubber stopper and tip cap. Container materials are compliant with Ph. Eur. The stability data support the proposed shelf life for the WFI in PFS.

The co-packaged prefilled syringe containing the solvent is used for reconstitution and administration and is therefore considered an integral medical device. A major objection was raised requesting the applicant to confirm full compliance with the relevant General Safety and Performance Requirements (GSPRs) in Annex I of Regulation (EU) 2017/745, in the form of a Declaration of Conformity, a CE certificate or a Notified Body opinion prior to the opinion. The applicant has provided a Declaration of Conformity confirming compliance with the relevant General Safety and Performance Requirements (GSPRs) in Annex I of Regulation (EU) 2017/745.

In conclusion, all issues have been adequately addressed. Therefore, based on the review of the quality data provided, the marketing authorisation application for Ixchiq vaccine is approvable from the quality point of view.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product, which pertain to additional testing of the WCB, mycobacteria testing on the unprocessed harvest and photostability study. These points are put forward and agreed as recommendations for future quality development.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical

performance of the product have been investigated and are controlled in a satisfactory way and comply with existing guidelines.

2.4.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends some points for investigation.

2.5. Non-clinical aspects

2.5.1. Introduction

Non-clinical studies have been conducted to characterize the pharmacology and toxicology profile of VLA1553. Pharmacology data were obtained from in vitro and in vivo studies which employed rodent and non-rodent species (non-human primates (NHPs)). To determine a surrogate of protection for VLA1553, a passive transfer study was conducted in cynomolgus macaques, and a passive transfer study in immunocompromised C57BL/6 mice assessed the quality of the immune response of individual human serum samples post-vaccination. Another study employed cynomolgus macaques to evaluate the potential of viral persistence of VLA1553. Furthermore, the genetic stability of VLA1553 was confirmed with human serum samples taken post-vaccination from the Phase 1 clinical trial VLA1553-101 subjects which were analyzed by Sanger sequencing. The transmission potential of VLA1553 through *Aedes albopictus* mosquitoes was also evaluated.

The toxicological program included repeat-dose studies in rabbits as well as pre- and post-natal development studies in rats. These pivotal toxicity studies were conducted in accordance with GLP regulations. All findings were transient and resolved within the 30 days recovery period, thus, there were no adverse findings.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Passive transfer studies: determination of surrogate of protection for VLA1553

Valneva conducted passive transfer studies in two animal models. In the **non-human primate model**, pooled human serum was used at different levels, allowing the selection of a neutralizing antibody titre as a surrogate of protection. An **immunosuppressed mouse model** offered complimentary data with survival following a lethal VLA1553 challenge as read out for protection, and the possibility to assess individual human sera from vaccinated individuals for comparability of antibody quality, which was not feasible in NHPs due to insufficient serum volumes.

Non-human primate model (NHP)

To establish a neutralizing antibody titre threshold for protection following VLA1553 vaccination, a passive transfer study (Study No. **VAC1816-02**) was conducted in NHPs (cynomolgus macaques; *Macaca fascicularis*) using human sera derived from study participants of the Phase 1 clinical trial (VLA1553-101). NHPs received human non-immune serum (control) or pooled VLA1553-101 serum from various time points following VLA1553 vaccination (Days 14, 28, 84, and 180) by intravenous

(i.v.) injection in the saphenous vein (3 mL/kg). There were 3 males and 3 females in the control group and 20 males and 20 females in the other experimental groups.

Experiments were performed in 4 rounds, each depending on the outcome of the previous round. The first two rounds used day 28 post-VLA1553 vaccination sera, where groups of 5 animals were exposed to ultralow, low, medium or high titre pooled human serum (ULS, LS, MS, HS). The third round was performed with sera derived from day 14 and day 84 and the fourth round was performed with sera from day 180 post-VLA1553 vaccination. Following sera administration, the animals were challenged with a high dose (7,000 to 10,000 plaque-forming units (PFU)) of WT CHIKV (LR006-OPY1; the parent strain of VLA1553; 100 AID_{50} (50% animal infectious dose) via subcutaneous administration. The challenge dose used was higher than the dose individuals may encounter when bitten by *Aedes aegypti* or *Aedes albopictus* mosquitoes.

The endpoints measured include quantitative measurements of neutralization titres (50% micro plaque reduction neutralization titre (µPRNT50)) in NHPs after serum transfer but prior to WT CHIKV challenge, monitoring viraemia and clinical parameters which include body temperature and blood cell counts as well as inflammation markers, viral load in tissues, and histopathology of joints. The threshold for protection was determined based on $\mu PRNT_{50}$ titres determined in NHPs after serum transfer and protection from viraemia and fever. The µPRNT assay for measurement of neutralizing titres in NHPs prior to challenge was also used for the phase 3 clinical trial VLA1553-301. This assay and the RT-qPCR to detect viral load post challenge were retrospectively validated.

CHIKV RNA was detected via RT-qPCR as early as Day 1 post-exposure (p.e.) and peaked on Days 2 to 3 in NHPs which had received the human non-immune serum. In comparison, NHPs treated with VLA1553-101 sera resulted in at least 3-5 log lower viraemia levels (maximum of ~3.5×10^5 GCE (genome copy equivalents)/mL in an animal that received d28 ULS), with no virus detected at all in 4 out of 5 animals that received d28 HS.

Presence of replicating CHIKV in NHP plasma samples after challenge was assessed in a $TCID_{50}$ assay, as part of a retrospective study (report RR-0089-02). None of the tested vaccinated animals showed any replicating CHIKV in the $TCID_{50}$ assay with pre-amplification step in mosquito cells (16/40 animals tested). In control animals for which the $TCID_{50}$ assay without pre-amplification step was most valuable as used for the highest number of samples (4/6), replicating CHIKV was detected at the peak viraemia in all tested samples.

To determine the potential of VLA1553-101 serum transfer to protect NHPs from developing clinical symptoms after WT CHIKV challenge, clinical parameters were also evaluated. For NHPs receiving nonimmune human sera prior to WT CHIKV exposure, fever was persisting from d1 to d7. In contrast, NHPs treated with pooled VLA1553-101 sera resulted in little (non-significant increase in animals receiving d28 ULS) to no increase in body temperature. Control animals showed lymphopenia and neutrophilia post-challenge. These clinical symptoms were consistent with the observed plasma viraemia and increased body temperature, and they were synchronized with the CHIKV RNA peak. In contrast, in all VLA1553 phase I serum–treated NHPs, such an effect was not observed, except for 1 animal treated with ULS d28 prior to WT CHIKV challenge that showed signs of low lymphopenia. This animal also showed the highest viraemia peak at 3.5×10^5 copies/mL among all animals treated with VLA1553 phase I serum.

All VLA1553-101 serum treated NHPs were protected from strong inflammatory responses as observed in the control animals, with the exception of some animals receiving the human d28 ULS and LS serum pools. In these animals, a significant expression (e.g. of IL-1Ra, MCP-1 and/or IL-8) was observed but delayed in time and at a lower expression level than in animals of the control group.

Viral load in selected tissues on d28 post-challenge was assessed by RT-qPCR analysis. For NHPs treated with the non-immune serum, CHIKV RNA was detected in all lymph nodes, spleen and muscle tested, while one animal also had detectable CHIKV RNA in the liver. Moreover, CHIKV RNA was detected sporadically in the capsula of the knees or the reproductive tract. In comparison, NHPs treated with VLA1553-101 sera were generally protected from CHIKV persistence, with the level of CHIKV RNA below the limit of detection or at titres ~2 to 5 log lower than in control animals across the analyzed tissues, expect for one animal that received d14 serum where RNA was detectable at levels comparable to control.

Histopathology analysis of joint tissues from all animals after sacrifice was also conducted, showing that joint lesions were rare, with minimal chondral erosion, mixed ligament and synovial infiltrations observed, without any group correlation noted. Neither control animals nor vaccinated animals showed typical CHIKV-induced chronic arthritis.

To derive a threshold for protection based on the NHP passive transfer study, the Applicant initially chose to examine the relationship between the neutralizing antibody titre before challenge (as measured by $\mu PRNT_{50}$) and protection from viraemia, defined as infectious particles. For the calculation of the protective titre the total RNA level was therefore converted into $TCID_{50}$ using a conversion factor of 200. This was considered important since the measurement of RNA copies (GCE/mL) by RT-qPCR does not only reflect the presence of live, replicating virus, but also non-viable virus particles, whereas the $TCID_{50}$ measurement determines only viable virus capable of infecting Vero cells. Besides the conversion factor converting measured viral genome copies to calculated estimates for replicating virus (set at 200 GCE/ml ~1 $TCID_{50}$/ml), the calculation of the surrogate is also influenced by cut-off viraemia, which was set at ≤ 150 $TCID_{50}$/mL. The protective threshold of a $\mu PRNT_{50} \geq 50$ corresponded to a titre at which ≥ 90% of NHPs were regarded as protected (assuming a viraemia threshold of ≤ 150 $TCID_{50}$/mL). At this titre, animals were protected from fever and no replicating virus was detected by $TCID_{50}$ assay.

However, upon interactions with regulators, the Applicant agreed to reconsider the threshold for protection. Complete prevention of viraemia, i.e. undetectable viral RNA (below the detection level of the RT-qPCR assay) based on the applied sensitive RT-qPCR assay, was proposed as evidence of protection. With this new definition of protection there is no need to include the conversion factor (200 GCE ~1 $TCID_{50}$) and the conservative but arbitrary cut-off viraemia of ≤150 $TCID_{50}$/mL for calculation of the surrogate. This strategy led to the selection of a $\mu PRNT_{50}$ titre of ≥ 150 as a surrogate of protection based on the NHP passive transfer study. The proposed surrogate of protection can be considered as conservative, as it is considerably higher than the titre required to protect animal from fever or detection of replicating CHIKV by $TCID_{50}$ assay.

The surrogate of protection based on the NHP passive transfer study is supported by a second independent source of evidence. Yoon et al. (2015, 2020) presented data generated with sera derived from a seroepidemiology Philippine Cohort study and concluded that detectable CHIKV neutralizing antibody titre at baseline may correlate with protection from symptomatic CHIKV infection and subclinical seroconversion, supporting the potential use of the neutralizing antibody titre as a surrogate endpoint for protection. The surrogate of protection identified in this study was a $PRNT_{80}$ titre ≥ 10. A number of samples were reanalysed with Valneva's validated $\mu PRNT_{50}$ assay, allowing translation into a $\mu PRNT_{50}$ titre ≥ 139.3 even when applying the most stringent conditions; i.e. using the highest individual $\mu PRNT_{50}/PRNT_{80}$ ratio. The proposed threshold of $\mu PRNT_{50}$ titre ≥150 also largely exceeds (3-fold) the titre of 48.7, obtained when applying the upper boundary of the 99% confidence interval around the geometric mean ratio (GMR) of $\mu PRNT_{50}/PRNT_{80}$ (3.73; 99%CI 2.86-4.87) to the $PRNT_{80}$ titre of ≥10 (see section 3.3. Clinical Aspects).

The aim of the investigations summarized in Study No. **RR-0089-02** was to confirm the conversion factor of 200 used in the NHP passive transfer study to convert viraemia measured by RT-qPCR and expressed in GCE/ml to $TCID_{50}$/ml, as Valneva initially chose to define protection from viraemia by the calculated infectious particles (see above). Several $TCID_{50}$ assays (in BHK-21 or Vero cells, with and without pre-amplification step in mosquito cells) were used to determine the conversion factor of GCE/mL to $TCID_{50}$/mL specifically for the for WT CHIKV used in the NHP passive transfer study, in culture medium and in NHP plasma. The obtained conversion factors (GCE/mL to $TCID_{50}$/mL) from analyses of: (1) the virus stock LR2006-OPY1 used for challenge in Study No. VAC1816-02, (2) NHP plasma from Study No. VAC1816-02 control animals and (3) from WT CHIKV–- spiked NHP plasma samples, support the selected conversion factor of 200 initially used by Valneva in the NHP passive transfer studies to calculate the protective titre. However, as indicated above, CHMP has expressed its preference during scientific advice procedures, to avoid the use of calculated viraemia levels and an arbitrary (although conservative) cutoff for viraemia. The conversion factor is no longer relevant in view of the final strategy to derive a surrogate of protection from the NHP passive transfer study, based on absence of genomic RNA.

Immunosuppressed mouse passive transfer model

In the immunosuppressed mouse passive transfer model (study **RR-0065-01**), the interferon alpha and beta receptor subunit 1 (IFNAR-1) was inhibited by the monoclonal antibody (mAb), MAR1-5A3, which led to blockade of the type I IFN signalling cascade and immunosuppression in mice. Consequently, mice should become susceptible to infection with CHIKV, since lack of type I IFN response allows for viral replication.

Samples (serial dilutions of sera or CHIK-specific monoclonal antibody) were administered intraperitoneally at day -2, monoclonal antibody MAR1-5A3 was administered intraperitoneally at day -1 and subcutaneous challenge with VLA1553 occurred on d0 (10^4 50% tissue culture infectious dose ($TCID_{50}$)). Survival of the animals was monitored for 14 days.

Human sera derived from Phase 1 clinical trial study participants 12 months after VLA1553 vaccination with similar neutralizing antibody titres (μNT_{50}) were transferred to assess person-to-person differences regarding antibody quality and their capacity to protect from lethal VLA1553 challenge. Serial dilutions of 10 human serum samples were injected in the mice, and the 50% protective titre (PT50) was calculated and compared to the result for serum derived from a previously infected individual. The study demonstrated a correlation between titres of human VLA1553-101 sera and survival with comparable levels of protection across the human sera tested, with the highest percentage of survival achieved at higher titres. There are no significant differences between the individual sera in terms of PT_{50} observed. Similarly, the CHIKV IgG positive serum from a naturally (wild type (WT) CHIKV) infected individual (with comparable μNT_{50}) protected mice at similar titres as 12 months post-vaccination sera from Valneva's Phase 1 study VLA1553-101.

In conclusion, the antibody quality in terms of protective neutralizing antibody titre induced by VLA1553 in individual human subjects is comparable at similar μNT_{50} titres. This is demonstrated in the immunosuppressed mouse passive transfer model where no significant differences in terms of protection were observed when individual human sera were compared. In addition, a transferred human serum sample from a convalescent subject after natural wild type CHIKV infection showed a very similar level of protection in this passive transfer mouse model.

Cross-protection to heterologous CHIKV strains has not been specifically addressed in non-clinical development. Challenge in the mouse and NHP passive transfer studies was done with VLA1553 and a homologous (ECSA) strain (LR2006-OPY1, the parent strain VLA1553 is derived of) respectively. Protection against a range of heterologous CHIKV strains was addressed in *in vitro* neutralisation assays with human post-vaccination serum samples (see section 2.6. Clinical Aspects).

Genetic stability of VLA1553

The genetic stability of VLA1553 was tested in vitro and in vivo.

After up to 20 in vitro passages on Vero cells, the 183-nucleotide deletion in nsP3 and the linker substituting the deleted viral sequence remain unchanged. The risk of reversion due to second site mutations in the genome, that might complement the deletion in the nsP3 gene, was assessed, and showed sequence heterogeneities in non-structural proteins (nsP2, nsP3 and nsP4) and E2. The occurrence of minor sequence heterogeneities led to a reduction of immunogenicity in mice with no evidence of compensating the effect caused by the 61 amino acid deletion in nsP3. For this reason, the number of passages in Vero cells is limited to 3 during vaccine production, which is agreed.

For in vivo testing, the genetic stability and emergence of second-site mutations in the Δ5nsP3-deleted region of VLA1553 using sera from study participants of the Phase 1 clinical trial VLA1553-101 (Study No. **RR-0048-02**) were evaluated using Sanger sequencing analysis of the nsP3 CHIKV region. Analysis of 20 human sera confirmed the Δ5nsP3-deletion and presence of the substituting linker sequence, which is analogous to the sequence of VLA1553 administered to the study participants. Sequencing revealed no mutations or sequence heterogeneity in the region of interest in serum samples derived from 2 study participants. Sequence heterogeneities identified outside the region of deletion (nsP1, nsP2 or C) were detected at negligible levels or attributable to an artifact.

In conclusion, there is no indication that replication of the VLA1553 vaccine virus in human subjects leads to the accumulation of mutations, which could ameliorate the attenuation caused by the deletion in the nsP3 gene.

2.5.2.2. Secondary pharmacodynamic studies

As part of an overall environmental risk assessment to evaluate the potential for transmission of the VLA1553 virus from vaccinated to non-vaccinated individuals by mosquitoes, studies were conducted which evaluated the transmission potential of VLA1553 to mosquitoes (Study No. **RR-0066-01**). The aims of the studies were to assess the virus uptake and dissemination profile of VLA1553 to the body and salivary glands of *Aedes albopictus* mosquitoes compared to the WT CHIKV strain (LR2006-OPY1) and to determine a threshold titre at which VLA1553 is no longer detectable in mosquito salivary glands, thus cannot be transmitted to another host. A small-scale experiment with immunocompromised mice also evaluated the transmissibility of WT CHIKV and VLA1553 from mosquitoes to mammalian hosts.

Briefly, using an in vitro membrane feeding method, female *Aedes albopictus* mosquitoes were fed on defibrinated sheep blood spiked with the wild-type (WT) CHIKV strain (LR2006-OPY1) or VLA1553 resulting in a high blood meal titre (>7 log10 TCID50)/mL (3 independent experiments)) or a range of decreasing titres of VLA1553 (4, 5 and 6 log10 TCID50/mL; 3 independent experiments). Mosquito bodies and saliva were analysed for the presence of CHIKV.

The study results indicate a low potential for VLA1553 transmission by mosquitoes because (1) a lower percentage of VLA1553 positive mosquitoes salivary glands after feeding at a high blood meal titre compared to WT CHIKV was observed; possibly attributable to VLA1553' s reduced replication capacity, and (2) the minimum blood meal titre of ≤ 3.875 log10 TCID50/mL required for VLA1553 transmission as determined in this study is significantly higher than the level of viraemia detected in vaccinated humans in Phase 1 clinical trial (mean peak viraemia of 8.9 x 104 GCE/mL by RT-qPCR, ~ 2.5 log10 TCID50/mL).

A small-scale experiment with immunocompromised mice suggests reduced transmissibility of VLA1553 compared to WT CHIKV, but the impact of the study is considered low.

The provided data propose a viraemia threshold at below which dissemination via mosquitoes is unlikely. The limitations of the studies include the numbers of mosquitoes tested for presence of virus (body/saliva) and number of mice exposed to fed mosquitoes. Moreover, this threshold is compared to mean viraemia observed in phase I trial participants, while maximum exposure would be more relevant, and more data (phase III) are available since. However, additional non-clinical testing is not deemed necessary to reach a higher degree of certainty.

It is agreed that the available data do not raise concerns regarding VLA1553 dissemination through mosquitoes feeding on a vaccinated subject and appears unlikely, but the study does not have the power to exclude it.

2.5.2.3. Safety pharmacology programme

No safety pharmacology studies were performed, which is acceptable in accordance with guidance on non-clinical development of vaccines.

2.5.2.4. Pharmacodynamic drug interactions

No studies on the pharmacodynamics drug interactions have been performed, which is in accordance with applicable guidelines.

2.5.3. Pharmacokinetics

A dissemination and persistence study, PHY1802-02 in NHP was provided. This study has shown that the administration of a high dose (3.2×10^6 $TCID_{50}$) of live-attenuated CHIKV VLA1553 to NHP, administered at a 2 logs higher dose than used in the Phase 3 clinical trials, was associated with only a short period of viraemia 3 logs lower than observed for WT CHIKV and induced only a limited inflammation detectable in peripheral blood. Despite a dispersion of VLA1553 through the blood circulation and demonstration of low amounts in tested tissues (the main target tissues of CHIKV were selected for testing) and saliva or vaginal fluid at early time points, mostly several logs lower than for WT CHIKV, no significant viral persistence could be observed except for immune organs.

VLA1553 RNA was detected in the spleen from day 2 to day 90 p.e., but RNA levels were 2 logs lower than in WT CHIKV exposed animals and replicating VLA1553 ($TCID_{50}$) was only detected on day 2 p.e. In the axillary lymph node (draining lymph node close to the inoculation site), VLA1553 RNA was consistently detected until day 90, but already on day 6 p.e. no replicating VLA1553 could be detected. Due to the expected and observed strong neutralizing immune response induced by exposure to VLA1553, it was also considered very unlikely that replicating VLA1553 would be present on day 90 in the axillary lymph node. VLA1553 RNA was not detected in any brain tissue (encephalon, mesencephalon, cerebelum, plexus choroïd), whereas WT CHIKV RNA was detected in the choroid plexus on day 6 p.e. at low titres. The study suggests that the risk for neurovirulence of VLA1553 is low.
In conclusion, no concerns in regard to safety could be identified in this study.

2.5.4. Toxicology

The toxicological assessment of VLA1553 consisted of two Good Laboratory Practice (GLP) compliant studies, namely, a repeat-dose toxicology study in rabbits, and a pre- and post-natal developmental study in rats.

No fertility, carcinogenicity, genotoxicity or mutagenic potential studies were performed which is acceptable.

The applicant did not perform a neurological assessment. However, the results of the biodistribution and persistence study in NHP discussed in section 2.5.3. indicate that VLA1553 disseminated through blood circulation in the body with no VLA1553 RNA detected in the brain while WT CHIKV RNA was detected in the choroid plexus at low titre on day 6 post-exposure. It was agreed during a CHMP Scientific Advice (EMADOC-1700519818-731365, dated 14/10/2021) that the results of this study provide sufficient evidence that the vaccine candidate does not represent an increased risk regarding neurovirulence. Further neurovirulence testing was therefore not considered necessary.

2.5.4.1. Repeat dose toxicity

In the repeat dose toxicity study (study No. 505078), two intramuscular injections of 3.8 x 10^5 TCID50/dose of VLA1553 at a 2-week interval to rabbits were well-tolerated both systemically and locally. VLA1553 was associated with transient inflammatory response and reactions at the administration sites. Those findings resolved after a 30-day recovery period. The dose, schedule and route of administration cover those proposed for administration in human. The presence of neutralizing anti-Chikungunya virus antibodies was assessed in a Virus Replicon Particle (VRP)- based neutralization assay, confirming the suitability of the rabbit species for the toxicological assessment. The lot used in this repeat dose toxicity study is batch #B3007457. This lot is comparable to the Phase 1 material (liquid formulation). The comparability between the liquid and lyophilised presentations (phase 3 and commercial lots) has been confirmed. The excipients used in the formulation of the Chikungunya vaccine are not considered to be novel and therefore, no further studies are required.

2.5.4.2. Reproductive and developmental toxicity

In the pre- and post-natal developmental study (study No. 490901), two administrations of VLA1553 at the intended human dose of 1.9 × 10^4 TCID50/0.5 mL by intramuscular injections in female rats, once before mating and once in early organogenesis (Gestation Day 6) were well-tolerated. In addition, there was no adverse effects noted in the dams, or in developing fetuses or pups. There were no relevant test item effects on delivery or on reproductive parameters. All animals treated with VLA1553 developed VLA1553-specific antibodies also detected in the milk of the VLA1553-treated female rats and in sera obtained from their offspring as early as during the embryofoetal development. The batch used was lot #2005040029, which was used in the Clinical Phase 3, pivotal study, VLA1553-301.

2.5.5. Ecotoxicity/environmental risk assessment

Ixchiq, a live attenuated chikungunya virus strain, has been obtained by reverse genetics of the parent LR2006-OPY1 CHIKV strain resulting in a deletion of 61 amino acids. No other foreign genetic material or genes expressing antibiotic resistance are present in the viral genome.

CHIKV Δ5nsP3 can be passaged up to 20 times on Vero cells with no changes in the deletion responsible for the attenuation of CHIKV Δ5nsP3. The stability of VLA 1553 has been assessed in vitro and in vivo and no second - site mutations were revealed during replication in human that could overcome the attenuation by the 61 amino acid deletion. The observed E168K heterogeneity following cell culture passaging was found to lead to further attenuation based on findings in mice.

Wild-type Chikungunya virus (CHIKV) is an enveloped, positive-sense RNA arbovirus of the genus Alphavirus (family Togaviridae) of which the dispersal relies on vector-borne transmission by mosquito

species. Therefore, information on Ixchiq related to the degree of viraemia in a vaccinee and the ability of mosquitoes to transmit Ixchiq are key to evaluate its potential for dissemination in the environment. Regarding the first aspect, Ixchiq replication capacity in non-human primates was shown to be reduced by 3 to 4 logs and was cleared more readily than observed for wild-type CHIKV. In a phase I clinical trial a transient mean peak viraemia level was measured at 8.9×104 GCE/mL (measured using RT-qPCR) which the applicant estimates to be equivalent to approximately 2.5 log10 CCID50/mL. It was also demonstrated that viraemia peaked at Day 3 (with clearance after D14) which leaves a limited time window after vaccination at which viraemia may be sufficiently high in humans for a possible transmission of Ixchiq to mosquitoes. With respect to mosquito transmission studies, data suggest a less efficient infection of *A. albopictus* for Ixchiq vaccine virus than for wild-type CHIK virus (approximately 1 log lower body titre). Also, infection of mice by mosquitoes fed on Ixchiq blood meals was not observed (0/3 mice), whereas for wild-type CHIKV all 3 of 3 mice were infected. Furthermore, by analysing saliva of mosquitoes fed on descending blood meal titres, the applicant expects the minimum titre required in salivary gland for transmission to be higher for Ixchiq as compared to the virus titre determined for CHIKV of 7.1 log10CCID50/mL.

In summary, considering the viraemia data and mosquito transmission studies, the probability of mosquitoes transmitting Ixchiq from a vaccinee appears to be low to negligible, since the level of viraemia after vaccination in humans was lower than the minimum titre required in a blood meal to allow transmission by mosquitoes.

Analysis of virus shedding data in non-human primates indicates the presence of VLA1553 (Ixchiq) in saliva and vaginal fluids and not in urine, whereas analysis of urinary shedding was detected in one study participant of the Phase 1 clinical study (1.1×104 GCE/mL) at a single timepoint (Day 7 after vaccination). However, should shedding occur, it will not contribute to the dissemination in human population as alphaviruses are fragile, lipid-enveloped viruses, sensitive to desiccation and thus relatively unstable outside their hosts. Moreover, mosquitoes do not feed on urine. The presence of Ixchiq in semen has not been studied. However, Martins et al., 2022 reported the presence of wt CHIKV RNA in 14,3% of semen samples up to 56 days after symptom onset and suggested, yet without proof of presence of infective particles, the potential for sexual transmission. Without data on the presence of Ixchiq in semen, uncertainty remains as to whether there is a possibility of sexual transmission.

An uncertainty remains as to whether recombination events could occur in vivo upon simultaneous presence in one cell of VLA1553 and replicons harbouring replicase encoding genes originating from alphaviruses, such as self-amplifying mRNA replicons used as a vaccine (e.g. ARCT-154) or for cancer therapy. Although the likelihood of simultaneous presence in one cell is currently deemed negligible owing to the small number of marketing authorized samRNA vaccines, the theoretical concern is that potential in vivo recombination and/or complementation events could possibly lead to (partial) complementation of the attenuated phenotype of VLA1553 and/or the formation of new and uncharacterized viral particles with unknown properties. The biodistribution or persistence of VLA1553 or samRNA is not fully characterised in human, hence co-localization in one cell (like for example in lymph nodes or spleen) can currently not be ruled out if both are administered in one single host. If the administration of samRNA based (investigational) medicinal products to receivers of VLA1553 becomes likely (for example when more medicinal products based on samRNA technology get authorized on the market), it should prompt a reconsideration of the environmental risk assessment focusing on biodistribution and persistence properties and likelihood of interaction between both VLA1553 and (investigational) medicinal products using samRNA technology. This reconsideration could lead to a precautionary measure such as the implementation of a safety time interval between administration of VLA1553 and the samRNA replicon in order to minimize the likelihood of having both entities present in one cell.

Considering all of the above elements, the overall risk linked to the intended use of Ixchiq for both humans and the environment is considered negligible. If a possible presence of infectious particles of Ixchiq in semen can be demonstrated or when a simultaneous presence in a cell with samRNA becomes likely, this may prompt to consider precautionary measures to alleviate any concern regarding possible sexual transmission or the formation of uncharacterized and harmful viral particles, respectively.

2.5.6. Discussion on non-clinical aspects

The applicant conducted passive transfer studies in two animal models, using human sera derived from study participants of the phase I clinical trial VLA1553-101. In the NHP model, pooled human serum was used at different levels, allowing the selection of a neutralizing antibody titre as a surrogate of protection. An immunosuppressed mouse model offered complimentary data with survival following a lethal VLA1553 challenge as read out for protection, and the possibility to assess individual human sera from vaccinated individuals for comparability of antibody quality, not feasible in NHPs.

To derive a threshold for protection based on the NHP passive transfer study, the relationship between the neutralizing antibody titre before challenge and protection from viraemia post-challenge was examined. A µPRNT50 titre $\geq$ 150 was associated with protection against wild-type CHIKV infections, defined by absence of viraemia in the 14 days following challenge, i.e. no detectable viral RNA (<LOD in the RT-qPCR assay). The applicant initially proposed to derive a protective threshold based on $\geq$ 90% of NHP protected from viraemia, defined by lack of infectious particles ($TCID_{50}$). These $TCID_{50}$ values are not measured but calculated from measured RNA levels using a conversion factor (set at 200 GCE ~1 $TCID_{50}$; justification provided in a separate study report (RR-0089-02)), and a cutoff threshold for viraemia was applied ($TCID_{50}$ >150). A protective threshold of a µPRNT50 $\geq$ 50 was derived, but the Applicant agreed with the more stringent approach described above, during scientific advice.

The surrogate of protection based on the NHP passive transfer study is supported by a second independent source of evidence, a seroepidemiology study described by Yoon et al. (2015, 2020). The strategy to obtain a surrogate marker for protection to be used in phase III has been discussed and agreed by CHMP during several scientific advice procedures. Given the existing sero-epidemiological information on neutralizing antibody titres correlating with human protection against symptomatic CHIKV disease, it was highly recommended to include these data to determine a threshold value to infer protection from the phase III immunogenicity data. While recognizing the limitations of both approaches, it is considered supportive that the correlate of protection identified in the NHP passive transfer study and based on the seroepidemiology data are of the same order of magnitude. As the threshold (1) has been set to correlate with the absence of viral RNA in the NHP passive transfer study, and (2) is 3- fold the $\mu PRNT_{50}$ titre derived from the sero-epidemiology data taking into account the upper-limit of the 99% CI around the GMR of the validated $\mu PRNT_{50}$ vs the $PRNT_{80}$ of AFRIMS used by Yoon et al., it is considered conservative. Therefore, CHMP agreed that a $\mu PRNT_{50}$ titre $\geq$150 (based on total absence of viral RNA) is a marker that is reasonably likely to predict clinical benefit against infection with CHIKV.

Both in the NHP passive transfer study (Study No. VAC1816-02) and the persistence study (Study No. PHY1802-02), infection with WT CHIKV in the control animals led to rather modest signs compared to literature. Rash and other clinical symptoms were not observed in the WT CHIKV exposed control animals in the persistence study or in the NHP passive transfer study. There does not seem to be a clear explanation for the observed differences based on the source or dose of the WT CHIKV, but the route of administration cannot be excluded to play a role. In the context of the determination of a surrogate of infection, sterilizing immunity (absence of RNA, below LOD) was chosen as a marker for protection, and this is considered a stringent protection criteria.

The humoral and cellular responses immune responses in mice and non-human primates (NHPs) have been assessed after s.c. vaccination with CHIKV Δ5nsP3, as published by Hallengärd et al., 2014 and Roques et al., 2017. Immunization with a single dose of VLA1553 generated high titres of binding and neutralizing antibodies. The strong humoral immune responses induced by VLA1553 were comparable to WT infection, and were long-lasting. In mice, vaccination induced similarly high CD8+ T cell responses as WT infection. In NHPs, T cell responses generated after vaccination slowly declined, and after challenge, there was no clear expansion of T cells. The observed T cell responses were consistent with the observed antibody responses.

The passive transfer studies support the important role of neutralizing antibodies for protection, as do human sero-epidemiology data. T cells have been suggested to play only a limited role in protection against CHIKV infection (Chu et al., 2013), but both, CD4 and CD8 T cell subsets, are required to develop functional and long-lived immune responses and to clear CHIKV-infected cells, respectively (Lum et al., 2013, Chu et al.,2013, Swain et al., 2012). Hence, the strong T cell response elicited by VLA1553 probably plays a significant role in the formation of the long-lived protective antibody response.

Non-clinical data reveal no special hazard for humans based on conventional studies of repeated dose toxicity, local tolerance, and pre and post-natal developmental studies, all conducted according to GLP requirements and the relevant WHO and EMA guidelines. Both the rabbit and the rat species showed an immune response to the vaccine.

No fertility, carcinogenicity, genotoxicity or mutagenic potential studies were performed which is acceptable.

Finally, the applicant did not perform neurovirulence testing, as the results of the biodistribution and persistence study in NHP did not show the presence of the VLA1553 in the CNS in non-human primates. This was agreed during scientific advice.

The overall environmental risk linked to the intended use of Ixchiq for both humans and the environment is considered negligible. If a possible presence of infectious particles of Ixchiq in semen can be demonstrated or when a simultaneous presence in a cell with samRNA becomes likely, this may prompt to consider precautionary measures to alleviate any concern regarding possible sexual transmission or the formation of uncharacterized and harmful viral particles respectively.

2.5.7. Conclusion on the non-clinical aspects

The pharmacology and toxicology studies performed are in line with the WHO and EMA guidelines, supporting the dose, dosing schedule and route of administration of VLA1553 to humans. The applicant has provided acceptable responses to the concerns raised by the Committee; therefore, the non-clinical package is considered acceptable in support of the MAA.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

The current application includes data from three completed clinical studies conducted in generally healthy adults in the US (VLA1553-101, VLA1553-301, and VLA1553-302), and an ongoing long-term extension study (VLA1553-303) conducted in healthy adults in the US. Preliminary (Day 29) safety and immunogenicity data from a study conducted in adolescents (VLA1553-321) in Brazil were also provided.

A list of the completed and ongoing clinical studies conducted to support the marketing authorization of VLA1553 is provided in Table 1.

Table 1. List of Clinical Studies Supporting Efficacy of VLA1553 as Active Immunization for the Prevention of Disease Caused by CHIKV

Study ID	Number of Sites, Location	Study Dates, Status	Study Design and Type of Control	Treatments	Number of Participants Per Arm [1]	Gender M/F Age Median (Range)	Primary Endpoint(s)
VLA1553-301	43, United States	First participant in: 17-Sep-2020 Last participant out: 15-Oct-2021 Completed	Placebo-controlled, randomized, double-blinded, multicenter	**VLA1553**: 1×10E4 $TCID_{50}$ per 0.5 mL	3,093 (3,045 planned) Immunogenicity subset: 375	1,864 M / 2,251 F 45.0 years (18-94)	Proportion of participants with a seroprotective CHIKV antibody level defined as $\mu PRNT_{50} \geq 150$ for baseline negative participants 28 days post-vaccination
				Placebo: 0.5 mL	1,035 (1,015 planned) Immunogenicity subset: 126		
VLA1553-302	12, United States	First participant in: 22-Feb-2021 Last participant out: 10-Dec-2021 Completed	Lot-to-lot, randomized, double-blinded, multicenter	**Lot 1 VLA1553**: 1×10E4 $TCID_{50}$ per 0.5 mL	136 (134 planned)	185 M / 223 F 34.0 years (18-45)	GMT of CHIKV-specific neutralizing antibodies as determined by µPRNT assay on Day 29 post-vaccination.
				Lot 2 VLA1553: 1×10E4 $TCID_{50}$ per 0.5 mL	137 (134 planned)		
				Lot 3 VLA1553: 1×10E4 $TCID_{50}$ per 0.5 mL	136 (134 planned)		
VLA1553-101	3, United States	First participant in: 05-Mar-2018 Last participan	**Sentinel dosing**: Dose-escalation, open-label, single center	**VLA1553 low dose**: 3.2×10E3 $TCID_{50}$ per 0.1 mL + re-vaccination at Month 12	31 (30 planned) Sentinel: 5 Randomized: 26	106 M / 14 F 33.0 years (19.0-45.0)	Frequency and severity of solicited injection site and systemic reactions within 14 days of

Study ID	Number of Sites, Location	Study Dates, Status	Study Design and Type of Control	Treatments	Number of Participants Per Arm [1]	Gender M/F Age Median (Range)	Primary Endpoint(s)
		t out: 23-Jul-2019 Completed	**Randomized dosing**: Three parallel doses, randomized, observer-blinded, multicenter	3.2×10E5 TC ID_{50} per 1 mL			the single vaccination.
				VLA1553 medium dose: 3.2×10E4 TC ID_{50} per 1 mL + re-vaccination at Month 12 3.2×10E5 TC ID_{50} per 1 mL	30 (30 planned) Sentinel: 5 Randomized: 25		
				VLA1553 high dose: 3.2×10E5 $TCID_{50}$ per 1 mL + re-vaccination at Month 6/12 3.2×10E5 $TCID_{50}$ per 1 mL	59 (60 planned) Sentinel: 10 Randomized: 49		

Study ID	Number of Sites, Location	Study Dates, Status	Study Design and Type of Control	Treatments	Number of Participants Per Arm [1]	Gender M/F Age Median (Range)	Primary Endpoint(s)
VLA1553-303	10, United States	First participant in: 02-Apr-2021 Last participant completing Year 1: 15-Apr-2022 Ongoing	Open-label uncontrolled	**VLA1553**: 1×10E4 $TCID_{50}$ per 0.5 mL (dosing in VLA1553-301)	363 (375 planned)	156 M / 207 F 49.0 years (18-78)	Proportion of subjects with a seroprotective CHIKV antibody level defined as $\mu PRNT_{50} \geq 150$ at Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination
VLA1553-321	10, Brazil	First participant in: 14-Feb-2022 Last participant completing Day 29: 14-Mar-2023 Ongoing	Double-blind, randomized placebo-controlled	**VLA1553** 1×10E4 $TCID_{50}$ per 0.5 mL	510 (500 planned)	348 M / 406 F 15.0 years (12-17)	Proportion of subjects with a seroprotective CHIKV antibody level defined as $\mu PRNT_{50} \geq 150$ for baseline negative participants 28 days post-vaccination
				Placebo: 0.5 mL	255 (250 planned)		

Abbreviations: CHIKV=Chikungunya virus; F=female; GCP=Good Clinical Practice; GMT=Geometric Mean Titre; ID=identification; IMM=immunogenicity (population); M=male; µPRNT=micro plaque reduction neutralization test; $\mu PRNT_{50}$=50% plaque reduction in a micro plaque reduction neutralization test; $TCID_{50}$=50% tissue culture infectious dose

[1] Number of participants per arm includes all randomized/enrolled participants while demographic information is summarized for participants in the safety population. Among randomized participants, ten participants in study VLA1553-301 (9 randomized to VLA1553, 1 randomized to placebo) and one participant in study VLA1553-302 (randomized to lot 3) did not receive vaccination and are not included in the safety population. One additional participant in the VLA1553 arm of study VLA1553-301 who was randomized and vaccinated twice, was excluded from the safety population due to lack of GCP compliance. One participant randomized to placebo in study VLA1553-301 actually received vaccination with VLA1553-301. This participant is included in the placebo arm for immunogenicity analyses conducted in the IMM population and in the VLA1553 arm for analyses performed in the safety population.

Additional studies are planned in adults (VLA1553-304 in moderately immunocompromised patients infected with HIV and a co-administration study VLA1553-305) and in children from birth to 12 years (VLA1553-221, VLA1553-322, VLA1553-222, and VLA1553-323).

There are also two effectiveness studies VLA1553-402 and VLA1553-404 that are currently planned post-marketing.

2.6.2. Clinical pharmacology

Different bioanalytical methods were applied to address clinical pharmacology aspects of VLA1553. Table 2 summarizes which assays were applied for clinical development in the different clinical studies.

Table 2. List of Bioanalytical Methods applied for the clinical development of VLA1553

Name	Description and comments	Clinical Study
RT-qPCR assay (nsP1)	To assess vaccine viraemia and shedding, CHIKV RNA was quantified from RNA extracted from human plasma samples (VLA1553-101, VLA1553-301, VLA1553-302, VLA1553-321) and from urine samples (VLA1553-101). A one-step quantitative reverse transcription PCR (RT-qPCR) assay targeting the coding sequence of the non-structural protein nsP1 of CHIKV was applied. **Result expression and definitions** • Results are reported in GCE/mL (or GCE/rxn) with one decimal point. • The LLOQ was established at 3,214.2 GCE/mL (30.0 GCE/rxn). • Samples >LLOQ are considered positive for the presence of CHIKV RNA. **STATUS – Laboratory** • Qualified assay for human plasma and urine samples for VLA1553-101 (Genuity) • Validated assay for human plasma samples for VLA1553-301, VLA1553-302, VLA1553-321 (Nexelis).	VLA1553-101 VLA1553-301 VLA1553-302 VLA1553-321
CHIKV µPRNT assay (CHIKV 181/clone 25)	The CHIKV µPRNT assay is a microplate plaque reduction neutralization test. CHIKV µPRNT quantifies the levels of antibodies that neutralize CHIKV infection of Vero cells in human serum samples. The target CHIKV used is the serially passaged, live-attenuated CHIKV vaccine (CHIKV 181/25, TSI-GSD-218 or 181/clone 25). **Result expression and definitions** • Results are expressed in µPRNT50 titres, which correspond to the reciprocal of the serum dilution that yields a 50% neutralization in the number of viral plaques compared to the average virus control. • LLOQ: µPRNT50 titre of 20 • For VLA1553-301, VLA1553-302, VLA1553-303, seroconversion was defined as CHIKV-specific µPRNT50 ≥20 for baseline negative subjects and as a >4-fold increase over baseline for µPRNT baseline positive subjects. • For VLA1553-321, seroconversion was defined as a >4-fold increase over baseline for µPRNT baseline negative and µPRNT baseline positive subjects.	VLA1553-101 (post-hoc) VLA1553-301 VLA1553-302 VLA1553-303 VLA1553-321

Name	Description and comments	Clinical Study
	STATUS – Laboratory Validated assay for human plasma samples for VLA1553-101 (post-hoc analysis), VLA1553-301, VLA1553-302, VLA1553-303 and VLA1553-321 (Nexelis).	
CHIKV µNT assay (CHIKV Δ5nsP3)	The CHIV µNT assay is a microplate neutralization test. CHIKV µNT quantifies the levels of antibodies that neutralize CHIKV infection of Vero cells in human serum samples. The target CHIKV used is the CHIKV Δ5nsP3 vaccine strain. **Result expression and definitions** • Results are expressed in µNT50 titres, which correspond to the reciprocal of the highest serum dilution that results in 50% reduction of virus induced cytopathic effect (CPE) compared to virus control. • The lower limit of quantitation of the assay was defined as µNT50 titre of 20. • Seroconversion was defined as reaching a µNT50 ≥ 20 for baseline seronegative subjects. **STATUS – Laboratory** Valneva in-house developed assay.	VLA1553-101
CHIKV PRNT assay (7000 LR / strain 37997 / M109)	The CHIKV PRNT assay is a plaque reduction neutralization test. CHIKV PRNT quantifies the levels of antibodies that neutralize CHIKV infection of Vero cells in human serum samples. Three different CHIKV PRNT assays were applied, each one targets a different wt CHIKV strain. Namely: • La Reunion strain (7000 LR / LR2006_OPY1 strain) of the Indian Ocean/ECSA lineage • strain 37997 of West African lineage • clone M109 Caribbean strain from Asian lineage **Result expression and definitions** Results are expressed in PRNT50 titres, which correspond to the reciprocal of the highest serum dilution that yields a 50% neutralization in the number of viral plaques compared to the average virus control. **STATUS – Laboratory**	VLA1553-101 VLA1553-301

Name	Description and comments	Clinical Study
	• Developed assay for human plasma samples for VLA1553-101 at University of Texas Medical Branch (UTMB) • Qualified assay for human plasma samples from VLA1553-301 at University of Texas Medical Branch (UTMB)	
Alphavirus PRNT assay	The alphavirus PRNT assay is a plaque reduction neutralization test. Alphavirus PRNT quantifies the levels of antibodies that neutralize alphavirus infection of Vero cells in human serum samples. Different alphavirus PRNT assays were applied, each one targets a different alphavirus strain. Namely: • $CHIKV_{181/25}$ (attenuated strain, Asian Lineage), • $CHIKV_{LR2006}$ (wt strain, ECSA Lineage), • $CHIKV_{Brazil\text{-}7124}$ (wt strain, ECSA Lineage), • $MAYV_{BeAr505411}$, • $ONNV_{GMP30}$, • RRV_{T48} **Result expression and definitions** Results are expressed in PRNT50 titres, which correspond to the reciprocal of the highest serum dilution that yields a 50% neutralization in the number of viral plaques compared to the virus control. **STATUS – Laboratory** • Developed assay at Oregon Health and Science University (OHSU)	VLA1553-301 VLA1553-303
Anti-Chikungunya Virus IgG ELISA assay	The Anti-Chikungunya Virus IgG ELISA assay is an enzyme-linked immunosorbent assay that provides semiquantitative or quantitative determination of levels of CHIKV-specific binding IgG antibodies in human serum samples. **Result expression and definitions** • Results are expressed qualitatively into negative, borderline or positive based on reference intervals of the ratio of the extinctions from the patient sample (or control) and the cut-off calibrator. **STATUS – Laboratory** Validated at CERBA Laboratory	VLA1553-101 VLA1553-301 VLA1553-302 VLA1553-321
Anti-Mayaro Virus IgG ELISA assay	The Anti-Mayaro Virus IgG ELISA assay is an enzyme-linked immunosorbent assay that provides semiquantitative or quantitative determination of	VLA1553-301 VLA1553-302 VLA1553-321

Name	Description and comments	Clinical Study
	levels of MAYV-specific binding IgG antibodies in human serum samples. **Result expression and definitions** • Results are expressed qualitatively into negative, borderline or positive based on reference intervals of the ratio of the extinctions from the patient sample (or control) and the cut-off calibrator. **STATUS – Laboratory** CE marked –validated at CERBA Laboratory	
Anti-Dengue Virus IgG ELISA assay	The Anti-Dengue Virus IgG ELISA assay is an enzyme-linked immunosorbent assay that provides semiquantitative or quantitative determination of levels of DENV(1-4)-specific binding IgG antibodies in human serum samples. **Result expression and definitions** • Results are expressed qualitatively into negative, borderline or positive based on reference intervals of the ratio of the extinctions from the patient sample (or control) and the cut-off calibrator. **STATUS – Laboratory** CE marked –validated at CERBA Laboratory	VLA1553-301 VLA1553-302 VLA1553-321
Anti-Zika Virus IgG ELISA assay	The Anti-Zika Virus IgG ELISA assay is an enzyme-linked immunosorbent assay that provides semiquantitative or quantitative determination of levels of Zika-specific binding IgG antibodies in human serum samples. **Result expression and definitions** • Results are expressed qualitatively into negative, borderline or positive based on reference intervals of the ratio of the extinctions from the patient sample (or control) and the cut-off calibrator. **STATUS – Laboratory** CE marked –validated at CERBA Laboratory	VLA1553-301 VLA1553-302 VLA1553-321

2.6.3. Discussion on clinical pharmacology

Pharmacokinetic

Pharmacokinetic studies are in general not required for clinical evaluation of new vaccines. However, for live-attenuated vaccines based on genetically modified organisms such as VLA1553, it is required to assess aspects related to potential risks to human health (including safety for the vaccinee and transmission to third parties) and to potential risks to the environment by evaluating vaccine viraemia

and vaccine shedding during clinical development. Such data can provide information contributing to adequate dosing recommendations.

Vaccine viraemia is defined as presence of the vaccine virus in the blood stream and vaccine shedding is defined as presence in secretions or excretions. Vaccine viraemia of VLA1553 was investigated in all plasma samples for studies VLA1553-101 and in a limited number of plasma samples in VLA1553-301, VLA1553-302 and VLA1553-321. Vaccine shedding of VLA1553 was investigated only in urine samples in study VLA1553-101. Data are described in section 2.6.8.

Following RNA extraction, VLA1553 viral loads in clinical samples were determined using a single assay, namely a one-step quantitative reverse transcription PCR (RT-qPCR) assay. This assay is comparable to the assays described by Panning et al. (Emerg Infect Dis. 2008 Mar;14(3):416-22) and amplification targets the nsP1 gene, which is encoded on viral genomic RNA and not on sub-genomic viral RNAs.

The RT-qPCR assay applied for samples of VLA1553-101 (both plasma and urine samples) was qualified for plasma and urine samples and the one applied for samples of VLA1553-301, VLA1553-302 and VLA1553-321 (only plasma samples) was validated for plasma samples. Based on urinary shedding results obtained in study VLA1553-101, the Applicant decided to not further explore shedding in the following clinical studies.

The applicant submitted a validation report for the RT-qPCR assay. With the exception of the specificity analyses, it is considered that validation parameters and corresponding acceptance criteria were defined and validated appropriately. The validated CHIKV RT-qPCR is deemed suitable for its intended purpose to measure CHIKV RNA concentrations in human plasma. As specificity analyses only included Mayaro virus (MAYV) and did not include the closest related alphavirus O'nyong nyong virus (ONNV), additional cross-reactivities analyses will be needed if clinical trials will be conducted in regions of CHIKV and ONNV co-circulation.

Pharmacodynamic

Pharmacodynamic studies are required for clinical evaluation of new vaccines and comprise the characterisation of the vaccine induced immune responses.

For VLA1553, clinical studies were designed to characterise levels, kinetic and persistence of CHIKV-specific neutralizing antibodies. These were mainly evaluated with the Nexelis CHIKV µPRNT assay (also referred to as the Applicant assay in this report), which measures *in vitro* neutralizing antibody responses specific to the attenuated CHIKV 181/25 clone of Asian lineage.

Key immunogenicity data provided for assessment of this MAA were from the validated CHIKV µPRNT assay and are described in section 2.6.5. . Indeed, as agreed with CHMP (EMEA/H/SA/4412/1/2020/III), the conduct of classical vaccine efficacy field trials pre-authorisation was considered not feasible for CHIK. A threshold of CHIKV µPRNT50 titre ≥150 was used as a marker likely to predict clinical benefit of VLA1553 against CHIK.

This threshold CHIKV µPRNT50 value was established in the NHP passive transfer study using samples from the Phase 1 VLA1553-101 study and is in agreement with the proposed threshold determined in the sero-epidemiological study of Yoon et al. Data from the correlation analysis between CHIKV PRNT and CHIKV µPRNT assay results obtained with clinical samples from the Yoon's sero-epidemiological analysis were used to translate the Yoon threshold into a threshold defined in the µPRNT assay. Overall, it is agreed that the Deming regression correlation data submitted by the Applicant support a good level of agreement between neutralizing antibody responses measured with the PRNT assay described by Yoon et al. and those measured with the validated Nexelis CHIKV µPRNT assay. By analysing the positive sera (n=39), the Geometric Mean ration (GMR) µPRNT50/PRNT80 was at 3.73

(99% CI, 2.86–4.87). Applying the 99% upper CI, a PRNT80 of 10 in the Yoon assay would translate into a µPRNT50 of 48.7 in the Applicant assay. Applying the maximal individual ratio of the titre obtained by the Applicant assay over the titre obtained by the Yoon assay (13.9), a PRNT80 of 10 in the Yoon assay would translate into a µPRNT50 of 139.3 in the Applicant assay. The proposed threshold of 150 thus slightly exceeds the threshold based on the maximal individual ratio but largely exceeds (3x) the titre based on the upper boundary of the 99% confidence interval around the GMR. More details on the definition of the CHIKV µPRNT50 titre ≥150 as a marker likely to predict clinical benefit of VLA1553 against CHIK are provided in Clinical Efficacy.

Concerning validation of the Nexelis CHIKV µPRNT assay, the Applicant submitted bioanalytical method validation documentation, which was assessed in detail. With the exception of the specificity analyses, it is considered that validation parameters and corresponding acceptance criteria were defined and validated appropriately. It is agreed that the CHIKV µPRNT assay is precise, accurate, linear, and stable. In addition, it is considered that the submitted data indicate that the validated CHIKV µPRNT assay is also suitable to test NHP serum samples. However, concerning specificity analyses, these only included Mayaro virus (against which the limited data submitted indicate cross-reactivity of the CHIKV µPRNT assay) and distant arboviruses (Dengue/Zika). Cross-reactivity of the assay to ONNV was not tested, against which an even stronger cross-reactivity of the CHIKV µPRNT assay is expected based on published results. For trials conducted in regions where CHIKV and MAYV or CHIKV and ONNV co-circulate cross-reactivities of the CHIKV µPRNT assay should be considered, as these could bias immunogenicity, safety and efficacy analyses. Stratified analyses should be provided for studies conducted in such areas (REC).

In addition to analyses by CHIKV µPRNT assay, CHIKV-specific neutralizing antibodies were also characterised against the vaccine strain by a developed µNT assay (only for study VLA1553-101) and by cross-neutralization analyses with developed or qualified classical PRNT assays against different wt CHIKV strains and against 3 other arthritogenic alphaviruses (ONNV, MAYV and RRV) (selected samples from VLA1553-101 and VLA1553-301) at UTMB (University of Texas Medical Branch) and OHSU (Oregon Health and Science University). Results of these analyses are described in Clinical Efficacy.

The µNT assay applied in the VLA1553-101 was developed by the Applicant to evaluate VLA1553-specific neutralizing antibody responses in early clinical trials. The applicant also submitted a post-hoc analysis addendum reporting data obtained by retesting of selected samples from study VLA1553-101 with the validated CHIKV µPRNT assay. No formal statistical comparison between the two methods was submitted, but scatter plots indicate a good agreement between both assays. Antibody titres are higher when measured with the µPRNT assay as compared to the µNT assay, but a comparable kinetic of neutralizing antibody response up to 3 months post-vaccination is determined with both assays. An (unexpected) trend for increased GMTs at Month 6 and Month 12 was observed when neutralizing antibody titres were measured with the µNT assay but was not observed when using the µPRNT assay.

Finally, besides CHIKV-specific neutralizing antibody responses, the Applicant did not submit any additional data pertaining with the characterisation of the immune response induced by VLA1553 (e.g. innate immune responses, binding antibody responses, cell-mediated immune responses). Although characterisation beyond binding and neutralizing antibody responses were not discussed in the different scientific advice at CHMP level, this is considered a limitation in the clinical development of VLA1553.

Additional Bioanalytical Methods

Definition of CHIKV baseline serostatus

In some of the clinical trials, baseline immunity to CHIKV was measured by ELISA assay with the aim to exclude volunteers that were seropositive at baseline from the PP immunogenicity population, or to stratify enrolled participants by CHIKV baseline serostatus and/or to better characterize the population. Baseline serostatus was also confirmed by CHIKV µPRNT analysis in the Phase 3 studies.

The applied validated anti-Chikungunya Virus IgG ELISA assay (of commercial source) is considered suitable for its intended purpose to screen anti-CHIKV baseline immunity, provided cross-reactivities of this assay to other alphaviruses are considered when analysing immunogenicity, safety and efficacy of trials conducted in regions of co-circulation of CHIKV with other alphaviruses (i.e. MAYV in Latin America and ONNV in Africa).

Definition of baseline serostatus specific to other alphaviruses, Dengue and Zika

Retrospective investigation of pre-existing antibodies including but not limited to other alphaviruses (i.e. Mayaro) or Dengue and Zika was foreseen for different clinical studies, which was conducted by applying validated ELISA assays for the semiquantitative detection of IgG specific to MAYV, DENV(1-4) and Zika virus. Collectively, these ELISA assays suffer from cross-reactivities that should be considered for analyses of the results.

2.6.4. Conclusions on clinical pharmacology

The main assays that were applied for the clinical development of VLA1553 (nsP1 RT-qPCR and CHIKV µPRNT assays) are deemed suitable for their intended purpose to measure CHIKV RNA concentrations in human plasma and circulating CHIKV-specific neutralizing antibody. Notable limitations of these assays are related to their cross-reactivities, in particular to related alphaviruses co-circulating with CHIKV in some regions (i.e. MAYV in Latin America and ONNV in Africa). These cross-reactivities should be considered when analysing immunogenicity, safety and efficacy of trials conducted in these regions (REC).

2.6.5. Clinical efficacy

It has been agreed by CHMP that efficacy trials are currently not feasible pre-authorization due to unpredictable and short-lived outbreaks. Therefore, the approach to rely on a threshold value of neutralizing antibodies to infer efficacy is acceptable. The basis for establishing the threshold is discussed below.

2.6.5.1. Establishment of the threshold- clinical aspects

1. Introduction

CHIKV neutralizing antibodies have been suggested as a potential immune correlate of protection based on both animal and human data, after both natural infection or vaccination (reviewed in Milligan et al.;2019). The main evidence comes from animal passive transfer studies of human sera and challenge studies in various species (mice and NHPs). Although data are very limited in human, findings converge to a major role of neutralizing antibodies in the protection against CHIKV infection (Kam et al.; J Infect Dis 2012, Yoon et al.; 2015; Srikiatkhachorn et al.; 2016, Galatas et al. 2016, Jain et al. 2017, Auerswald et al.2018, Yoon et al. 2020). Some studies indicate a role of neutralizing antibodies in the resolution of the acute infection by showing lower viraemia in convalescent patients with higher neutralizing antibodies titres (Kam et al., 2012; Jain et al., 2017). Only the Yoon study evidences the association with (a certain level of) antibody and a decrease risk of CHIKV symptomatic infection.

2. Yoon et al., 2015

Citation: Yoon I-K, Alera MT, Lago CB, Tac-An IA, Villa D, Fernandez S, et al. (2015) High Rate of Subclinical Chikungunya Virus Infection and Association of Neutralizing Antibody with Protection in a Prospective Cohort in The Philippines. PLoS Negl Trop Dis 9(5): e0003764. doi:10.1371/journal. pntd.0003764.

Yoon *et al.* conducted a prospective longitudinal community-based sero-epidemiological cohort study in Cebu City (Philippines) during the period 2012-13. The study was initiated primarily to evaluate the incidence of influenza and dengue virus infections and secondarily to evaluate the incidence of other causes of acute febrile illness.

Study design

A total of 1007 subjects ≥6 months of age were recruited within the community. Approximately 200 subjects were recruited in each of five age groups: 6 months-5 years, 6-15 years, 16-30 years, 31-50 years, and >50 years old. Participants ranged in age from 6 months to 83 years.

Blood samples, demographic and health data were collected at enrolment (baseline) and approximately 1 year later. Neutralizing antibodies were assessed at these timepoints. The neutralisation assay used was a plaque reduction neutralization test (PRNT80) based on the attenuated strain 181/clone 25 derived from a CHIKV Asian strain isolated from Thailand in 1962. The 181/clone 25 (181/25) was developed by the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). CHIKV PRNT titre used 80% plaque reduction. The validation status of the assay is not known.

PRNT of 10 corresponds to the LOD (first sera dilution). This value is defined as cut-off of positivity in the study.

Baseline PRNT titre ≥10 was considered to indicate past CHIKV infection.

An active surveillance of acute febrile illness was set up within the cohort during the 12-months study period (participants were instructed to report any event of fever and were monitored for acute febrile illnesses by weekly telephone calls or home visits). Over the study period, whenever an event of fever was reported, clinical data and blood samples were collected. The initial blood sample was collected within 7 days of onset of the acute febrile illness (Day 0, acute blood sample). Follow up visits were performed at Day 2, Day 5, Day 8 and Day 21. RT-PCR to detect CHIKV RNA was performed on the acute samples. IgM and IgG ELISA were performed at Day 0 and Day 21.

Acute symptomatic CHIKV infection was defined as a febrile illness with: (i) positive CHIKV PCR in the acute sample, and/or (ii) rising IgM levels between the acute and convalescent samples, and/or (iii) ≥4-fold rise in CHIKV IgG ELISA between the acute and convalescent samples. The definition of acute infection is overall in line with the current clinical knowledge on CHIKV infection.

Subclinical CHIKV infection was defined as an at least 8-fold rise in PRNT titre (which corresponds to a difference of 2 dilutions) between baseline and Month 12, in the absence of acute symptomatic CHIKV infection detected during the active surveillance period.

Results

Of the 1007 enrolled participants, 853 'per-protocol subjects' completed all study procedures per-protocol including enrollment and 12-month blood collections. Among the 853 per-protocol subjects, 19 acute symptomatic CHIKV infections occurred. All were confirmed by RT-PCR.

A substantial proportion of the cohort (28% [239/853]) presented PRNT titres ≥10 at baseline. There was a clear trend for this percentage to increase with age.

Incidence in participants with negative baseline PRNT (PRNT titre <10)

All 19 acute symptomatic CHIKV RT-PCR confirmed infection cases occurred in participants (n=614) with negative baseline PRNT. The incidence of symptomatic CHIKV infection was 3.01 per 100 person-years (95% CI: 1.87-4.61) in participants with negative baseline PRNT.

An additional 87 subclinical CHIKV infections occurred in the per-protocol subjects with negative baseline PRNT, which resulted in an incidence of subclinical infection of 13.79 per 100 person-years (95% CI: 11.12-16.92). It is noted that since the active surveillance focused on detecting febrile illness, cases of symptomatic disease (such as arthralgia without fever) might have been missed leading to an overestimation of the number subclinical vs. symptomatic infections.

Incidence in participants with positive baseline PRNT (PRNT titre ≥10)

No acute symptomatic CHIKV RT-PCR confirmed infection occurred in the 239 participants with positive baseline PRNT.

The paper describes 4 participants with a 4 to 8-fold rise in PRNT titre at 12 months (increasing from 10 to 42, 59 to 286, 83 to 555, and 162 to 799, respectively) and 1 participant with a ≥8-fold rise (from 95 to 959). Because of dilution 4, the authors consider that a difference <8-fold rise could be due to assay variability, while a difference ≥8-fold rise may be a true difference due to immunological boosting after CHIKV re-exposure. Thus, out of the 239 subjects with baseline positive CHIKV PRNT, only 1 might have presented a subclinical infection. It is noted that most participants (n=234) did not mount any antibody response at all, suggesting that a positive PRNT titre probably confers a certain degree of sterile immunity.

Association between symptomatic CHIKV infection and baseline titre

In order to estimate the association between baseline CHIKV PRNT titres and the frequency of acute symptomatic CHIKV RT-PCR confirmed infection, exact odds ratio (OR) and confidence interval (CI) for baseline PRNT status (<10 vs. ≥10) were calculated using conditional maximum likelihood estimates. Percent protection attributable to PRNT status was calculated as 100x(1-OR). A baseline CHIKV PRNT titre ≥10 vs. <10 was associated with 100% (95%CI 46.1-100.0) protection from acute symptomatic CHIKV RT-PCR confirmed infection.

Considering that 1/239 and 87/614 subclinical infections might have occurred respectively in participants with baseline positive vs. negative CHIKV PRNT, a similar trend is found for subclinical infections as for symptomatic infections.

3. Yoon et al. 2020, 2 years FU data

Yoon Int J Infect Dis 2020. Pre-existing chikungunya virus neutralizing antibodies correlate with risk of symptomatic infection and subclinical seroconversion in a Philippine cohort.
https://pubmed.ncbi.nlm.nih.gov/32247051/

Yoon *et al.* 2020 presents data from the previous cohort study updated with a follow up visit at 2 years, including blood collection. The authors describe both symptomatic CHIVK infection and subclinical seroconversion. In addition, the neutralizing antibody response to natural CHIKV infection was further characterized. Active fever surveillance continued over the 2-year period (including weekly face-to-face or telephone contacts). Any history of fever identified triggered an acute and 3-week visit with blood collection, similar as for the first year. Symptomatic CHIKV infection was defined as a febrile illness with positive CHIKV PCR in the acute sample. Subclinical seroconversion was defined as a ≥8-fold rise in CHIKV PRNT80 titre between annual visits without an intervening symptomatic infection. Same assays than described in Yoon 2015 were used.

Incidence of symptomatic CHIVK infection and subclinical seroconversion

The results of the FU study are presented for 854 participants (instead of 853 in the original paper).

At baseline, 28.0% (239/854) of participants had detectable PRNT80 titres (i.e., ≥10). Of those 239 baseline neutralizing antibody-positive participants, 20.1% (48/239) had low PRNT80 titres (PRNT80 titre of 10 to <100), 58.2% (139/239) had medium titres (100 to 500), and 21.8% (52/239) had high titres (>500).

A total of 25 symptomatic CHIKV infections occurred during the 2-year study period (19 in Year 1 and 6 in Year 2). All 25 cases occurred in the 615 participants with no detectable neutralizing antibody at baseline (PRNT80<10). The case which occurred during the second year also had no detectable neutralizing antibody pre-year 2 for those).

During Year 1 and Year 2 respectively, 89 (instead of 87 as initially reported) and 15 subclinical seroconversions occurred in the participants with no detectable baseline neutralizing antibody. Only 1 subclinical infection occurred in participants with positive baseline titre (during Year 1). The baseline titre of this participant was low (PRNT80 titre of 95), and increased to 959 at 12 months. A sensitivity analysis using PRNT50 instead of PRNT80 data yielded consistent results with a total of 104 subclinical seroconversions, all but one of which occurred in those with no detectable pre-year neutralizing antibody.

Neutralizing antibody response to natural CHIKV infection

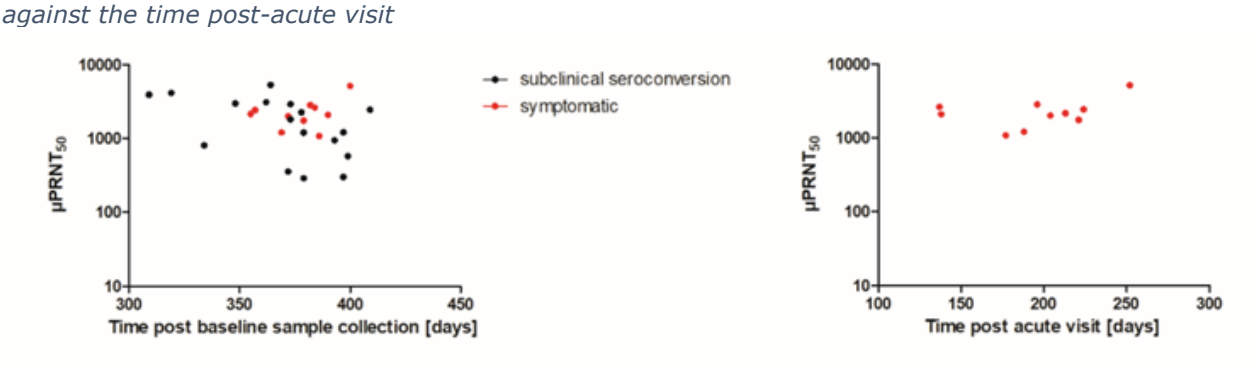
The geometric mean neutralizing antibody titre (PRNT80 of 450) in participants experiencing a symptomatic CHIKV infection was similar at the initial and latest post-infection timepoints (estimated at 90 days and 600 days post-infection respectively). Data shortly after infection where the peak of neutralizing antibodies could be expected (around 28 days post-infection) are not available. In addition, the geometric mean neutralizing antibody titre at baseline in participants with baseline positive PRNT was only slightly lower (PRNT80 of 223). Geometric means of neutralizing antibodies post-symptomatic infection or post-subclinical infection are comparable (450 and 413 PRNT80).

4. Immune responses to VLA1553 vs. natural disease

Participants from study VLA1553-301 with no detectable antibodies at baseline had GMT of 3361.6 (2993.8-3774.4) at 28 days post-vaccination. At Day 85, GMT (95%CI) was 1083.1 (968.3-1212.6) and Day 180 GMT (95% CI) was of 752.1 (665.9-849.5). Magnitude and kinetics of the humoral response observed in study VLA1553-302 were consistent with results of study VLA1553-301. At year 1 post-vaccination (VLA1553-303 [follow-up study of VLA-1553-301]), GMT was 1070.3 (935.35-1224.64). At year 2 post-vaccination (VLA1553-303 [follow-up study of VLA-1553-301]), GMT was 819.5 (713.74-917.81).

Several samples of participants from the Yoon study were tested in the µPRNT of the Applicant, results are provided for 27 baseline negative individuals who became positive post-baseline within the first year of follow-up. Among these subjects, 10/27 had symptomatic CHIK confirmed by PCR and 17/27 had subclinical seroconversion. All baseline samples were confirmed negative by the µPRNT assay (µPRNT50<20) and the post-baseline GMT was of 1657 (min-max of 289-5297). The time interval between infection and sampling is not described in detail, but ranges from approximately 3 months to 1 year according to Figure 2.

Figure 2. Dot plot of µPRNT50 titre measured for baseline negative participants with symptomatic infection (red) or subclinical seroconversion (black). On the left neutralization titre are blotted against the time post baseline sample collection, on the right titre after symptomatic infection are blotted against the time post-acute visit



Additional data to support that VLA1553 induces neutralizing antibody responses are comparable to the one induced by natural infection are expected from ongoing studies (refer to section 2.6.5.6.).

5. Conclusion and limitations of Yoon et al. study

The Yoon *et al.* (2015 and 2020) prospective cohort study was conducted in the Philippines in participants ≥6 months of age. Of the participants with PRNT titres ≥10 at baseline (n=239), none presented an acute symptomatic RT-PCR confirmed CHIKV infection over a 1-year period. In contrast, in participants with PRNT titres <10 (n=614), the incidence of acute symptomatic RT-PCR confirmed CHIKV infections (19 cases) was 3.01 per 100 person-years (95% CI: 1.87-4.61). A PRNT titre ≥10 (vs. <10) was associated with a 100% (95% CI 46.1-100.0) protection from acute symptomatic CHIKV infections over a 1-year follow up period. During the second year of follow up, still no acute symptomatic CHIKV laboratory confirmed infection occurred in participants with baseline PRNT titres ≥10, while an additional 6 cases occurred in participants with baseline PRNT titres <10.

A similar trend was found for subclinical infections (based on serological evidence of a rise in neutralizing antibody titres from baseline). During Year 1 and Year 2, 89 and 15 subclinical infections occurred in the participants with no detectable baseline PRNT (titre <10). In addition, 1 subclinical infection occurred in participants with a PRNT titre ≥10 over the 2 years.

Results are based on PRNT80, but consistent results were obtained when using PRNT50.

In conclusion, Yoon *et al.* data show that individuals with PRNT titre ≥10 resulting from a prior natural infection (which could be an old infection) experienced a lower frequency of acute symptomatic PCR confirmed CHIKV infections for at least two years. Data also suggest they were protected from subclinical/asymptomatic infection (based on a rise in neutralizing antibody titres from baseline), suggesting a certain degree of sterilizing immunity.

According to the authors the results support CHIKV PRNT titre ≥10 as a potential immune correlate of protection from symptomatic and subclinical infection. The fact that the lowest measurable PRNT titre was found to correlate with a decreased risk of infection in this study using either PRNT80 or PRNT50 suggests that the presence of any detectable CHIKV-specific neutralizing antibody using other assays may be able to differentiate individuals at lower risk of infection.

It is acknowledged that PRNT80 titre of ≥10 may correlate with protection from symptomatic infection and subclinical seroconversion. However, it should be kept in mind that this study presents many limitations. The main limitations are as follows:

- The data are from a single study in a defined geographic area (one endemic area endemic for CHIKV, where a single lineage of CHIKV circulated, and with no other alphavirus circulation

reported). Additional studies would be needed to validate the ICP and ensure external validity of the results.

- The study estimate is very imprecise. Although the protection level associated with PRNT (≥10) is estimated to be 100%, the LB 95% CI is 46%.
- The study included a limited number of baseline positive PRNT (≥10) participants in all 3 baseline PRNT titres categories: low (10 to <100), medium (100 to 500), and high (>500). There were only 48 participants with baseline PRNT titres between 10 and 100. Therefore, it is in fact uncertain whether a threshold between 10 and 100 would be needed to offer protection.
- The Yoon *et al.* study evidenced an immune correlate of risk. Whether the criteria for an ICP are met is unknown (see Prentice criteria, and WHO guidance on correlate of vaccine induced protection, https://iris.who.int/bitstream/handle/10665/84288/WHO_IVB_13.01_eng.pdf?sequence=1). Usually, ICP are derived from efficacy trial data.
- Titres shortly after vaccination (at the peak titre) are used in ICP analyses. In contrast, in the Yoon *et al.* study, titres used in the analysis reflect a prior infection which may have occurred several years before. The magnitude of the response to reach shortly after infection in order to be protected is not known. Therefore, the actual Day 29 post-vaccination titre to be reached in order to infer protection is not known. However, with respect to the Valneva vaccine, the majority of the participants of VLA1553-301 maintain a titre well above the threshold over 2 years, which is reassuring.
- It is unknown whether the level of neutralizing antibodies determined by Yoon et al. as associated with protection would suffice to protect from (symptomatic) CHIKV infection if induced by a vaccine rather than by the natural disease. The contribution of other immune parameters participating to the protection (or optimal induction of the protection) is not taken into account. The magnitude and quality of immune responses (including of memory) may vary according to the type of priming. The fact that VLA1553 is a live-attenuated vaccine is however reassuring as it might be expected to induce similar immune responses vs. a natural infection.
- The CHIKV strain identified in this study was from Asian genotype which could behave differently from other CHIKV genotypes. The threshold may not be applicable to other genotypes.
- Whether this threshold is associated with protection from arthritis is not known (for ex. if subclinical infections could still occur and be associated with arthritis).

6. Link between the Yoon and Valneva PRNT

The applicant proposed a threshold of 150 µPRNT50 as being a marker that is reasonably likely to predict clinical protection from Chikungunya. This threshold of 150 µPRNT50 was established in the NHP passive transfer study using samples from the Phase 1 VLA1553-101 study.

Sera from participants of the Yoon study were tested in the µPRNT of the Applicant. By analyzing the positive sera (n=39), the Geometric Mean ration (GMR) µPRNT50/PRNT80 was at 3.73 (99% CI, 2.86–4.87). Applying the 99% upper CI, a PRNT80 of 10 in the Yoon assay would translate into a µPRNT50 of 48.7 in the Applicant assay. Applying the maximal individual ratio of the titre obtained by the Applicant assay over the titre obtained by the Yoon assay (13.9), a PRNT80 of 10 in the Yoon assay would translate into a µPRNT50 of 139.3 in the Applicant assay. The proposed threshold is thus in agreement with the proposed threshold determined in the sero-epidemiological study of Yoon *et al.*

Please refer to section 2.6.3. for more details about the bridge between results obtained with the Yoon and the Applicant assay.

7. Threshold, non-clinical approach

To determine a surrogate of protection for VLA1553, a NHP passive transfer study was conducted using human sera derived from study participants of the Phase I clinical trial VLA1553-101. Please refer to section 2.5.2.1. for further details.

8. General conclusion

The approach was agreed during several SA.

The use of a neutralizing antibody threshold reasonably likely to predict protection after vaccination with the live-attenuated vaccine candidate VLA1553 is agreed. Even if the exact mechanisms of protection are unknown, neutralizing antibodies have a major role in protecting against CHIKV infection and/or disease. This is supported by animal and human data, both after natural infection and vaccination.

The threshold proposed by the Applicant to infer 'likely protection' is supported by both animal and sero-epidemiological data. Non-human primates with human (transferred) VLA1553-antibody titres above the threshold of 150 µPRNT50 were shown to be protected from viraemia after a challenge with the wild-type La Reunion strain. Yoon *et al.* showed in a prospective study in the Philippines that individuals with PRNT titre ≥10 resulting from a prior natural infection experienced a lower frequency of symptomatic PCR confirmed CHIKV infections and of subclinical/asymptomatic infection (based on a rise in neutralizing antibody titres from baseline) for at least two years. A PRNT80 of 10 in the sero-epidemiological study of Yoon corresponds to a value of approximately 50 µPRNT50 in the Applicant's µPRNT assay.

However, even though a conservative approach has been taken, the µPRNT threshold of 150 cannot be considered as an established ICP as it was not derived from efficacy trials. For both the animal and the human data, there are several limitations, resulting in uncertainties around the protection that VLA1553 might offer against disease (including chronic chikungunya) and/or infection caused by homologous and/or heterologous strain.

It is deemed more correct to refer to the neutralizing µPRNT50 antibody threshold of 150 as a threshold reasonably likely to predict protection rather than to a seroprotective threshold (see also assessment of study VLA1553-301) and the term "seroresponse" instead of the term "seroprotection" is therefore used hereafter.

Ultimately, effectiveness studies are needed to confirm and characterize the clinical protection offered by the vaccine.

2.6.5.2. Dose response study

The selection of the dose was based on the results obtained in the Phase 1 VLA1553-101 trial.

Study VLA1553-101

Methods

The assessment of the methods is based on Protocol Version 5.0 dated 20 April 2018.

VLA1553-101 was a randomized, observer-blinded, multicentre, dose-escalation trial investigating 3 dose levels of a liquid formulation of VLA1553 after a single immunization given intramuscularly. The

dose levels tested were of 3.2x103 TCID50/dose (low dose, 0.5mL), 3.2x104 TCID50/dose (medium dose, 1mL) and 3.2x105 TCID50/dose (high dose, 1mL).

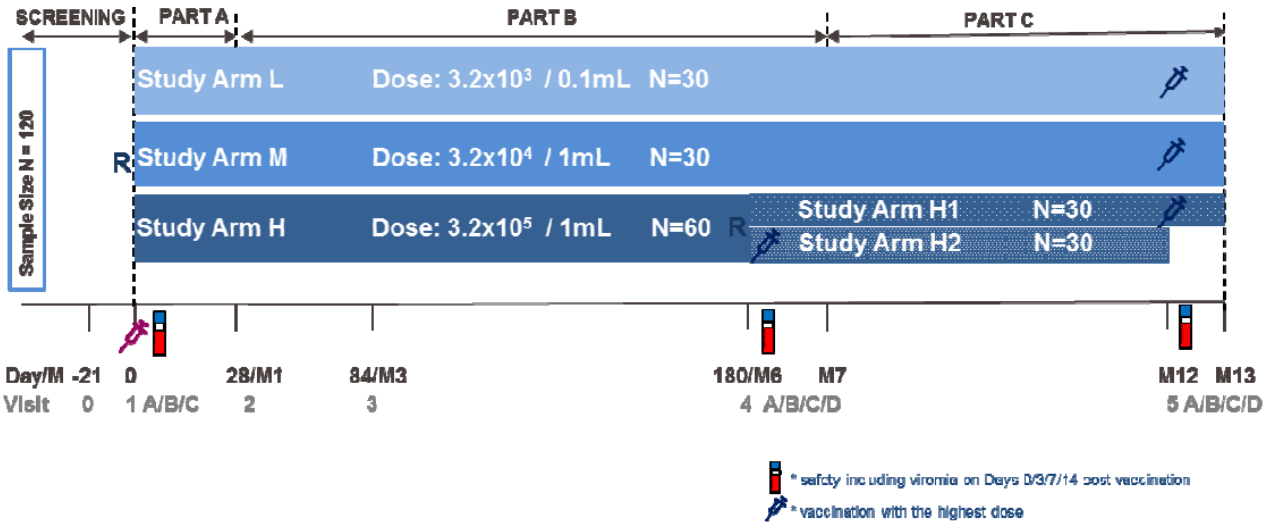
The study was conducted at 2 study sites in the U.S. One study site was selected for the dose-escalation phase.

The study was also designed to evaluate the booster effect following a second vaccine dose (re-vaccination) at 6- or 12-months post-dose 1. The re-vaccination dose level was the high dose level (3.2x105 TCID50/dose), allowing to demonstrate the potential capability of the antibodies induced by one single dose of the vaccine to neutralize the vaccine virus and protect the participants from vaccine virus viraemia, up to 6- or 12-months post-vaccination, and therefore the potential absence of anamnestic response.

The study also allows to gather, although limited, immunogenicity and safety data up to 12 months post-single dose.

The overall study design is displayed in Figure 3.

Figure 3. VLA1553-101: Overall study design



The vaccine administered in this study was liquid formulated, which is different from the lyophilized formulation tested in the Phase 3 trials (VLA1553-301, VLA1553-302 and VLA1553-321) and used in the final formulation. A dose of 1×10E4 TCID50 per 0.5 mL lyophilized formulation (final formulation) was considered comparable to the dose of 3.2×10E4 TCID50 per 1 mL liquid formulation (medium dose). This was mainly based on analytical results and stability profile comparisons, as well as limited non-clinical data.

Overall, 120 participants aged 18-45 years were to be enrolled into the study. Thirty were to receive the low dose level (study arm L), 30 the medium dose level (study arm M) and 60 the high dose level (study arm H, sub-divided in H1 and H2 arms).

All the participants from the L and M arms were to receive a second vaccine (high) dose at Month 12. Half of the participants of the high dose arm were to receive a second vaccine (high) dose at Month 12 (H1 arm), and the other half at Month 6 (H2 arm) (re-randomization of the participants with a ratio of 1:1).

The vaccine was prepared and administered by unblinded study staff. The unblinded staff members were not involved in any other study procedures/assessments. The participant, the investigator and

other staff involved in general study conduct and safety assessments were blinded. All laboratory personnel at laboratories for immunogenicity and safety assessments were also blinded.

Participants were followed up for approximately 13 months following vaccination (up to 1 month post-re-vaccination or until the viraemia was resolved).

Immunogenicity blood samples were taken from all the participants at baseline, 3 days, 7 days, 28 days, 84 days, 180 days, and 365 days post-vaccination. Blood samples were also obtained 28 days post-re-vaccination (either at Month 6 or Month 12).

Immunogenicity assessment was performed at a central laboratory. A µNT was developed by the Applicant for the measurement of neutralizing antibodies. The assay is based on the same principle than a PRNT assay. The assay allows testing with higher throughput. The strain used in the assay is the vaccine virus strain. The LLOQ was defined as 20 NT50. This assay is not the same assay than the one used for the Phase 3 trials in which a µPRNT was used (with a different virus strain). Selected stored samples were further re-analyzed by using the µPRNT assay as for the Phase 3 trials. Post-hoc analysis performed on selected samples from 91 participants (PP population Part C) is presented in this report.

GMTs, SCRs and GMFI's were compared overall and pair-wise (Tukey's HSD test) between VLA1553 study arms at all time points. GMTs between the VLA1553 study arms across all doses at Day 28 (i.e. 28 days after vaccination) by ANOVA (factors study arm, covariate study site) were also compared.

The immunogenicity analyses were performed on the Per Protocol (PP) population and the Intent-To-Treat (ITT) population. The ITT population was defined to include all enrolled participants who received at least one vaccination. Participants were analyzed according to the dose arm they had been allocated to, rather than by the actual treatment they received. The PP population was defined as all ITT participants who had no major protocol violations that might have an impact on the immunogenicity. Two PP populations were defined. PP populations Part B and Part C were determined based on major protocol deviations observed up to Visit 4D (28 days after the Day 180 re-vaccination) and up to Visit 5D (28 days after the Day 365 re-vaccination), respectively.

Study participants

Participants were, generally healthy adults 18 to 45 years of age. In addition, participants were excluded if they had history of known CHIKV infection, if they had travelled or planned to travel in an endemic CHIKV area within 4 weeks prior to study enrolment or during the study course, and if they had received an investigational CHIKV vaccine. Participants with history of immune-mediated or clinically significant arthritis/arthralgia were also excluded. Other inclusion and exclusion criteria are standard criteria for a first-in-human trial. Criteria for re-vaccination were also standard.

Objectives and endpoints

The primary objective was to assess the safety and tolerability of the vaccine. There were several secondary objectives for both safety and immunogenicity.

Immunogenicity objectives were to assess the immunogenicity of the vaccine after a single dose (short- and longer-term) and after a re-vaccination (6 or 12 months post-first dose).

Immunogenicity endpoints comprised measurement of neutralizing antibodies (as measured with µNT assay) at 28 days post-vaccination, but also the kinetics and persistence of the response (7, 14, 84, 180 and 365 days) after single vaccination.

GMTs, seroconversion rates, fold-increase of specific neutralizing antibodies post-vaccination or post-re-vaccination as compared to baseline or prior re-vaccination were to be assessed.

Assessment of cross-neutralization of heterologous CHIKV strains was performed on 47 samples collected from 12 participants at different timepoints. Cross-neutralization capacity was tested against virus strains representing the ECSA lineage (LR2006_OPY1 strain [also referred as 7000 CHIKV-LR]), West African lineage (37997 strain) and Asian lineage (Caribbean M109 strain). Results are presented in section 2.6.5.6. together with the results generated on samples from the pivotal study VLA1553-301.

Results

Study participants and baseline characteristics

A total of 120 adults (18 to 45 years), CHIKV seronegative at baseline were vaccinated, including 31 in the low dose group, 30 in the medium dose group and 59 in the high dose group.

A total of 25 participants of arm H2 were re-vaccinated at Month 6. At Month 12, a total of 23, 23 and 20 participants of arm L, arm M and arm H1, respectively, were re-vaccinated. A total of 91 completed the visit 28 days after re-vaccination (visit 5D).

There were 95 major protocol deviations recorded for 29 participants. Twenty-six participants did not received re-vaccination, all at Month 12. Twenty-eight participants had missing sample for immunogenicity assessment (representing a total of 68 samples). One participant (arm H1) had taken a concomitant treatment deemed to impact the immune response.

Out of the 120 participants, 106 and 91 did not have major protocol deviation at Visit 4D or Visit 5D and constituted the PP population Part B and Part C, respectively.

There were no major differences in the demographic characteristics between the safety/ITT population and the PP population Part C. Demographic characteristics for PP population Part B were not provided but no difference is expected.

More male participants (80/91 [87.9%]) than female participants were included in the PP population, due to inclusion of participants of non-childbearing potential only. Median age (Q1-Q3) of the participants was 34.0 years (28.0-38.0 years). Median body mass index (Q1-Q3) was 25.8 kg/m^2 (23.3-38.7 kg/m^2). Characteristics were overall comparable between study arms ,taking into account the limited number of participants per arm.

Immunogenicity results

A summary of GMTs for CHIKV-specific neutralizing antibodies by study day is provided in Table 3 for the PP population.

Table 3. GMTs for CHKIV-specific neutralizing antibodies by visit (PP population) (Part B and Part C)

	VLA1553				
	Arm L M12 re-vacc. (NB=27) (NC=23) GMT (SD [log10]) [95% CI]	**Arm M M12 re-vacc. (NB=29) (NC=23) GMT (SD [log10]) [95% CI]**	**Arm H (NB=50) (NC=45) GMT (SD [log10]) [95% CI]**	**Arm H1 M12 re-vacc. (NC=20) GMT (SD [log10]) [95% CI]**	**Arm H2 M6 re-vacc. (NB=26) (NC=25) GMT (SD [log10]) [95% CI]**
Visit 1 – Day 0	10.0 (0.00) [10.0, 10.0]	10.0 (0.00) [10.0, 10.0]	10.0 (0.00) [10.0, 10.0]	N/A	10.0 (0.00) [10.0, 10.0]
Visit 1A – Day 3	10.0 (0.00) [10.0, 10.0]	10.0 (0.00) [10.0, 10.0]	10.3 (0.09) [9.7, 10.9]	N/A	10.0 (0.00) [10.0, 10.0]
Visit 1B – Day 7	11.4 (0.17) [9.8, 13.2]	13.1 (0.30) [10.1, 17.2]	16.0 (0.30) [13.2, 19.5]	N/A	18.0 (0.36) [12.9, 25.1]
Visit 1C – Day 14	247.5 (0.36) [179.0, 342.3]	383.0 (0.26) [302.6, 484.7]	475.5 (0.30) [389.7, 580.3]	N/A	545.4 (0.27) [423.0, 703.3]
Visit 2 – Day 28	592.6 (0.35) [431.7, 813.3]	656.0 (0.34) [483.2, 890.8]	686.9 (0.34) [549.3, 858.9]	N/A	606.8 (0.31) [456.5, 806.6]
Visit 3 – Day 84	274.3 (0.37) [196.4, 383.2]	297.9 (0.35) [218.5, 406.1]	302.4 (0.34) [241.3, 379.0]	N/A	303.4 (0.35) [219.0, 420.2]
Visit 4 – Day 180	413.7 (0.40) [286.9, 596.4]	469.1 (0.59) [278.7, 789.5]	485.0 (0.51) [346.8, 678.3]	N/A	452.5 (0.47) [292.8, 699.5]
Visit 4D – Day 180 plus 28 days	N/A	N/A	N/A	N/A	490.2 (0.36) [350.1, 686.5]
Visit 5 – Month 12	602.6 (0.39) [406.7, 892.7]	1005.8 (0.32) [729.7, 1386.4]	N/A	735.2 (0.47) [440.9, 1225.9]	588.9 (0.25) [464.1, 747.3]
Visit 5D – Month 12 plus 28 days	600.9 (0.31) [439.3, 822.0]	964.0 (0.34) [678.9, 1368.6]	N/A	870.9 (0.36) [576.1, 1316.7]	N/A

CHIKV = Chikungunya virus; PP = per-protocol; M12 = Month 12; re-vacc. = re-vaccination; NB = PP Part B population (for Visits 1 to 4D); NC = PP Part C population (for Visits 5 and 5D); GMT = geometric mean titer; SD = standard deviation; M6 = Month 6; N/A = not applicable
Visit 4D was only applicable for Arm H2 and Visit 5D was only applicable for Arms L, M, and H1.
Source: Section 14, Tables 2.3.1.2, 3.3.1.2, Post Hoc analysis 21FEB2024, Tables 2.3.1.2, 3.3.1.2

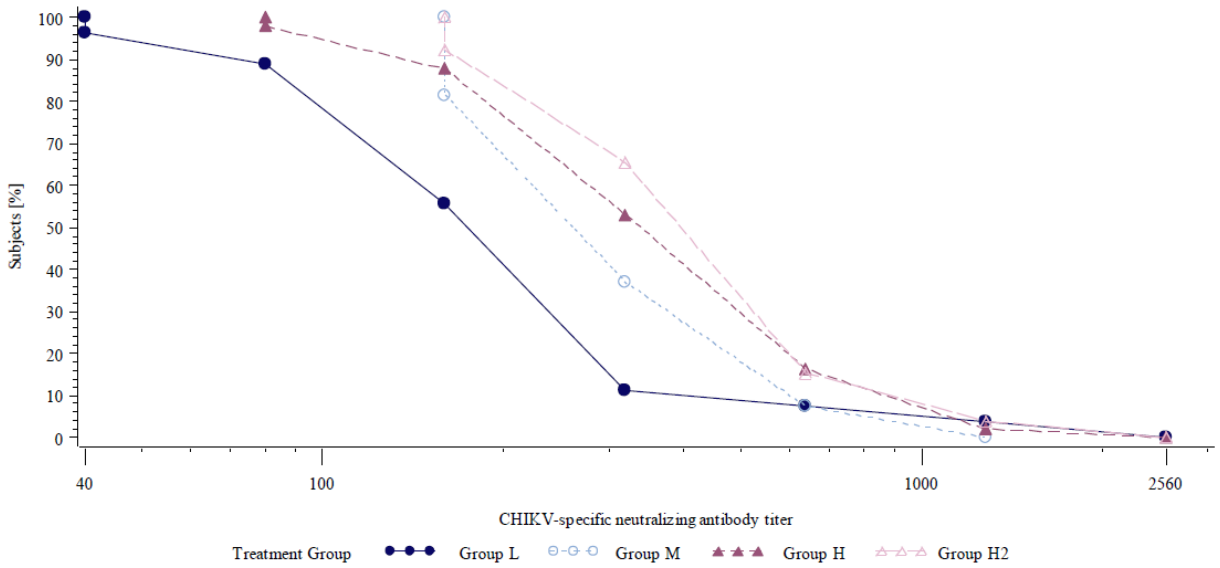
All participants were seronegative for CHIKV-specific neutralizing antibody at baseline. Response were low/absent at Day 3 and at Day 7. On Day 14, GMTs (95% CI) ranged from 247.5 (179.0 - 342.3, arm L) to 475.5 (389.7 - 580.3, arm H). The peak of neutralizing antibody titres was observed at Day 28 with GMTs (95% CI) of 592.6 (431.7 – 596.4), 656.0 (483.2 – 890.8) and 686.9 (549.3 – 858.9) for L, M and H arm respectively (PP population Part B). GMTs were decreased at Day 84 and Day 180. At Day 365, in the non re-vaccinated participants, GMTs tend to be higher than at Day 84/Day 180 and comparable than at Day 28 timepoint (ranging from 602.6 [L arm] and 1005.8 [M arm]) (PP population Part C). This kinetics of the response is unexplained. GMFIs as compared to baseline are consistent with these results. Similar results are obtained for the ITT population.

Seroconversion rates (defined as μNT50 ≥ 20) were 100% from Day 14 to Day 365 in all study arms. Three out of 27, 5/28 and 20/50 participants seroconverted 7 days post-vaccination. At Day 14 all but one participants had at least a 8-fold increase in neutralizing antibody titres when compare to baseline. At Day 365, all participants had at least a 8-fold increase in neutralizing antibody titres when compare to baseline, and 74% had still a 64-fold increased as compared to baseline.

Only 2 participants had an increase in antibodies ≥ 4-fold 28 days post-re-vaccination vs. pre-vaccination, reflecting an anamnestic response as defined by the Applicant (one in each arm H1 and H2). Results of re-vaccination are however considered hardly interpretable (PP population Part C). Nevertheless, no viraemia was detected after the re-vaccination in any of the 23 participants within 14 days after re-vaccination (in contrast with 27/30 after a single dose of VLA1553 [medium dose, equivalent to the final dose]), which suggest protection against a challenge with the vaccine virus. Whether participants would be protected against a virulent wild-type strain and/or a more distant infection is not known.

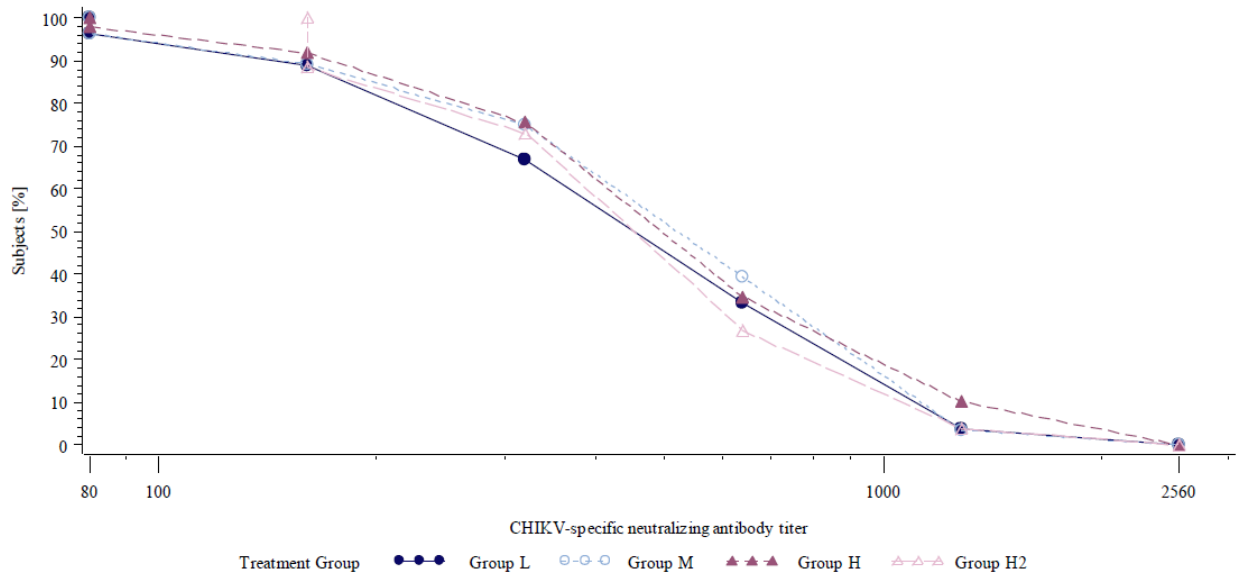
A trend for lower response at 14 days post-vaccination was observed for the low dose when compared to both other dose levels. This is illustrated by the reverse cumulative distribution curves for neutralizing antibodies specific to the virus vaccine (*Figure 4*, Figure 5 and *Figure 6*, PP population).

Figure 4. Reverse cumulative distribution curves for CHIKV-specific neutralizing antibodies 14 days after a single vaccination by study arm (PP population - Part B)



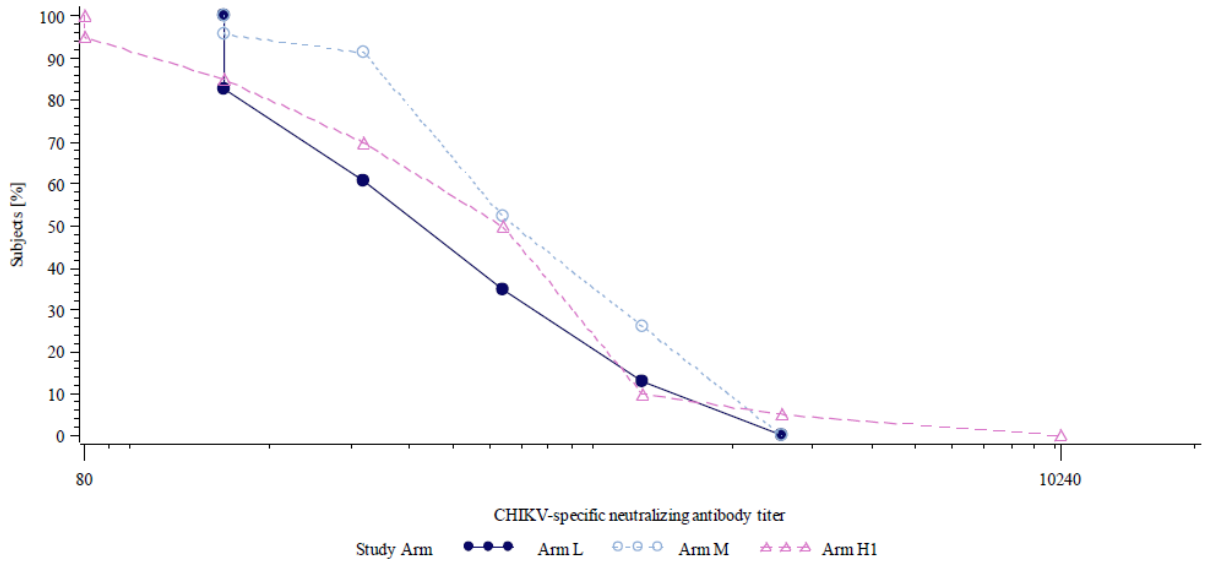
CHIKV = Chikungunya virus; PP = per-protocol
Source: Section 14, Figure 2.1.5

Figure 5.Reverse cumulative distribution curves for CHIKV-specific neutralizing antibodies 28 days after a single vaccination by study arm (PP population - Part B)



CHIKV = Chikungunya virus; PP = per-protocol
Source: Section 14, Figure 2.1.5

Figure 6. Reverse cumulative distribution curves for CHIKV-specific neutralizing antibodies 365 days after a single vaccination by study arm (PP population - Part C)



Together with the reactogenicity/safety profile (including in terms of viraemia), the medium dose was selected by the Applicant for further clinical development. The low and medium dose levels were better tolerated than the high dose level.

Comparison of µNT and µPRNT results – post-hoc analysis

A post-hoc analysis was performed on selected samples from 91 participants (PP population Part C) to compare neutralizing antibody titres obtained by using the µNT (assay used in this study) versus by using the µPRNT (assay used in the Phase 3 studies).

All the samples up to Day 365 post-dose 1 of the 91 participants included in the PP population Part C were re-tested in the validated µPRNT at Nexelis laboratory. Day 3 and Day 7 samples were not tested.

The same principles, calculations and populations were used as in the original analysis. 'Seroresponse' rate was added, defined as reaching µPRNT50 ≥ 150. µPRNT50 <20 was set to 10 for GMT and GMFI summaries.

A summary of GMTs over time is shown in the following Table 4.

Table 4. GMTs for CHIKV-specific neutralizing antibodies by visit (PP population)

			VLA1553				
		Statistic	**Arm L M12 re-vacc. (N=23)**	**Arm M M12 re-vacc. (N=23)**	**Arm H (N=45)**	**Arm H1 M12 re-vacc. (N=20)**	**Arm H2 M6 re-vacc. (N=25)**
Visit 1 - Day 0							
		Geometric Mean	10.7	10.0	10.0	10.0	10.0
		[95% CI] GM	[9.3, 12.4]	[10.0, 10.0]	[10.0, 10.0]	[10.0, 10.0]	[10.0, 10.0]
		n	23	23	45	20	25
Visit 1C - Day 14							
		Geometric Mean	1815.2	2773.5	3013.0	2752.2	3227.6

			VLA1553				
		[95% CI] GM	[1222.7, 2694.9]	[1858.4, 4139.1]	[2455.2, 3697.5]	[2002.9, 3781.8]	[2428.5, 4289.7]
		n	23	23	44	19	25
Visit 2 - Day 28							
		Geometric Mean	4471.1	4209.4	5033.5	3992.6	6058.5
		[95% CI] GM	[3238.2, 6173.4]	[2770.8, 6395.1]	[4105.3, 6171.6]	[2816.3, 5660.3]	[4791.3, 7660.8]
		n	23	22	45	20	25
Visit 3 - Day 84							
		Geometric Mean	1222.6	1534.8	1522.1	1405.8	1622.0
		[95% CI] GM	[863.4, 1731.1]	[1189.4, 1980.7]	[1220.8, 1897.7]	[1039.1, 1901.9]	[1163.3, 2261.6]
		n	23	23	45	20	25
Visit 4 - Day 180							
		Geometric Mean	957.7	1167.6	1018.9	990.4	1042.4
		[95% CI] GM	[747.5, 1227.0]	[856.7, 1591.3]	[823.9, 1260.2]	[708.5, 1384.3]	[776.1, 1400.0]
		n	23	23	45	20	25
Visit 5 - Day 365							
		Geometric Mean	851.2	1137.6	856.5	856.7	856.3
		[95% CI] GM	[654.0, 1108.1]	[834.8, 1550.3]	[700.6, 1046.9]	[584.9, 1254.7]	[684.9, 1070.6]
		n	23	23	45	20	25
n: number of subjects with available result VLA1553-101 Post Hoc analysis 21FEB2024, Table 3.3.1.2							

Proportion of participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (μPRNT50 ≥150) by visit is shown in Table 5.

Table 5. Proportion of participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) by visit (PP population) (Table R.3.3.1.14.1)

		VLA1553				
	Statistic	Arm L M12 re-vacc. (N=23)	Arm M M12 re-vacc. (N=23)	Arm H (N=45)	Arm H1 M12 re-vacc. (N=20)	Arm H2 M6 re-vacc. (N=25)
Visit 1C - Day 14	n /Nm (%) [95% CI]	23 /23 (100) [85.7, 100]	23 /23 (100) [85.7, 100]	44 /44 (100) [92.0, 100]	19 /19 (100) [83.2, 100]	25 /25 (100) [86.7, 100]
Visit 2 - Day 28	n /Nm (%) [95% CI]	23 /23 (100) [85.7, 100]	22 /22 (100) [85.1, 100]	45 /45 (100) [92.1, 100]	20 /20 (100) [83.9, 100]	25 /25 (100) [86.7, 100]
Visit 3 - Day 84	n /Nm (%) [95% CI]	23 /23 (100) [85.7, 100]	23 /23 (100) [85.7, 100]	45 /45 (100) [92.1, 100]	20 /20 (100) [83.9, 100]	25 /25 (100) [86.7, 100]
Visit 4 - Day 180	n /Nm (%) [95% CI]	23 /23 (100) [85.7, 100]	23 /23 (100) [85.7, 100]	45 /45 (100) [92.1, 100]	20 /20 (100) [83.9, 100]	25 /25 (100) [86.7, 100]
Visit 5 - Day 365	n /Nm (%) [95% CI]	23 /23 (100) [85.7, 100]	23 /23 (100) [85.7, 100]	44 /45 (97.8) [88.4, 99.6]	19 /20 (95.0) [76.4, 99.1]	25 /25 (100) [86.7, 100]

n... number of subjects
Percentages are based on the number of non-missing observations within each stratum (Nm).
Two-sided 95% confidence intervals calculated according to Altman
SPR is defined as proportion of subjects achieving a CHIKV-specific neutralizing antibody titer of µPRNT50 ≥150

All but one (L arm) participants were seronegative for CHIKV-specific neutralizing antibody at baseline. On Day 14, GMTs (95% CI) ranged from 1815.2 (1222.7 – 2694.9, arm L) to 3013.0 (2455.2 – 3697.5, arm H). The peak of neutralizing antibody titres was observed at Day 28 with GMTs (95% CI) of 4471.1 (3238.2 – 6173.4), 4209.4 (2770.8 – 6395.1) and 5033.5 (4105.3 – 6171.6) for L, M and H arms respectively. GMTs decreased from Day 28 to Day 365. At Day 365, GMTs (95% CI) were of 851.2 (654.0 – 1108.1), 1137.6 (834.8 – 1550.3) and 856.7 (700.6 – 1046.9) for L, M and H1 arms respectively. The kinetics of the response is not the same as observed by using the µNT to measure the neutralizing antibodies. This might be explained by the differences in the assays such as the qualification/validation status, the cells and the strain used.

Proportion of participants with antibody titres post-vaccination above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) was 100% from Day 14 to Day 180. At Day 365, only 1 participants had antibody titres below this threshold (arm H1).

Samples post-re-vaccination were not tested which is a missing information. As the kinetics of the antibody results differ between both assays, in particular for Day 365 timepoint, it might be that results post-re-vaccination be also different. It would have been of interest to confirm the absence of booster effect by using the µPRNT.

These limited results confirmed findings obtained by using the µNT to measure the neutralizing antibody titres, i.e. that a trend for higher GMTs was observed at 14 days post-vaccination for the medium and the high dose levels as compared to the low dose level (significant for the high dose level, but with 95% CI still overlapping). However, no difference was observed between dose levels in terms of proportion of participants with antibody titres post-vaccination above the defined threshold reasonably likely to predict protection, at any timepoint.

Conclusion

A trend for lower response at 14 days post-vaccination was observed for the low dose when compared to both other dose levels. Based on the reactogenicity/safety profile (including in terms of viraemia), the medium dose was selected for further clinical development. The low and medium dose levels were better tolerated than the high dose level. The choice of the dose was further supported by re-assessment of the antibody titres by using the validated CHIKV µPRNT assay (against the attenuated strain 181/clone 25), however performed after the start of the Pivotal Phase 3 study.

Viraemia results are presented in section 2.6.8.8.2.4. No viraemia was detected after the re-vaccination in any of the 23 participants within 14 days after re-vaccination (in contrast with 27/30 after a single dose of VLA1553 [3.2x104 TCID50, equivalent to the final dose]), suggesting protection against a challenge with the vaccine virus. Immunogenicity results are hardly interpretable in terms of boostability (anamnestic antibody response).

2.6.5.3. Main study(ies)

Study VLA1553-301

Methods

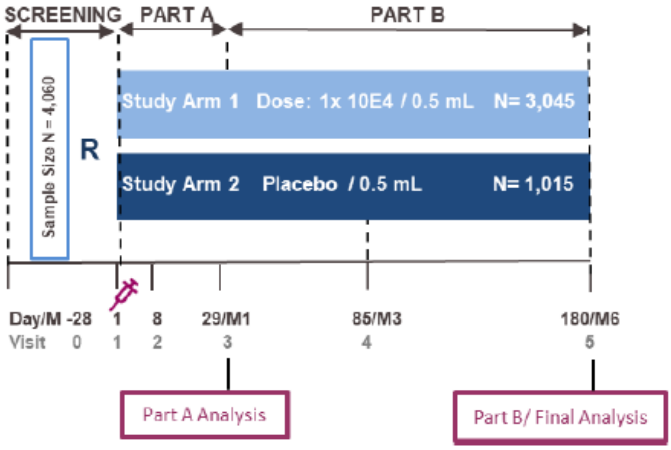
The assessment of the methods is based on Protocol version 6.0 dated 23 Mar 2021.

- **Overall study design**

VLA1553-301 is a randomised, placebo-controlled, double-blind, multicentre, Phase 3 trial designed to assess the immunogenicity and safety of the final dose of VLA1553 (1x10E4 TCID50 per 0.5 mL) in comparison to a placebo control in generally healthy adult participants. The study was conducted at 43 sites in the US and started mid-Sept 2020.

The overall study design is displayed in the figure below.

Figure 7. Overall study design



Approximately 4,060 participants were planned to be randomly allocated in a 3:1 ratio to VLA1553 (n = approximately 3,045) or placebo (n= approximately 1,015).

Randomization was stratified by age using two age strata: Stratum A (18–64 YOA) and Stratum B (≥65 YOA). Numbers of participants to be recruited in each age stratum were pre-defined. Approximately 3,653 and 407 participants were planned to be enrolled respectively in Stratum A and in Stratum B.

Participants received a single intramuscular administration of either VLA1553 or placebo in the deltoid region of the arm on Day 1.

Participants were followed up for approximately 6 months following vaccination.

Vaccinated participants had immunogenicity blood samples taken at pre-vaccination baseline (Day 1), 7 days (Day 8), 28 days (Day 29), 84 days (Day 85), and 179 days (Day 180) after vaccination. Samples were taken from all participants. However, immunogenicity evaluations were only planned to be performed in the Immunogenicity subset.

The first approximately 500 participants enrolled at approximately 15 pre-selected study sites were planned to be part of the Immunogenicity subset (approximately 346 subjects in Stratum A and 154 subjects in Stratum B). Following difficulties in recruiting elderly participants (≥65 years), the age-stratified recruitment caps were adapted in the protocol in December 2020. This resulted in a number of elderly participants in the Immunogenicity subset lower than initially planned (decreased from n=154 to n=111), with the age distribution of the Immunogenicity subset different than initially planned The total number of elderly participants in the safety population (n=463) was however reached and was even higher than the initially planned number (n=407). In order to compensate for the lower number of elderly participants in the Immunogenicity subset, elderly participants not part of the Immunogenicity subset were to be selected to also be analyzed for immunogenicity. This was made possible because all participants had blood samples taken for immunogenicity at all timepoints. So, a newly defined Immunogenicity subset of elderly participants was determined, including the initial approximately 111 elderly participants in the Immunogenicity subset and the 43 newly selected elderly participants.

- **Study Participants**

A total of approximately 4,060 generally healthy participants aged ⩾18 years were targeted for enrolment into this study. Participants were recruited at 43 study sites distributed throughout the US.

Participants with well-controlled (defined as stable and on therapy for the past 6 months) chronic conditions such as hypertension, type 2 diabetes mellitus, or hyperlipidemia were allowed to be enrolled. Participants immunocompromised due to medical condition or due to immunosuppressive treatments were excluded. History of (suspected) CHIKV infection and history of immune-mediated or clinically relevant arthritis or arthralgia were also part of the key exclusion criteria, as well as administration of any inactivated vaccine within 2 weeks before vaccination, or any live vaccine within 4 weeks before vaccination.

- **Treatments**

- VLA1553

Description

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

One dose (0.5 mL) of the VLA1553 vaccine contains between 1.6 x10E3 and 2.5 x10E4 TCID50 per dose. The active ingredient is suspended in a formulation buffer of pH 7.3, before freeze drying.

VLA1553 must be stored at +2-8°C (35.6 °F to +46.4 °F) in a refrigerator. Storage at room temperature or higher should be avoided because of potential impairment to immunogenicity and tolerability.

VLA1553 administered in the study

Only 1 batch was used in the trial and had potency of 2.0x10E4.The commercial release specifications are 4.0x10E3 – 4.0x10E4 (and stability lower limit is 1.0x10E3). Refer to the CMC assessment.

Lot is representative of (and very similar to) commercial lots.

- Placebo

VLA1553 placebo consists of a PBS buffer based on Dulbecco's PBS media formulation without Calcium and Magnesium. The concentration of the PBS is 1x and is produced with raw material classified as free from animal origin. The filling volume is 0.6 mL, ensuring an extractable volume of 0.5 mL. All reagents and material were provided in kit.

- ***Concomitant therapies***

All medications (including vaccinations) received from 2 weeks prior to study enrolment until completion/termination were reported. Concomitant medications were those with a start or end date on or after date of vaccination.

Any of the following treatments were documented as a protocol deviation: (i) Any blood products or immunoglobulins during the course of the study, (ii) immunosuppressive therapies (e.g. systemic or high dose inhaled [>800 µg/day of beclomethasone dipropionate or equivalent] corticosteroids, radiation treatment or other immunosuppressive or cytotoxic drugs) during the course of the study, (iii) Prophylactic administration of antipyretics within 4 hours prior to and during the first 72 hours after vaccination. The later treatment was not a reason for exclusion from the PP population.

- ***Study assessments***

Assessment of immunogenicity

The primary immunogenicity assay to measure CHIKV-specific neutralizing antibodies was a validated micro Plaque Reduction Neutralization Test (µPRNT), in order to allow for testing with higher throughput (vs. classical PRNT). The µPRNT was performed at a central laboratory (Nexelis Laboratories, Canada). The primary immunogenicity assay was the same in the Lot-to-Lot consistency study. LLOQ is defined as 20 µPRNT50. µPRNT50 was preferred over µPRNT80.

The strain used in the µPRNT assay is an attenuated strain from the Asian genotype (181/clone 25). The µPRNT assay differs from the micro neutralization assay (µNT) assay used for the assessment of neutralizing antibodies during Phase 1 clinical testing. The µNT assay used the vaccine strain.

The primary immunogenicity µPRNT assay is thus using an heterologous strain, which is the same as the one used in the Yoon et al. study (sero-epidemiological study used as a basis for the primary endpoint's threshold). This approach is supported.

The applicant indicated that the aim of using an heterologous strain is to show that vaccine-induced antibodies have the capacity to neutralize an heterologous strain. However, the strain is an attenuated one, and it might be that neutralization of this attenuated strain be easier compared to a virulent wild-type strain. It is thus important to show that the vaccine-induced antibodies can neutralize virulent wild-type strains (including recently circulating ones) and to compare the neutralizing antibody titres specific to those strains with the neutralizing antibody titre specific to the attenuated strain, in the same assay set-up.

In the context of exploratory analyses, a subset of samples were analysed in classical PRNT assays to quantify circulating levels of neutralizing antibodies specific to the wild-type CHIKV La Reunion strain, 37997 strain, and Caribbean M109 strain. Testing was done by use of 3 qualified classical PRNT at the University of Texas Medical Branch (UTMB). Additional testing for cross-neutralisation against a more recent CHIKV isolates and against other alphaviruses were performed the Oregon Health and Science

University (OHSU), with classical PRNT assays (developed status). Additional data in PRNT assays specific to additional CHIKV strains and with additional samples (including samples isolated from naturally infected subjects) will be generated. Refer to section 2.6.5.6.

Neither µPRNT nor 'classical PRNT' data are available with respect to neutralisation of the vaccine strain. Only limited µNT data from the Phase 1 are available with respect to the vaccine strain.

Baseline samples were also tested for CHIKV, Mayaro virus, Zika virus and Dengue virus by ELISA. Testing was performed at Cerba Research.

- ***Objectives and endpoints***

- Primary objective and endpoint

The primary objective was to assess the immunogenicity and safety of VLA1553 28 days following vaccination with a single dose of VLA1553 in a population aged 18 years. However, only an immunogenicity primary endpoint is provided (safety endpoints are classified as secondary).

The primary endpoint was the proportion of baseline CHIKV seronegative participants in the VLA1553 arm with a Day 29 CHIKV neutralizing antibody titre (as determined by µPRNT50) ≥150 (threshold pre-determined as reasonably likely to predict protection).

The term seroresponse is used in the report to refer to a CHIKV µPRNT50 antibody titre ≥150.

The primary endpoint was considered met if the lower bound of the 95% confidence interval (CI) around the proportion is >70%. This acceptance criterion is acceptable.

The primary endpoint is evaluated based on the VLA1553 vaccinated participants only. In general, in the absence of an established ICP, the immunogenicity conclusion should be based on the difference in response rates between treatment groups. Immunogenicity results from the treatment group alone may be prone to bias and their external validity is unclear. In contrast to between-group comparisons for which statistical inference can be based on a preplanned and well controlled randomization process, statistical inference of group-wise results can only rely on assumptions about the sampling properties of the recruitment process. For the present study, however, this may not be of major concern as the prevalence of natural immunity and likelihood to develop corresponding titres without active immunization in the course of the study duration can be considered minimal. Moreover, the variability of the assay is well below the applied threshold, such that the expectation of a placebo response rate of zero is plausible. Consequently, treatment group only results can be expected to closely resemble corresponding between group comparisons. Furthermore, the Applicant provides corresponding between group comparisons as a secondary endpoints which support the immunogenicity conclusions based on the primary endpoint.

- Secondary objectives and endpoints

The secondary objectives were to assess the immunogenicity and safety of VLA1553 up to 180 days following vaccination. Longer follow-up data were obtained in study VLA1553-303.

Secondary immunogenicity endpoints comprise the proportion of participants reaching at least the seroresponse titre threshold (µPRNT50 ≥150) at the other timepoints (Day 8, Day 85 and Month 6 post-vaccination). These endpoints are supported. The Day 8 early endpoint is supported as this vaccine may be used in the context of an outbreak.

The proportion of participants with seroconversion at Day 29 and Month 6 was also part of the secondary endpoints. For baseline CHIKV seronegative participants, seroconversion (i.e., presence of CHIKV neutralizing antibodies) was defined as CHIKV-specific µPRNT50 neutralizing antibody titre of ≥20 (lower assay limit of quantitation of µPRNT50=20). For baseline CHIKV seropositive participants,

seroconversion was defined as a more than 4-fold increase over baseline. An alternative definition was used for analyses performed on the sPP population in which another cut-off (>40) was used for CHIKV seropositivity (see below). In these sPP population analyses, seroconversion was defined as >4-fold increase of µPRNT50 compared to baseline (Day 1) for both baseline positive participants and baseline negative participants.

CHIKV-specific µPRNT50 neutralizing antibody titres (GMTs), fold increases of CHIKV-specific µPRNT50 neutralizing antibody titres compared with baseline and proportion of participants reaching an at least 4-fold, 8-fold, 16-fold or 64-fold increase, at Days 8, 29, 85 and Month 6 post-vaccination were also part of the secondary endpoints. Reverse cumulative distribution plots of the proportion of participants by titres value were produced. This was done by arm and by age strata, at Day 29 and Day 180.

The characterization of the neutralizing antibody response to VLA1553 at various timepoints, as part of the secondary immunogenicity endpoints is supported and overall in line with the EMA advice. Some of the endpoints (such as fold antibody titre increases) are considered of less relevance.

Exploratory objectives and endpoints

Subgroups analyses by age were planned as well as descriptive analyses of immune responses in baseline CHIKV seropositive participants.

Measurement of pre-existing antibodies was planned on the baseline blood samples for a panel of alphaviruses (in practice, only Mayaro [alphavirus circulating in South America] serology was tested) and flaviviruses (Dengue and Zika viruses) in order to characterize the population at baseline and to investigate possible interference with the vaccine-induced response in the presence of these specific antibodies. These viruses are not endemic in the US regions where the study was conducted. Mosquitos that can transmit Dengue virus and CHIKV are present in the South/South-east states of the US. Limited CHIKV local transmission has been reported in Florida and Texas (very limited for Dengue), where respectively 8 and 4 study sites are located. There is co-circulation of CHIKV and Dengue, Zika and Mayaro viruses in Brazil where the study VLA1553-321 is conducted.

- ***Sample size***

The sample size of approximately 3,000 VLA1553-vaccinated participants was calculated to allow for the detection of at least one vaccine-attributable uncommon event (incidence rate 1/1000) with a probability of 95%. Overall, this sample size was meant to meet the requirement of the former EMA vaccine guidance to include at least 3,000 participants in the safety database (EMEA/CHMP/VWP/164653/2005). This approach is considered acceptable.

The Immunogenicity subset of 375 participants vaccinated with VLA1553 was calculated to allow for sufficient statistical power when applying a one-sided exact binomial test with a significance level of 2.5% against a non-acceptance threshold of 70% on the proportion of participants with a seroresponse level (defined as µPRNT50 titre $\geqslant$150 for baseline seronegative participants) 28 days post-vaccination. A seroresponse rate of 80% was assumed on the basis of previous results, and 200 VLA1553-vaccinated participants were therefore necessary for a statistical power of 90%. With an expected dropout and major protocol deviations rate of approximately 10%, 225 VLA1553 vaccinees needed to be allocated to the Immunogenicity subset. To account for placebo participants, to achieve a meaningful number of participants in both age strata, and for long-term follow-up in a subsequent trial, 501 participants were deemed required for enrolment into the Immunogenicity subset. This approach is acceptable.

- ***Randomisation and Blinding (masking)***

Treatment allocation was performed using block randomization using a fixed block size of 4. This choice can be criticized, especially in light of the 3:1 allocation ratio, which would mean that in case of

inadvertent (or protocol justified) unblinding of a treated subject, the treatment assignment of the following subjects in the corresponding block could be determined. Considering, however, that blocks were not stratified by centre the actual risk of unblinding is considered negligible.

According to the SAP randomization was stratified by age group (Stratum A: 18-64, Stratum B: 65 and older) and inclusion in the Immunogenicity subset. Numbers of participants to be recruited in each age stratum were pre-defined. Approximately 3,653 and 407 participants were planned to be enrolled respectively in Stratum A and in Stratum B.

The first approximately 500 participants enrolled at approximately 15 pre-selected study sites were planned to be enrolled in the Immunogenicity (IMM) subset. The CSR refers to 12 pre-selected sites for the recruitment of the Immunogenicity subset. Approximately 346 participants in Stratum A and 154 participants in Stratum B were planned to be enrolled.

As recruitment of elderly participants was slower than anticipated changes were implemented with Version 6.0 of the study protocol. The maximum number of participants allocated to Stratum A of the IMM subset was increased in order to reach the preplanned size of 500 subjects in the IMM subset, effectively changing the ratio of older to younger subjects in the IMM subset compared to preplanned proportions. Within strata, the allocation ratios between treatment and control subjects was maintained by this approach. Consequently, the resulting treatment allocation can be considered stratified by age.

In order to compensate for the lower number of elderly participants in the Immunogenicity subset (43 participants lacking versus the initial plan), elderly participants not part of the Immunogenicity subset were to be selected to also be analyzed for immunogenicity. For that purpose, the Protocol V.6.0 states that 43 elderly participants had to be randomly selected (while maintaining the approximate 3:1 ratio of treatment arms) from the safety analysis population from the 15 sites initially contributing to the Immunogenicity subset. It is noted that if less than 43 participants could be identified in the 15 sites, then the additional number of participants needed to reach 43 could be randomly selected from the other sites (than pre-selected for the Immunogenicity subset). So, a newly defined Immunogenicity subset of elderly participants was determined, including the initial approximately 111 elderly participants in the Immunogenicity subset and the 43 newly selected elderly participants.

Randomization was not stratified by site or region. The "Guideline on adjustment for baseline covariates in clinical trials (EMA/CHMP/295050/2013)", however, states that randomization should be stratified by site and deviation from this needs to be justified. The justification provided by the Applicant for the non-implementation of site stratification is to avoid unbalanced randomization due to many small sites, especially in combination with age stratification. Considering that a majority of sites recruited more than 50 participants with the smallest site still recruiting 6 participants, it remains debatable to what extent this risk applied. The distribution of study sites by arms was provided, but these data are very difficult to interpret given the large number of sites. As stratification mainly reduces between site variability (and thereby improves sensitivity to demonstrate treatment effects) this is not considered of major relevance with respect to immunogenicity results. However, it may hamper identification of between group differences with respect to safety endpoints.

Participants, investigators, site staff performing the safety assessments, laboratory personnel and the biostatistician (except the one involved in DSMB) were blinded to the assignment into study arms. The vaccine was prepared by unblinded site staff.

VLA1553 was supplied in prefilled vials under a freeze-dried presentation. Vaccine reconstitution was performed by unblinded site staff. For both the VLA1553 and placebo, identical syringes were supplied (provided in kits) for vaccine reconstitution or to re-filled with PBS. The content of the syringes was masked by use of a label wrapped around the syringes and of identical appearance for both arms. The

appearance of the dissolved vaccine can vary between a colorless clear solution to a slightly yellowish solution. The adhesive tape attached to the syringes is a yellow-colored transparent tape. As the color of the tape is yellow, it should not allow to distinguish a yellowish solution (vaccine) from a clear solution (placebo, PBS in water). As the content in the syringes was concealed, vaccination of the participants could be performed by either unblinded or blinded study staff.

The study is double-blinded (more specifically observer-blinded). The process for blinding described in the protocol is deemed appropriate.

- **Statistical methods**

Analysis Cohorts

The Immunogenicity (IMM) population includes all vaccinated participants from the IMM subset who were CHIKV seronegative at baseline (defined as µPRNT50 titre <20) and have at least one evaluable post-baseline titre measurement.

The Immunogenicity Elderly (eIMM) population is constituted of the IMM population (younger adults and older adults) and of randomly selected elderly participants from the safety population who were CHIKV seronegative at baseline (defined as µPRNT50 titre <20) and have at least one evaluable post-baseline titre measurement. The eIMM population is in fact derived from a newly defined Immunogenicity subset constituted of the initial participants in the Immunogenicity subset and newly selected elderly participants randomly selected from the from the safety population. The intention is to reach the original number of elderly in the subset.

The per protocol (PP) population contains all vaccinated participants part of the IMM subset who were CHIKV seronegative at baseline (defined as µPRNT50 <20), have at least one evaluable post-baseline titre measurement, and who have no major protocol deviation related to Visit 3 (major protocol deviations are defined as those that could affect the assessment of immune responses).

Some major protocol deviations were pre-specified in the protocol (those which were anticipated based on previous vaccine trial experiences), such as receiving the wrong IMP (not according to randomization), missing Visit 3, sample testing issues, use of concomitant medication which could influence the immune response, known or suspected defect of the immune system. Other types of protocol deviations could also be classified as major as per the discretion of the Sponsor, if they may in the opinion of the Sponsor impact the immune response. This was decided on a case-by-case basis and had to be done in a blinded manner (prior to study unblinding).

The sensitivity per protocol (sPP) population is defined in a similar way as the PP population, but using another threshold to define CHIKV seronegative at baseline (µPRNT50 ≤40). Results from the VLA1553-301 Part A analysis showed that a small proportion of baseline samples and of the post-vaccination samples from placebo participants had tested positive with µPRNT50 values close to the µPRNT lower limit of quantitation of 20. This justifies the conduct of a sensitivity analysis in sPP population. A similar sensitivity analysis was done in VLA1553-302 (sPP2 population).

For the primary and secondary analyses (conducted in a CHIKV seronegative population), the cut-off of <20 was used to defined baseline seronegativity. For exploratory descriptive analysis in CHIKV seropositive participants, the cut-off of >40 was used to define baseline seropositivity. The approach of the Applicant is endorsed, as the most clinically relevant cut-off was used for the respective analyses.

The sensitivity per protocol (sPP2) population was also defined in a similar way as the PP population but using visit windows to report the results rather than visit labels. Visits (including any unscheduled or planned visits) or immunogenicity samples were to be assigned to the Visit number of the window they fall into. If there were multiple results falling into one visit after re-windowing, then a conservative approach was to be taken, and the lower titre value was to be used. Participants with

major protocol deviations around immunogenicity results were not excluded if a result was found within the allowed visit window.

Immunogenicity analyses

The primary endpoint was evaluated using an exact binomial test and corresponding exact confidence interval to demonstrate statistical significance in terms of a seroresponse rate in excess of 70%. The corresponding analysis does not appear to be stratified by age cohort (as would be required by the Guideline on adjustment for baseline covariates in clinical trials (EMA/CHMP/295050/2013)). However, results across individual strata are highly consistent, such that not substantial difference in estimates between adjusted and unadjusted analyses is to be expected.

Secondary endpoints concerning GMT ratios at different post-baseline visits were analysed using an ANCOVA model appropriately adjusted for randomization strata. This is endorsed. Secondary endpoints concerning the difference in seroresponse rates between treatment groups were evaluated using Fisher's exact test, which does not account for randomization strata. Wald CIs were used. Alternative estimates using an exact method results were also provided.

While the definition of the IMM population ensures that at least one post-baseline measurement is available for each subject in the IMM population, titre measurements at specific post-baseline visits may not be available for all participants. No imputation method for missing data was foreseen in the protocol, unless the amount of missing data exceeded a certain proportion (5%). Upon request, additional analyses of seroresponse rates and GMTs (per arm and between arms) according to a worst case imputation strategy where missing post baseline titre measurements are set to 10 (i.e. below the seroresponse threshold) were also provided.

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

A formal hypothesis test was defined for the primary immunogenicity analysis, using a one-sided significance level of 2.5%. No adjustment for multiplicity for any immunogenicity endpoints or across subgroups was preplanned or applied.

Results

- **Participant flow**

The disposition of subjects of the immunogenicity subset and the safety population is provided in Figure 10 and Figure 11, respectively.

A total of 4,128 participants were randomized, 3,093 participants in the VLA1553 arm and 1,035 participants in the placebo arm (3:1 ratio). Numbers are close to the pre-planned numbers of approximately 4,060 (approximately 3,045 and 1,015 in the VLA1553 and placebo arms).

Ten participants in the VLA1553 arm and one in the placebo arm did not receive vaccination. One participant was randomized twice and vaccinated twice with VLA1553. One participant randomized to placebo was vaccinated with VLA1553. The first participant (accounting for 2 participants) was excluded from the safety population and the second one was included in the VLA1553 arm. Thus, in total the safety population consisted in 4,115 participants, 3,082 in the VLA1553 arm and 1,033 in the placebo arm.

A total of 3,644 participants (88.6%) completed the study (Visit 6/Day 180). A total of 471 (11.4%) participants (358 [11.6%] and 113 [10.9%] in the VLA1553 and placebo arm, respectively) discontinued the study prior to Month 6. This is comparable to VLA1553-302 study. Main reasons were

lost of follow-up (6.0% and 6.1% respectively in the VLA1553 and placebo arm) and consent withdrawal (4.7% and 4.0% respectively in the VLA1553 and placebo arm). Reasons for discontinuation were similar across arms.

Out of the 4,115 participants included in the safety population, 3,923 participants (95.3%) completed the Visit 3/Day 29 (95.1% and 95.9% respectively in the VLA1553 and placebo arm).

Of the 4,128 randomized participants, 501 were allocated to the IMM subset (respectively 375 and 126 in the VLA1553 and placebo arm) of which 497 were vaccinated (respectively 371 and 126 in the VLA1553 and placebo arm).

From the 375 participants in the VLA1553 group and 126 in the placebo group of the IMM subset, 43 (11.5%) and 10 (7.9%) subjects discontinued the study, respectively. The main reasons for discontinuation from study in the IMM subset for both treatment groups were withdrawal by participant (5.3% and 4.0% respectively in the VLA1553 and placebo arm) and lost to follow-up (5.1% and 3.2% respectively in the VLA1553 and placebo arm).

The proportion of participants in the IMM subset who completed the respective study visits were overall comparable to the safety population.

Figure 8. Participant disposition (Immunogenicity subset)

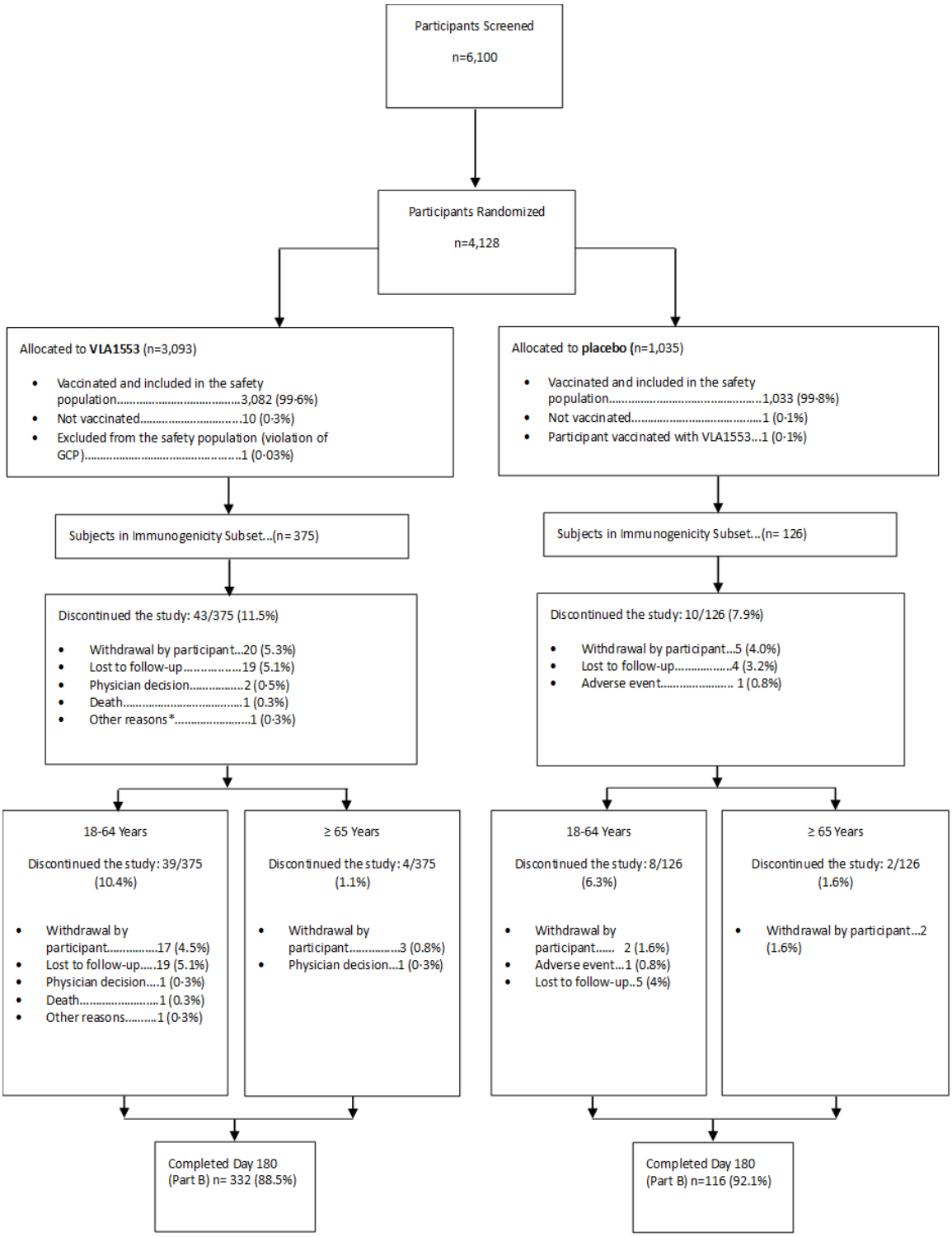
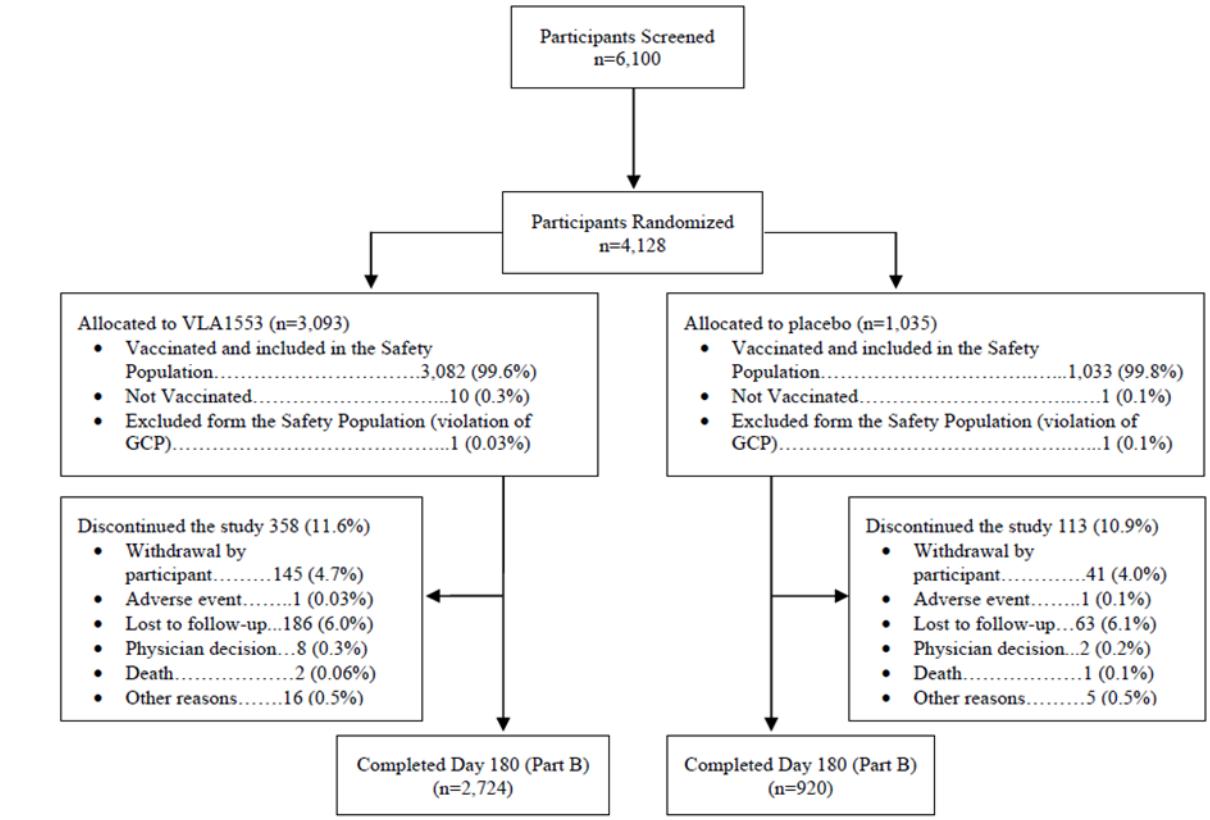


Figure 9. Participant disposition (Safety population)



eCRF=electronic case report form.
Note: Reasons for discontinuation are based on the End of Study/Early Termination eCRF page. Discontinued study includes all participants who discontinued prior to the Visit 5 (Day 180/Month 6). Three participants (participant 1553-1-18-073 in the VLA1553 arm, and participants 1553-1-25-044 and 1553-1-25-139 in the placebo arm) had end of study recorded as completed on the eCRF, however they did not complete Visit 5 as required. In addition, 3 participants (participants 1553-1-04-044, 1553-1-14-237, and 1553-1-18-055, all in the VLA1553 arm) actually completed Visit 5 but did not have end of study page completed in the eCRF.
Source: Table 14.1.1.1, Table 14.1.1.3.1, Table 14.1.1.6, and Listing 16.2.1.2

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Populations of analysis

A summary of the analysis sets is provided in the Table below.

Table 6. Summary of the analysis set

Populations of analysis	**VLA1553**	**Placebo**	**Total**
IMM subset	375	126	501
IMM population	344	118	462
PP population	266	96	362
Excluded from PP pop.	*109 (29%)*	*30 (24%)*	*139 (28%)*
Excluded for major protocol deviation	*78 (21%)*	*22 (17%)*	*100 (20%)*
eIMM population	376	127	503
sPP population	275	99	374
sPP2 population	267	96	363

For note, the primary endpoint and some of the secondary endpoints are estimated based only on the Active arm.

The IMM population consisted in 462 participants (344 and 118 in the VLA1553 and placebo arm, respectively). Of the 501 participants allocated to the Immunogenicity subset, 7.8% (n=39; respectively 8.3% [n=31] and 6.3% [n=8] in the VLA1553 and placebo arm) were excluded to constitute the IMM population.

Of the 375 participants of the IMM subset included in the VLA1553 arm, 31 were excluded from the IMM population for the following reasons: seropositive (n=16) or unknown (n=1) at baseline, no evaluable post-baseline titre (n=10), not vaccinated (n=4). Eight of 126 placebo participants were excluded from the IMM subset because of no evaluable post-baseline titre (n=4) and seropositivity at baseline (n=4).

The PP population consisted in 362 participants (266 and 96 in the VLA1553 and placebo arm, respectively). Therefore, of the 462 participants in the IMM population, 100 (21.6%) were excluded to constitute the PP population, due to major protocol deviations that could impact the immune response. This percentage of deviations leading to exclusion from the PP population is considered high and is much higher than in VLA1553-302.

The 103 major protocol deviations leading to exclusion from the PP population are presented in the Table 7 below.

A total of 75 participants (57 and 18 in the VLA1553 and placebo arm respectively) had major protocol deviations for Visit 3 (Day 29) attributable to either 'Missing endpoint data' or 'Out of range Visit window'. Overall, of the 75 participants, n=51 attended the Visit out of window, n=20 missed the Visit (Visit 3 not attended or withdrawal or lost to follow-up before Visit 3), and n=4 had immunogenicity sample not collected or not usable (although they attended Visit 3). The time window deviations up to +/- 8 days were captured as minor protocol deviations, whereas time window deviations exceeding +/- 8 days were considered major, resulting in the exclusion from the PP population. The window allowed around the Visit 3 of +/- 8 days is deemed acceptable.

A total of 24 participants (20 and 4 in the VLA1553 and placebo arm respectively) had an 'investigational product' major protocol deviation. The IMP had to be stored at +2°C to +8°C during shipment and storage. Any recording of temperature out of this predefined range (irrespective of the duration of the excursion) had to be reported as temperature deviation to Valneva. In such situation, the IMP had to be quarantined until finalization of the Product Qualify Assessment. If the IMP was administered unintentionally prior to completion of the Product Quality Assessment this was reported as an important protocol deviation. If the IMP was deemed not appropriate for further use, as a result from the Product Quality Assessment (i.e., unstable) the protocol deviation was further classified as major one.

Two participants (one in each arm) had taken a prohibited medication, one participant of the VLA1553 arm met an exclusion criteria and one participant of the VLA1553 arm had a protocol deviation 'safety assessment issue'.

The distributions of the different types of major protocol deviations are roughly balanced across arms.

Table 7. Major protocol deviations in study VLA1553-301 leading to exclusion of subjects from the PP population (Immunogenicity Population)

Major PD Category [n(%)]	VLA1553 N=344	Placebo N=118	Total N=462
Any major PD	78 (22.7)	22 (18.6)	100 (21.6)
Visit Window or Endpoint Data	57 (16.6)	18 (15.2)	75 (16.2)
Investigational Product	20 (5.8)	4 (3.4)	24 (5.2)
Prohibited Co-Medication	1 (0.3)	1 (0.8)	2 (0.4)
Exclusion Criteria	1 (0.3)	0	1 (0.2)
Assessment Safety	1 (0.3)	0	1 (0.2)

PD=protocol deviation

Note: For each category, subjects are included only once, even if they experienced multiple events in a category.

Source: VLA1553-301 CSR v4.0, Table 14.1.1.4.2

In addition, as mentioned in the methods section, additional populations were defined for further (sensitivity) analyses, namely eIMM, sPP and sPP2 populations.

The eIMM population included additional elderly participants (from the safety population) to reach the planned number of elderly participants in the IMM subset. The eIMM population includes 503 participants (respectively 376 and 127 in the VLA1553 and the placebo arms), i.e. 41 participants in addition to the IMM population.

The sPP population was defined similarly than the PP population but used the threshold of 40 µPRNT50 to define the baseline serostatus. The sPP2 population was also defined similarly than the PP population but used visit windows to report the results (instead of the visit label). Numbers were thus slightly different between the PP population and the sPP/sPP2 populations.

Strata vs age categories

The initial plan of the Applicant was to enrol approximately 4,060 participants, of which approximately 3,653 participants in Stratum A and approximately 407 participants in Stratum B. In total the safety population consisted in 4,115 participants, including 3,652 participants aged 18-64 years (2,736 and 916 participants in the VLA1553 arm and placebo arm, respectively) and 463 participants aged 65 years and above (346 and 117 participants in the VLA1553 arm and placebo arm, respectively) (see below, section on baseline demographics).

For the IMM subset, the initial plan was to include a total of 154 subjects in Stratum B. Finally,99 are included the IMM population (75 and 24 in the VLA1553 and placebo arms, respectively). Note that a total of 140 (107 and 33 in the VLA1553 and placebo arms, respectively) elderly participants are included in the eIMM.

Populations of analysis, by age category

A summary of the analysis sets is provided in the Table below.

Table 8. Summary of analysis set by age category

Populations of analysis	VLA1553	Placebo	Total
IMM subset	375	126	501
18 to 64 years	293	98	391
≥65 years	82	28	110
IMM population	344	118	462
18 to 64 years	269	94	363
≥65 years	75	24	99
PP population	266	96	362
18 to 64 years	207	73	280
≥65 years	59	23	82
eIMM population	376	127	503
18 to 64 years	269	94	363
≥65 years	107	33	140

- **Recruitment**

Participants were recruited in 43 centres between 17 Sept 2020 and 10 Apr 2021 (Schneider et al.; Lancet 2023 ; doi : 10.1016/S0140-6736(23)00641-4). The study was completed on 27 July 2022.

- **Conduct of the study**

Protocol amendments

Summary of changes to the VLA1553-301 Protocol version 5.0 (changes versus Protocol version 4.0)

Amendments introduced in Protocol version 5.0 are not always clearly described by the Applicant. This was in general clarified during the assessment. Due to difficulties of recruiting elderly participants (≥65 years) during the COVID-19 period, the recruitment caps were amended in the Protocol to allow recruitment of more participants 18–64 YOA started. This amendment was implemented from 14th of December 2020 to allow recruitment completion within a reasonable time frame.

Further changes include an update of the threshold to define CHIKV seropositivity following validation of the µPRNT assay (from CHIKV-specific neutralizing antibody titre of ≥ 1:tbd to ≥1:20).

Summary of changes to the VLA1553-301 Protocol version 6.0 (changes versus Protocol version 5.0)

This amendment was implemented due to feedback received from FDA on 16th of March 2021, including mainly agreement on surrogate of protection titre of 150 µPRNT50, and changes due to the ongoing SARS-CoV2 pandemic in the US that impacted recruitment of elderly.

Surrogate of protection titre

The primary endpoint was changed from 'Proportion of subjects with a seroprotective CHIKV antibody level defined as µPRNT50 ≥50 for baseline negative subjects 28 days post-vaccination' to 'Proportion of

subjects with a seroprotective CHIKV antibody level defined as µPRNT50 ≥150 for baseline negative subjects 28 days post-vaccination.' The threshold was changed accordingly in other parts of the protocol.

Number of study sites

The number of study sites was increased from 30 to approximately 44.

Updated number of participants in the IMM subset

The number of elderly participants (≥65 years) planned to be available in the Immunogenicity subset was reduced from 154 to 111 (from 115 and 39 to 83 and 28, respectively in the Active vs Placebo group). As the total number of participants in the Immunogenicity subset (approximately 500) was not amended, the amount of data in the elderly was reduced, while the amount of data in adults 18-64 years was increased.

eIMM

The selection of additional non-immunogenicity subset elderly participants to constitute the eIMM subset was described, as well as the statistical analysis of the eIMM population.

PP population

The PP population contains all IMM population participants who have no major protocol deviations that could impact the immune response. Some examples that may lead to exclusion from the PP population were provided already in version 5.0, such as 'Subject has received the wrong (not according to randomization) or no IMP'. Protocol version 6.0 added the following examples: 'Subjects who received an excluded concomitant medication which could influence the immune response'; 'Subjects missing Visit 3'.

Protocol deviations

Protocol deviations were to be classified in in important/non-important deviations and minor/major protocol deviations. The important/not-important PDs were defined according to Section 10.2 of the ICH E3 guideline "*Important Protocol Deviations are a subset of Protocol Deviations that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a subject's rights, safety, or well-being*." PDs were further classified into major or not major (minor), which was based on their possible impact on the immune response. Major PDs were those which led to exclusion from the PP analysis set. Only protocol deviations until Visit 3 were assessed into major or minor protocol deviations. The same approach was applied in VLA1553-301 and VLA1553-302.

A total of 353 participants (8.6%) of the safety population had at least a major protocol deviation until (and including) Visit 3 (278 [9.0%] and 75 [7.3%] respectively in the VLA1553 and placebo arm). A total of 2,593 (63.0%) participants had important minor protocol deviations and 1,516 (36.8%) had (not important) minor protocol deviations.

No **inspection** is mentioned in the submission.

- **Baseline data**

Demographic characteristics for the IMM subset and PP populations are presented in the tables below.

Table 9. Demographic Characteristics by Immunogenicity Subset Status (Randomized Population)

Characteristic	Immunogenicity Subjects			Non Immunogenicity Subjects		
	VLA1553 (N=375)	Placebo (N=126)	Total (N=501)	VLA1553 (N=2718)	Placebo (N=909)	Total (N=3627)
Age (years)						
n	375	126	501	2718	909	3627
Mean (std)	49.1 (15.75)	49.0 (16.15)	49.1 (15.84)	44.5 (15.32)	44.5 (15.44)	44.5 (15.35)
Median	49.0	51.0	50.0	45.0	44.0	44.0
Q1, Q3	36.0, 63.0	34.0, 63.0	36.0, 63.0	31.0, 57.0	32.0, 57.0	31.0, 57.0
Min, Max	18, 82	18, 76	18, 82	18, 88	18, 94	18, 94
Age Group [n (%)]						
≥ 18 years - 64 years	293 (78.1)	98 (77.8)	391 (78.0)	2452 (90.2)	819 (90.1)	3271 (90.2)
≥ 65 years	82 (21.9)	28 (22.2)	110 (22.0)	266 (9.8)	90 (9.9)	356 (9.8)
Weight (kg)						
n	369	126	495	2710	906	3616
Mean (std)	87.92 (21.590)	87.39 (21.351)	87.79 (21.509)	87.76 (22.751)	86.26 (21.918)	87.39 (22.552)
Median	86.50	85.95	86.30	84.80	83.15	84.33
Q1, Q3	73.08, 99.60	70.30, 98.90	72.60, 99.60	71.73, 100.35	70.30, 98.80	71.40, 100.00
Min, Max	44.9, 179.6	50.2, 142.4	44.9, 179.6	38.7, 247.7	43.8, 197.8	38.7, 247.7

std = standard deviation
a. BMI = Body Mass Index
Note: 'Not Reported' and 'Unknown' ethnicity categories are as recorded on the CRF.
Note: Immunogenicity Subjects are all subjects who were initially enrolled into the immunogenicity evaluation group (Immunogenicity Subset), regardless of any other factors.

Source: Listing 16.2.4.1, Dataset: ADSL, Program: t-demog-rand.sas, Output: T-14-01-02-06-demog-rand.rtf, Generated on: 2022-03-14T07:33
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Table 10. Demographic characteristics (PP population)

Characteristic	VLA1553 (N=266)	Placebo (N=96)	Total (N=362)
Sex [n (%)]			
Female	154 (57.9)	60 (62.5)	214 (59.1)
Male	112 (42.1)	36 (37.5)	148 (40.9)
Race [n (%)]			
American Indian or Alaska Native	2 (0.8)	0	2 (0.6)
Asian	2 (0.8)	2 (2.1)	4 (1.1)
Black or African American	41 (15.4)	1 (10.4)	51 (14.1)
Native Hawaiian or other Pacific Islander	1 (0.4)	1 (1.0)	2 (0.6)
White	212 (79.7)	82 (85.4)	294 (81.2)
Other	8 (3.0)	1 (1.0)	9 (2.5)
Ethnicity [n (%)]			
Hispanic or Latino	21 (7.9)	12 (12.5)	33 (9.1)
Not Hispanic or Latino	241 (90.6)	83 (86.5)	324 (89.5)
Not Reported	4 (1.5)	1 (1.0)	5 (1.4)
Unknown	0	0	0
Age (years)			
n	266	96	362
Mean (std)	49.1 (15.75)	49.5 (15.61)	49.2 (15.69)
Median	49.0	50.5	49.5
Q1, Q3	36.0, 63.0	35.5, 64.0	36.0, 64.0
Min, Max	18, 81	21, 75	18, 81
Age Group [n (%)]			
≥18 years – 64 years	207 (77.8)	73 (76.0)	280 (77.3)
≥65 years	59 (22.2)	23 (24.0)	82 (22.7)
Weight (kg)			
n	264	96	360
Mean (std)	87.8 (21.8)	86.7 (21.5)	87.5 (21.7)
Median	86.6	85.8	86.4
Q1, Q3	72.7, 99.8	69.1, 97.1	71.8, 99.6
Min, Max	46.5, 179.6	50.2, 142.4	46.5, 179.6
Height (cm)			
n	265	96	361
Mean (std)	169.2 (10.3)	169.2 (9.6)	169.2 (10.1)
Median	168.9	168.2	168.9
Q1, Q3	162.0, 176.5	162.9, 176.0	162.0, 176.5
Min, Max	128.8, 199.4	144.7, 190.0	128.8, 199.4
BMI (kg/m^2)			
n	264	96	360
Mean (std)	31.0 (8.6)	30.4 (7.2)	30.8 (8.3)
Median	29.5	29.5	29.5
Q1, Q3	25.7, 35.0	25.5, 34.0	25.6, 34.8
Min, Max	17.5, 102.3	17.1, 53.7	17.1, 102.3

eCRF=electronic case report form; PP=per protocol; std=standard deviation

a BMI=Body Mass Index

PP population versus IMM subset and safety population

There was a slightly higher percentage of female in the IMM subset (58.3%) and the PP population (59.1%) versus the safety population (54.7%).

Median age of the participants was 45.0 years (Q1-Q3: 32.0-57.0) and 49.5 years (Q1-Q3: 36.0-64.0) in the safety and PP populations, respectively. There were 11.3% of elderly adults (≥65 years) in the safety population versus 22.7% in the PP population. Median age of the IMM subset was 50.0 years

(Q1-Q3 of 36.0-63.0), which is similar than in PP population. Percentage of elderly adults was also comparable (22.0%).

Median body mass index was 29.3 kg/m^2 (Q1-Q3: 25.1-34.2) and 29.5 kg/m^2 (Q1-Q3: 25.6-34.8) in the safety and PP populations, respectively. Median body mass index was 29.8 kg/m^2 (Q1-Q3: 25.7-34.9) in the IMM subset.

Few study participants had a history of vaccination against yellow fever (48/4,115) and Japanese encephalitis (3/4,115), suggesting that the recruited population does not encompass many travellers. Data are not provided for the PP population. Because of the limited number of vaccinated participants, and because no cross-reactivity is expected, data are not requested.

In the safety population, 4,057 participants were tested at baseline for ELISA antibodies to CHIKV, Mayaro virus, Dengue virus and Zika virus.

Of those 4,057 participants, 36 (0.9%, 26 in the VLA1553 arm and 10 in the placebo arm) were seropositive for CHIKV ELISA antibodies while 22 (16 in the VLA1553 arm and 6 in the placebo arm) and 7 (4 in the VLA1553 arm and 3 in the placebo arm) tested positive for neutralizing CHIKV antibodies at baseline using the baseline threshold of respectively µPRNT50 ≥20 and µPRNT50 >40. Data suggest that false positive CHIKV ELISA results might have occur. On the other hand, participants tested positive by ELISA and negative by PRNT assay might have (true positive) detectable binding IgG antibodies which are not able to neutralize CHIKV.

For study VLA1553-301 (and for study VLA1553-302), conducted in non-endemic country for CHIKV, low proportion of baseline CHIKV seropositive participants is expected and therefore the number of false positive baseline CHIKV serostatus is expected to be very low. In contrast, the percentage of false positive might be much higher in endemic countries, which might have an impact on the results. Therefore, to ensure the most correct classification of the participants by baseline CHIKV serostatus, the threshold of 20 µPRNT50 should be used to identify seronegative participants and the threshold of 40 µPRNT50 should be used to identify the seropositive participants.

Of the 4,057 participants, 58 (1.4%) had detectable antibodies specific to Mayaro virus. Respectively 90 (2.2%) and 282 (7.0%) participants had baseline antibodies specific to Zika virus and Dengue virus. Of these participants, 27 tested positive for both CHIKV and Mayaro virus, 16 for CHIKV and Dengue virus, 19 for CHIKV and Zika virus, 64 for Dengue and Zika virus and 9 for the 4 viruses.

From the VLA1553-301 and VLA1553-302 studies a total of 31 participants tested positive for MAYV and CHIKV IgG antibodies in ELISA.

Given the well-documented cross-reactivities of these ELISA assays and the limited numbers of subjects with MAYV positivity at baseline as determined by ELISA, it is difficult to conclude on impact of single MAYV or double MAYV/CHIKV baseline seropositivity on VLA1553 induced immune responses.

Overall, participants of the PP population were thus more frequently female, and were older when compared to the safety population. The Immunogenicity subset (selected at 12 study sites) and the PP population are not representative of the overall study population (43 sites). This point is however not considered as an issue in terms of the internal validity of the findings.

Characteristics were roughly comparable between arms in the safety population. Small imbalances between arms were noted in the PP population. There was a higher percentage of female participants included in the placebo arm (62.5%) vs the VLA1553 arm (57.9%). However, these imbalances have no impact on the main analyses, given that the primary endpoint and most secondary endpoints are estimated in the active arm.

IMM and eIMM populations vs PP population:

Demographic characteristics of the IMM population were comparable to those of the PP population. Apart from age, demographic characteristics of the eIMM population were also comparable to those of the PP population.

By definition, the eIMM included more elderly participants (28.5% and 26.0% in the VLA1553 and placebo arms, respectively) when compare to the PP population (22.2% and 24.0% respectively) and IMM population (21.8% and 20.3% respectively).

- **Outcomes and estimation**

Primary immunogenicity endpoint results

Proportion of baseline negative participants with a CHIKV antibody level on Day 29 ≥ 150 µPRNT50

A summary of the seroresponse rate for CHIKV-specific neutralizing antibodies on Day 29 for the PP population is provided in the table below followed by results obtained in the IMM population and the eIMM population.

Table 11. Proportion of baseline seronegative participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) on Day 29, by age stratum (PP population)

	18 to 64 Years (Stratum A)		≥65 Years (Stratum B)		Total	
Statistic	VLA1553 (N=207)	Placebo (N=73)	VLA1553 (N=59)	Placebo (N=23)	VLA1553 (N=266)	Placebo (N=96)
Total[a] [n]	207	73	59	23	266	96
Participants with Seroprotection [n (%)]	204 (98.6)	0	59 (100.0)	0	263 (98.9)	0
95% CI for Seroprotection Rate	95.8, 99.7	0.0, 4.9	93.9, 100.0	0.0, 14.8	96.7, 99.8	0.0, 3.8
p-value[b]	*<0.0001*	>0.9999	*<0.0001*	>0.9999	*<0.0001*	>0.9999
Difference in Seroprotection Rate[c] [n]						
Difference	98.6		100.0		98.9	
95% CI	96.9, 100.0		100.0, 100.0		97.6, 100.0	
p-value[d]	*<0.0001*		*<0.0001*		*<0.0001*	

CHIKV=chikungunya virus; CI=confidence interval; µPRNT50=50% plaque reduction in a micro plaque reduction neutralization test; PP=per protocol.

a Number of µPRNT baseline negative participants (<20) with non-missing titers on Day 29.

b P-value from an exact binomial test for the null-hypothesis H0: seroprotection rate ≤70% against the alternative H1: seroprotection rate >70% with a one-sided significance level of 2.5%.

c Differences, p-values and associated CIs are presented for the VLA1553 arm minus placebo arm.

d P-value from Fisher's Exact test.

Percentages are based on the number of baseline negative participants with non-missing titers at the visit.

Seroprotection was defined as µPRNT50 ≥150 for µPRNT baseline negative participants (<20). Two-sided 95% exact (Clopper-Pearson) CIs presented. Where the upper bound of the CI would be greater than 100%, the upper confidence limit is restricted to 100.

Source: Table 14.2.1.1 and Listing 16.2.5.1

Table 12. Proportion of baseline seronegative participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) on Day 29 (IMM Population)

	18 to 64 Years (Stratum A)		≥65 Years (Stratum B)		Total	
Statistic	VLA1553 (N=269)	Placebo (N=94)	VLA1553 (N=75)	Placebo (N=24)	VLA1553 (N=344)	Placebo (N=118)
Total[a] [n]	251	88	74	24	325	112
Participants with Seroprotection [n (%)]	248 (98.8)	1 (1.1)	73 (98.6)	0	321 (98.8)	1 (0.9)
95% CI for Seroprotection Rate	96.5, 99.8	0.0, 6.2	92.7, 100.0	0.0, 14.2	96.9, 99.7	0.0, 4.9
p-value[b]	*<0.0001*	>0.9999	*<0.0001*	>0.9999	*<0.0001*	>0.9999
Difference in Seroprotection Rate[c] [n]						
Difference	97.7		98.6		97.9	
95% CI	95.1, 100.0		96.0, 100.0		95.8, 100.0	
p-value[d]	*<0.0001*		*<0.0001*		*<0.0001*	

CHIKV=chikungunya virus; CI=confidence interval; µPRNT50=50% plaque reduction in a micro plaque reduction neutralization test.

a Number of µPRNT baseline negative participants (<20) with non-missing titers on Day 29.

b P-value from an exact binomial test for the null-hypothesis H0: seroprotection rate ≤70% against the alternative H1: seroprotection rate >70% with a one-sided significance level of 2.5%.

c Differences, p-values and associated CIs are presented for the VLA1553 arm minus placebo arm.

d P-value from Fisher's Exact test.

Percentages are based on the number of baseline negative participants with non-missing titers at the visit.
Seroprotection was defined as µPRNT50 ≥150 for µPRNT baseline negative participants (<20). Two-sided 95% exact (Clopper-Pearson) CIs presented. Where the upper bound of the CI would be greater than 100%, the upper confidence limit is restricted to 100. Participant 1553-1-12- 013 in the placebo arm was incorrectly dosed with VLA1553, and met seroprotection threshold.
Source: Table 14.2.1.2 and Listing 16.2.5.1

Table 13. Proportion of baseline seronegative participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) at Day 29 (eIMM Population)

	18-64 Years		≥ 65 Years		Total	
Statistic	VLA1553 (N=269)	Placebo (N=94)	VLA1553 (N=107)	Placebo (N=33)	VLA1553 (N=376)	Placebo (N=127)
Total[a] [n]	251	88	104	33	355	121
Subjects with Seroprotection [n (%)]	248 (98.8)	1 (1.1)	103 (99.0)	0	351 (98.9)	1 (0.8)
95% CI for Seroprotection Rate	96.5, 99.8	0.0, 6.2	94.8, 100.0	0.0, 10.6	97.1, 99.7	0.0, 4.5
p-value[b]	*<0.0001*	>0.9999	*<0.0001*	>0.9999	*<0.0001*	>0.9999
Difference in Seroprotection Rate[c]						
Difference	97.7		99.0		98.0	
95% CI	95.1, 100.0		97.2, 100.0		96.1, 100.0	
p-value[d]	*<0.0001*		*<0.0001*		*<0.0001*	

CI = confidence interval.
a. Number of µPRNT baseline negative (<20) subjects with non-missing titers at Day 29.
b. P-value from an exact binomial test for the null-hypothesis H0: SPR ≤ 70% against the alternative H1: SPR > 70% with a one-sided significance level of 2.5%.
c. Differences, p-values and associated confidence intervals are presented for the VLA1553 arm minus Placebo treatment arm.
d. P-value from Fisher's Exact test.
Percentages are based on the number of baseline negative subjects with non-missing titers at the visit.
Seroprotection is defined as µPRNT50 ≥ 150 for µPRNT baseline negative (<20) subjects.
Two-sided 95% exact (Clopper-Pearson) confidence intervals presented.
Subject 12-013 in the placebo arm was incorrectly dosed with VLA1553, and met seroprotection threshold.

The primary endpoint was met. At 28 days post-vaccination, 98.9% (263/266) of the participants had an antibody titre of at least 150 µPRNT (95% CI: 96.7-99.8) in the VLA1553 arm (PP population). Similar proportions of younger adults (98.6% [204/207], 95% CI: 95.8-99.7) and of older adults (100% [59/59], 95% CI: 93.9-100.0) reached this threshold considered as reasonably likely to predict protection. None of the placebo participants reached the threshold. In total, 3 participants did not reach the threshold of 150 µPRNT in the VLA1553 arm. All 3 were younger adults. Two of them had no detectable antibody.

Results obtained in the IMM population and the eIMM population were similar.

Results obtained in the sPP population (based on a threshold for baseline serostatus of µPRNT50 ≤40) and the sPP2 population (using visit windows to report the results rather than visit labels) were similar.

Data are presented in respectively for 325 and 355 participants from the IMM and eIMM, while those population include in total respectively 344 and 376 participants. It is assumed that the gap is due to the absence of antibody data at Day 29 for the missing participants.

Secondary immunogenicity endpoint results

Proportion of baseline seronegative participants with CHIKV antibody level on Day 8, Day 29, Day 85, and Day 180 post-vaccination ≥150 µPRNT

Proportion of baseline seronegative participants with antibody titres post-vaccination above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) by visit and by age stratum for the PP analysis set are presented in the table below.

Table 14. Proportion of baseline seronegative participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) by visit and by age stratum (PP population)

	18 to 64 Years (Stratum A)		≥65 Years (Stratum B)		Total	
Time Point[a] [n] Statistic	VLA1553 (N=207)	Placebo (N=73)	VLA1553 (N=59)	Placebo (N=23)	VLA1553 (N=266)	Placebo (N=96)
Visit 2 – Day 8	198	70	53	23	251	93
Participants with Seroprotection [n (%)]	2 (1.0)	0	2 (3.8)	0	4 (1.6)	0
95% CI for Seroprotection Rate	0.1, 3.6	0.0, 5.1	0.5, 13.0	0.0, 14.8	0.4, 4.0	0.0, 3.9
Difference in Seroprotection Rate[b] [n]						
Difference	1.0		3.8		1.6	
95% CI	-0.4, 2.4		-1.4, 8.9		0.0, 3.1	
p-value[c]	>0.9999		>0.9999		0.5776	
Visit 3 – Day 29	207	73	59	23	266	96
Participants with Seroprotection [n (%)]	204 (98.6)	0	59 (100.0)	0	263 (98.9)	0
95% CI for Seroprotection Rate	95.8, 99.7	0.0, 4.9	93.9, 100.0	0.0, 14.8	96.7, 99.8	0.0, 3.8
Difference in Seroprotection Rate[b] [n]						
Difference	98.6		100.0		98.9	
95% CI	96.9, 100.0		100.0, 100.0		97.6, 100.0	
p-value[c]	*<0.0001*		*<0.0001*		*<0.0001*	
Visit 4 – Day 85	194	69	52	22	246	91
Participants with Seroprotection [n (%)]	189 (97.4)	0	52 (100.0)	0	241 (98.0)	0
95% CI for Seroprotection Rate	94.1, 99.2	0.0, 5.2	93.2, 100.0	0.0, 15.4	95.3, 99.3	0.0, 4.0
Difference in Seroprotection Rate[b] [n]						
Difference	97.4		100.0		98.0	
95% CI	95.2, 99.7		100.0, 100.0		96.2, 99.7	
p-value[c]	*<0.0001*		*<0.0001*		*<0.0001*	
Visit 5 – Day 180	184	68	58	23	242	91
Participants with Seroprotection [n (%)]	178 (96.7)	0	55 (94.8)	0	233 (96.3)	0
95% CI for Seroprotection Rate	93.0, 98.8	0.0, 5.3	85.6, 98.9	0.0, 14.8	93.1, 98.3	0.0, 4.0
Difference in Seroprotection Rate[b] [n]						
Difference	96.7		94.8		96.3	
95% CI	94.2, 99.3		89.1, 100.0		93.9, 98.7	
p-value[c]	*<0.0001*		*<0.0001*		*<0.0001*	

CHIKV=chikungunya virus; CI=confidence interval; $\mu PRNT_{50}$=50% plaque reduction in a micro plaque reduction neutralization test; PP=per protocol.

a Number of µPRNT baseline negative participants (<20) with non-missing titers at the specified time point.

b Differences, p-values and associated CIs are presented for the VLA1553 arm minus placebo arm.

c P-value from Fisher's Exact test.

Percentages are based on the number of participants with non-missing titers at the visit. Seroprotection was defined as $\mu PRNT_{50}$ ≥150 for µPRNT baseline negative participants (<20). µPRNT baseline positive participants (≥20) were not included in this summary. Two-sided 95% exact (Clopper-Pearson) CIs presented.

Source: Table 14.2.1.5 and Listing 16.2.5.1

Footnote regarding the estimate confidence intervals for the difference in seroresponse rates between treatment groups: alternative estimates based on an exact method are slightly wider, but do not impact the main conclusion of superior immunogenicity following Day 29 after vaccination. Conclusions of slightly (but irrelevant) improved immunogenicity as early as Day 8, however, would no longer be supported using this more conservative approach.

Only 1.6% (95% CI: 0.4-4.0, n=4/251 vs. none in the placebo arm) of the participants vaccinated with VLA1553 (PP population) had antibody level ≥150 µPRNT at Day 8. In contrast, 98.9% (95% CI: 96.7-99.8, n=263/266) of the participants reached this antibody level at Day 29. Proportions remain high up to Day 180, with a proportion of 96.3% (95% CI: 93.1-98.3, n=233/242) at Day 180. Comparable

proportions were observed in both age categories, for all the timepoint tested (all lower bound of the 95% CI are above 85%).

Results obtained with the IMM and eIMM were overall similar for this secondary endpoint. In the VLA1553 arm, proportions at Day 8 were slightly higher for both populations compared to the PP population (6.5% and 7.0% for IMM and eIMM, respectively, none in the placebo arm). Results obtained in the sPP and sPP2 populations were similar.

Post-hoc analysis of the seroresponse rates (SRRs) for CHIKV-specific neutralizing antibodies with imputation of missing post-baseline titre measurements were performed. SRRs were lower with imputation of missing post-baseline titre measurements when compared to the SRRs without imputation of missing post-baseline titre measurements, as expected. At Day 29 up to Day 180, the lower bound of the 95% CI still exceeds the non-acceptance criterion of 70%.

Immune response as measured by CHIKV-specific neutralizing antibody titres on Day 8, Day 29, Day 85, and Day 180 post-vaccination

A summary of GMTs for CHIKV-specific neutralizing antibody by visit for the PP analysis set is provided in Table 15 below and illustrated in Figure 10.

Figure 11 presents reverse cumulative distribution curves of CHIKV-specific neutralizing antibodies from Day 29 to Day 180, by study arm and age strata.

Table 15. Summary of GMTs for CHIKV-specific neutralizing antibody by visit and by age stratum (PP population) (Table 22, CSR)

	18 to 64 Years		≥65 Years		Total	
Time Point Statistic	VLA1553 (N=207)	Placebo (N=73)	Placebo (N=59)	Placebo (N=23)	VLA1553 (N=266)	Placebo (N=96)
Visit 2 – Day 8						
n[a]	198	70	53	23	251	93
Geometric Mean	13.6	10.2	13.4	10.0	13.6	10.2
95% CIs	12.22, 15.23	9.92, 10.50	11.05, 16.31	10.00, 10.00	12.36, 14.96	9.94, 10.38
Min, Max	10, 4099	10, 21	10, 173	10, 10	10, 4099	10, 21
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	1.34 (1.10)		1.34 (1.16)		1.34 (1.08)	
95% CIs[c]	1.11, 1.61		1.00, 1.80		1.14, 1.57	
p-value[c]	*0.0023*		0.0503		*0.0003*	
Visit 3 – Day 29						
n[a]	207	73	59	23	266	96
Geometric Mean	3273.7	10.1	3688.8	10.0	3361.6	10.1
95% CIs	2860.93, 3746.04	9.89, 10.33	2938.94, 4630.10	10.00, 10.00	2993.83, 3774.45	9.92, 10.25
Min, Max	10, 20749	10, 22	245, 18910	10, 10	10, 20749	10, 22
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	323.85 (1.12)		368.88 (1.20)		333.90 (1.10)	
95% CIs[c]	258.03, 406.47		256.53, 530.46		275.25, 405.05	
p-value[c]	*<0.0001*		*<0.0001*		*<0.0001*	
Visit 4 – Day 85						
n[a]	194	69	52	22	246	91
Geometric Mean	1068.7	10.0	1140.9	10.7	1083.6	10.2
95% CIs	934.77, 1221.82	10.00, 10.00	942.97, 1380.31	9.71, 11.78	968.27, 1212.59	9.94, 10.40
Min, Max	10, 7492	10, 10	227, 4574	10, 22	10, 7492	10, 22
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	106.87 (1.12)		106.66 (1.16)		106.82 (1.10)	
95% CIs[c]	85.39, 133.76		79.17, 143.69		88.72, 128.61	
p-value[c]	*<0.0001*		*<0.0001*		*<0.0001*	
Visit 5 – Day 180						
n[a]	184	68	58	23	242	91
Geometric Mean	755.1	10.0	742.8	10.0	752.1	10.0
95% CIs	656.01, 869.16	10.00, 10.00	578.36, 954.00	10.00, 10.00	665.91, 849.52	10.00, 10.00
Min, Max	10, 5871	10, 10	10, 4513	10, 10	10, 5871	10, 10
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	75.51 (1.12)		74.28 (1.22)		75.20 (1.11)	
95% CIs[c]	59.92, 95.16		49.97, 110.43		61.64, 91.74	
p-value[c]	*<0.0001*		*<0.0001*		*<0.0001*	

CHIKV=chikungunya virus; CI=confidence interval; GMT=geometric mean titer; LS=least squares; Max=maximum; Min=minimum; n=number of participants with available result; PP=per protocol; SE=standard error;

a n is the number of participants that contribute data at least once in the primary analysis model.

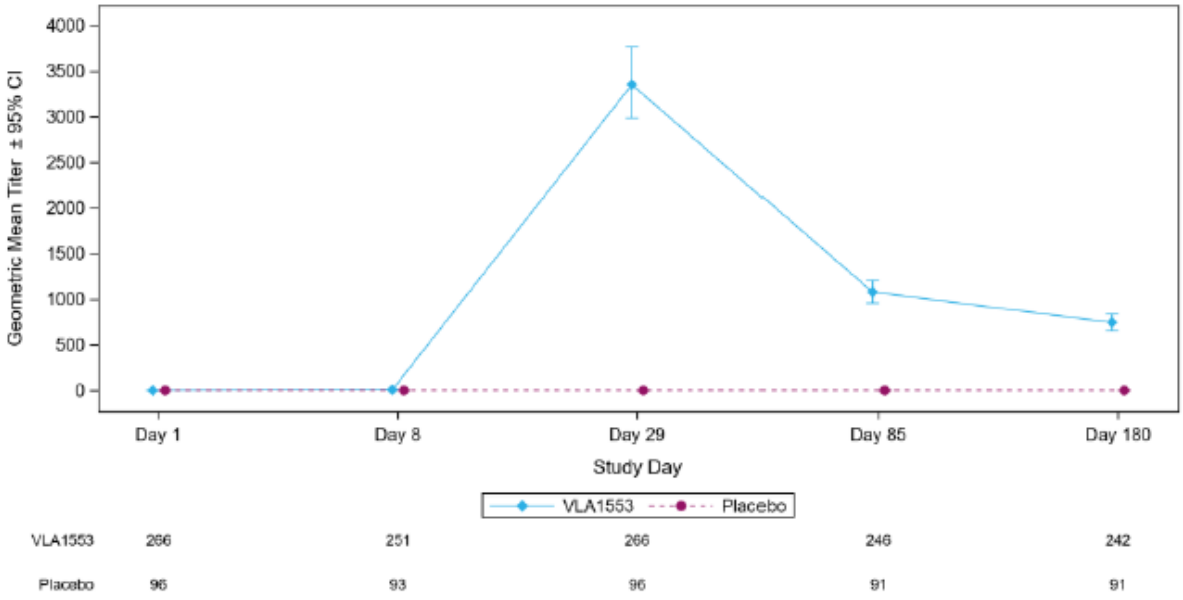
b LS means, standard errors, CIs, and p-values are from an analysis of covariance model (ANCOVA) with fixed factors for study arm and age group.

c p-values, LS mean differences and associated CIs are presented for the VLA1553 arm minus placebo arm.

Note: The ANCOVA model is applied to the log-transformed titers, and back-transformed results are displayed for the LS mean and difference. The difference in GMT is a ratio of the LS means.

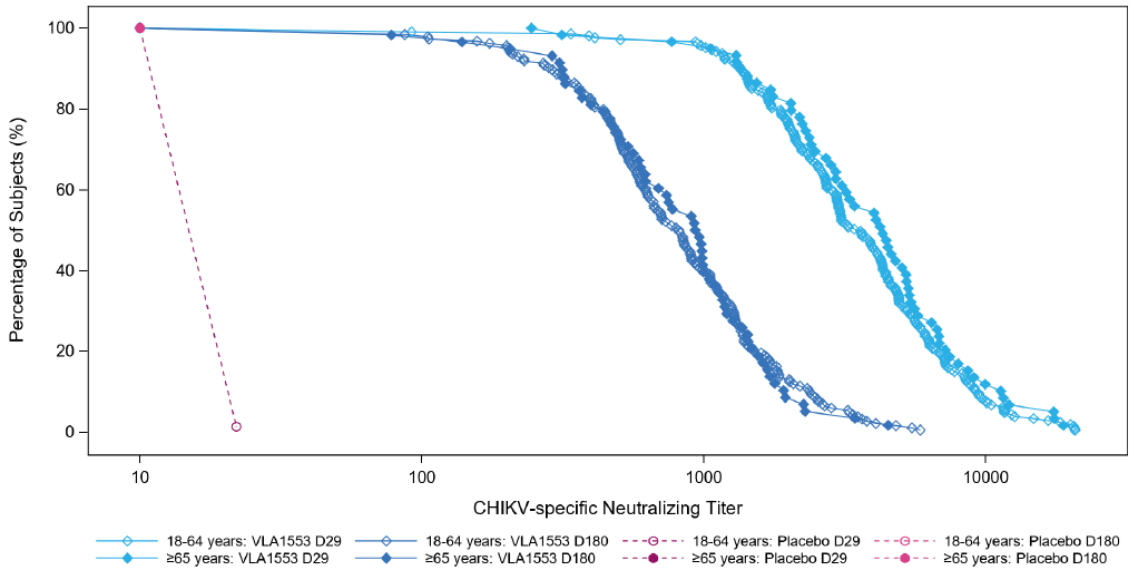
Source: Table 14.2.2.1 and Listing 16.2.5.1

Figure 10. Line plot of CHIKV-specific neutralizing antibodies (GMT) by study day and study arm (PP population)



CHIKV=chikungunya virus; CI=confidence interval; GMT=geometric mean titers; PP=per protocol.
Note: Counts below the chart show the number of participants in each arm at the timepoint.
Source: Table 14.2.2.1

Figure 11. Reverse cumulative distribution curves of CHIKV-specific neutralizing antibody on Day 29 and Day 180 by study arm and age strata (PP population)



Note: Due to all Placebo groups having identical results they are displayed on top of each other so only one is visible.
Source: Table 14.2.2.1, Dataset: ADIS, Program: f-rcdist-byage.sas, Output: F-14-02-01-03-02-01-rcdist-byage.rtf, Generated on: 15MAR2022 08:42
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In the PP population, Day 8-GMT was low both for the VLA1553 and placebo arms (13.6 [95% CI:12.4-15.0] and 10.2 [95% CI: 10.0-10.4] respectively) and peaked at Day 29 with GMT of 3361.6 (95% CI: 2993.8-3774.4) in the VLA1553-vaccinated group (versus GMT of 10.1 [95% CI: 9.9-10.2] in the placebo participants). In the active group, GMT decreased at Day 85 compared to Day 29-GMT, with a GMT of 1083.1 (95% CI: 968.3-1212.6), with no 95% CI overlap. Day 180-GMT continued to decrease

as compared to Day 85 (with no 95% CI overlap) but were still higher than GMT at baseline (Day 180-GMT of 752.1, 95% CI: 665.9-849.5). Antibody titres in the placebo group were low at each timepoint. However, the maximum value is higher than 20 µPRNT, i.e. the threshold for positivity, at Day 8, Day 29 and Day 85 with maximum values of 21 or 22. According to the Applicant, this justifies the sensitivity analysis performed on the sPP population, i.e. using a threshold of 40 µPRNT to define the baseline serostatus. This was also done for study VAL1553-302.

There were no difference between age strata, GMTs were similar at each timepoint (PP population). Day 29-GMTs were of 3273.7 (95% CI: 2860.9-3746.0) and of 3688.8 (95% CI: 2938.9-4630.1) for the younger and older vaccinees, respectively. On Day 180 GMTs were of 755.1 (95% CI: 656.0-869.2) and of 742.8 (95% CI: 578.4-954.0) for the for the younger and older adults, respectively. Reverse cumulative distribution curves confirm these observation. On Day 29, most participants in the VLA1553 arm achieved antibody titres between 1,000 and 10,000 µPRNT50. On Day 180, most participants still had antibody titres of at least around 500 µPRNT50.

Results obtained with the IMM and eIMM were overall similar for this secondary endpoint.

Results obtained in the sPP and sPP2 population were similar.

Post-hoc analysis of the GMTs for CHIKV-specific neutralizing antibodies with imputation of missing post-baseline titre measurements was performed. GMTs were lower with imputation of missing post-baseline titre measurements when compared to the GMTs without imputation of missing post-baseline titre measurements, as expected. At Day 29 up to Day 180, for the VLA1553 arm, GMTs were still high and largely above the threshold considered reasonably likely to predict protection (150 µPRNT50).

Other secondary endpoints

Proportion of participants with neutralizing antibody titre thresholds of µPRNT50 ≥50 and ≥250 post-vaccination, proportion of participants with seroconversion post-vaccination, proportion of participants with fold increase of CHIKV-specific neutralizing titres post-vaccination, and proportion of participants reaching an at least 4-fold, 8-fold, 16-fold, or 64-fold increase in CHIKV-specific neutralizing titres were also presented by visit and by age stratum for the PP population.

Proportions of baseline seronegative participants with post-vaccination antibody titres of at least µPRNT50 250 are overall similar to those found when the threshold was set at the µPRNT50 ≥150. On Day 180, 91.7% of the participants (222/242) had an antibody titre of at least 250 µPRNT50, which is slightly lower than the proportion of participants with at least a µPRNT50 of 150 (96.3%; 233/242). On Day 29, only 1 participant did not mount an antibody response of at least 250 µPRNT50. There were no difference between age strata.

Proportion of baseline seronegative participants with seroconversion at Day 29 and Day 180 post-vaccination was defined as CHIKV-specific neutralizing titre of µPRNT50 ≥20 for baseline negative µPRNT (<20) subjects. In the PP population, nearly all participants seroconverted at Day 29 (264/266 [99.2%]) in the VLA1553 arm, consistently with the primary endpoint results. Only one placebo participant seconverted at Day 29.

CHIKV-specific neutralizing antibody titres post-vaccination increased 3.9-fold (mean value) 7 days after vaccination when compared to baseline and peaked at Day 29 with a 470.8-fold increase versus baseline titres. At Day 180, titres were still 107.2-fold higher than baseline titres. Again, no differences were observed between age strata.

Results obtained with the IMM, eIMM, sPP and sPP2 were overall similar for all these secondary endpoints. Proportion of participants with neutralizing antibody titre thresholds of µPRNT50 ≥50 and ≥250 post-vaccination and proportion of participants reaching an at least 4-fold, 8-fold, 16-fold, or 64-fold increase in CHIKV-specific neutralizing titres were not presented for the sPP and sPP2 populations.

- **Ancillary analyses**

Analyses by age subgroup are described above.

- Concomitant Therapy

In the Safety population, 3,144/4,115 (76.4%) participants received concomitant medications (i.e. with a start or end date on or after date of vaccination).

The most common concomitant medications were vaccines (1,070/4,115 participants, respectively 25.5% and 27.4% in the VLA1553 and the placebo arm), anti-inflammatory and anti-rheumatic products (905/4,115 participants, respectively 24.2% and 15.4% in the VLA1553 and the placebo arm), and analgesics (905/4,115 participants, respectively 23.9% and 16.3% in the VLA1553 and the placebo arm). The most frequently used analgesics was paracetamol (11%). Anti-inflammatory and anti-rheumatic products taken as concomitant medications was mainly Ibuprofen (22.0%). The others were Naproxen and Meloxicam.

In the Safety population, a very low percentage of participants received concomitant systemic steroids or immunosuppressants (<2%).

None of the participants of study VLA1553-301 (and study VLA1553-302) were excluded from the PP population based on concomitant use of anti-inflammatory/anti-rheumatic products or analgesics.

Anti-inflammatory and anti-rheumatic products were recorded as concomitant medication in the VLA1553 group in 24.1% of the participants of the IMM population and 24.8% of the participants of the PP population. Analgesics were recorded as concomitant medication in the VLA1553 group in 25.6% of the participants of the IMM population and 28.2% of the participants of the PP population.

Trends for higher proportion of participants who used anti-inflammatory and anti-rheumatic products between Day 1 and Day 7, between Day 1 to Day 29, and between Day 30 and Day 180 post-vaccination in the VLA1553 group versus the placebo group was observed for the PP population (21.1% versus 10.4%, 23.3% versus 11.5%, and 17.3% versus 12.5% for the period between Day 1 and Day 7, between Day 1 to Day 29 and between Day 30 and Day 180 post-vaccination, respectively). These trends were also seen for the IMM population and the safety population (with the exception of the proportion of participants who used anti-inflammatory and anti-rheumatic products between Day 30 and Day 180 which was comparable between the treatment groups in the safety population).

Trend for higher proportion of participants who used analgesics in the VLA1553 group of the PP population between Day 1 and Day 7 and between Day 1 to Day 29 post-vaccination as compared to the placebo group was observed (26.3% versus 19.8% and 26.7% versus 19.8% between Day 1 and Day 7 and between Day 1 and Day 29 post-vaccination, respectively). The proportion of participants who used analgesics between Day 30 and Day 180 post-vaccination was comparable between the treatment groups (20.7% versus 18.8%). Same observations were also done for the safety population. Trends were less marked for the IMM population.

Analyses of immune responses (IMM and PP populations) stratified by use of those medications in relevant periods show that, overall, the GMTs and proportion of participants with seroresponse were not influenced by concomitant use of anti-inflammatory and anti-rheumatic products or analgesics in a respective time period. The immune response in participants who used these concomitant medications was comparable to participants who did not use this concomitant medication. No data are however provided with respect to the impact of prophylactic administration of antipyretics within 4 hours prior to and during the first 72 hours after vaccination on the immune responses (REC).

- Immunogenicity results in individuals tested positive for CHIKV at baseline using the baseline threshold of µPRNT50 >20 and >40

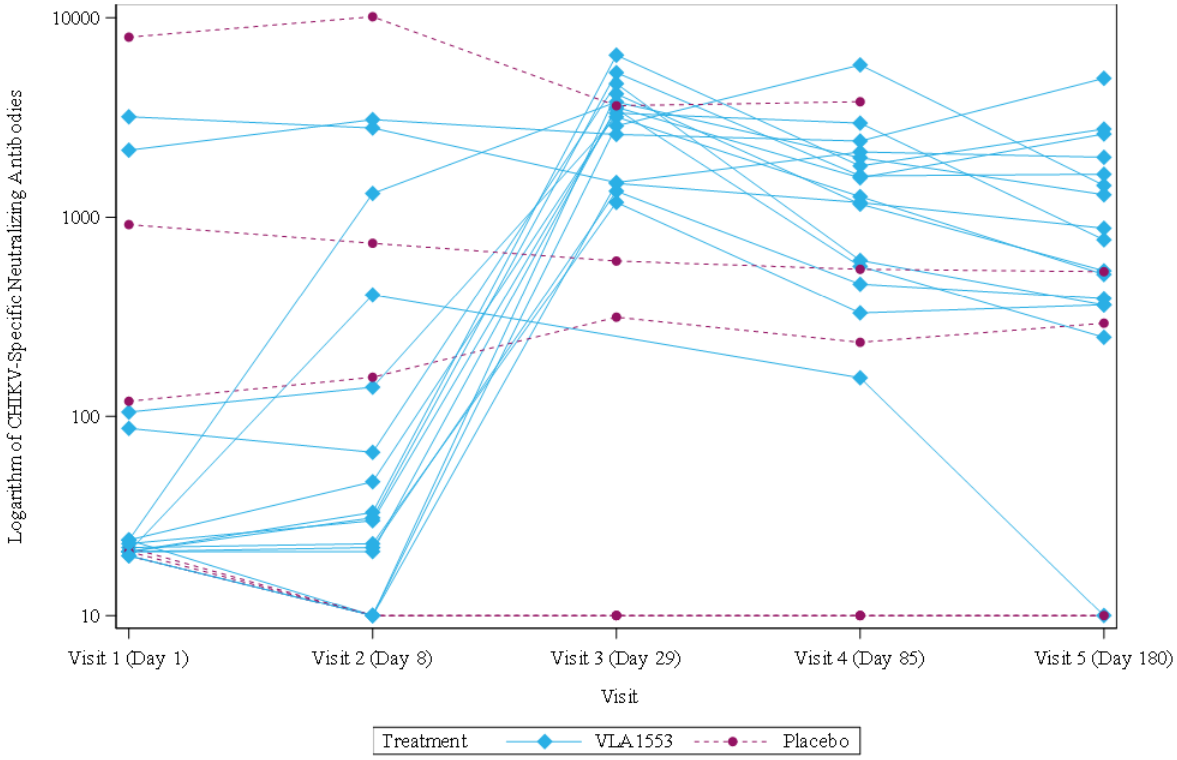
Overall, 22 (16 in the VLA1553 arm and 6 in the placebo arm), and 7 (4 in the VLA1553 arm and 3 in the placebo arm) participants from the Safety population tested positive for CHIKV at baseline using the baseline threshold of respectively µPRNT50 ≥20 and µPRNT50 >40. The threshold of µPRNT50 >40 is considered more appropriate for the immunogenicity and safety analyses of CHIKV baseline seropositive participants.

The very limited number (n=4 in the VLA1553 arm) of participants does not allow for a meaningful statistical analysis. Of the 4 participants in the VLA1553 arm, 2 seroconverted at Day 29 (defined as an at least a 4 fold increase in neutralizing antibody titre over baseline). None seroconverted in the placebo arm.

Individual CHIKV-specific neutralizing antibody curves for CHIKV participants with baseline µPRNT50 ≥20 suggests that, overall, a vaccine-induced immune response is observed in participants with baseline antibody titres <100 µPRNT50 (Figure 14). Participants with higher baseline antibody titres do not mount a response after vaccination, suggesting the neutralisation of the attenuated strain 181/clone 25 by the vaccine-induced neutralising antibody. This is in line with the findings observed in the Phase 1 study VLA1553-101. In this study, no viraemia was detected after the re-vaccination in any of the 23 participants primed with the medium dose level (a dose comparable to the final dose level) within 14 days after re-vaccination (in contrast with 27/30 after a single final dose of VLA1553), suggesting protection against a challenge with the vaccine virus.

It is noted that some of the participants with baseline µPRNT50 ≥20 threshold had no detectable antibody or had antibody titres <20 µPRNT50 20 at Day 9, pointing to the variability of the µPRNT assay for samples with a low concentration of neutralizing antibodies. This was also observed for 2 participants (1 of study VLA1553-301 and 1 of study VLA1553-302) when using the cut-off of 40 µPRNT50. The serostatus of participants within the 20-40 µPRNT50 stratum is thus uncertain.

Figure 12. Line plot of CHIKV-specific neutralizing antibodies for subjects who are seropositive at baseline in study VLA1553-301 (Safety Analysis Set, seropositive is µPRNT50 ≥20 at baseline)



- Immunogenicity results in individuals tested positive for CHIKV, Mayaro virus, Dengue virus or Zika virus at baseline

Given the limited numbers of subjects seropositive at baseline for Mayaro virus, Dengue virus and Zika virus for which µPRNT results post-vaccination were available, no conclusions can be drawn concerning potential immunological cross-reactivity or differential immune responses based on baseline serostatus specific to these viruses. It is anticipated that results of the VLA1553-321 study conducted in regions where Mayaro virus, Dengue virus and Zika virus should provide more solid data in that respect.

Study VLA1553-302

Methods

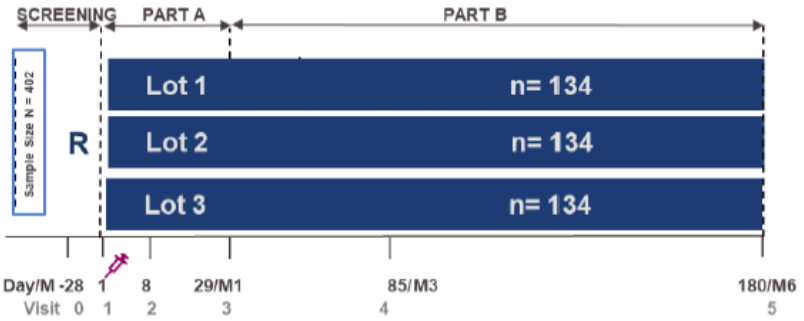
The assessment of the methods is based on on Protocol Version 3.0 dated 07 April 2021.

- **Overall study design**

VLA1553-302 is a randomized, double-blinded, multicentre Phase 3 clinical study investigating 3 Lots of VLA1553 at the final selected dose (target 1x10E4 TCID50 per 0.5 mL). Participants were recruited in 12 sites (instead of the approximately 4 sites planned in the protocol).

The overall study design is displayed in the Figure 16 below.

Figure 13. Overall clinical Phase 3 study design



This Lot-to-Lot Phase 3 study was designed to demonstrate manufacturing consistency of 3 manufacturing Lots. Consistency was met if GMT ratios were within the pre-defined acceptance margins of 0.67-1.5. The study was also designed to expand the safety and immunogenicity database of the final dose of VLA1553 administered as a single vaccination.

Approximately 402 participants 18-45 years of age were to be enrolled into the study, approximately 134 participants per VLA1553 Lot (randomization in a 1:1:1 ratio to each of the 3 Lot arms).

All participants received a single intramuscular administration of 1 of the 3 Lots of VLA1553 in the deltoid region of the arm.

As for study VLA1553-301, participants were followed up for approximately 6 months following vaccination. Immunogenicity blood samples were taken from all the participants at baseline (Day 1), 7 days (Day 8), 28 days (Day 29), 84 days (Day 85) and 179 days (Day 180) post-vaccination. Timepoints are the same as in study VLA1553-301.

- **Study Participants**

A total of approximately 402 generally healthy participants, 18 to 45 years of age, were targeted for enrolment into this study. Apart from the age range, inclusion and exclusion criteria are comparable to those of the pivotal study VLA1553-301.

- **Treatments**

All participants received a single i.m. vaccination in the deltoid region of the arm of the subject with VLA1553 in a 1:1:1 ratio to one of the 3 study arms to receive one of the 3 Lots.

The 3 Lots are from 3 different batches which is deemed appropriate. The actual potency were of 5 x 10E3 TCID50/0.5 mL, 8 x 10E3 TCID50/0.5 mL and 8 x 10E3 TCID50/0.5 mL for Lot 1, Lot 2 and Lot 3, respectively.

The potency of the 3 Lots are in the commercial release specification range (4.0x10E3 – 4.0x10E4), as the dose used in the pivotal VLA1553-301 study. All clinical lots are representative of commercial lots.

- **Concomitant therapies**

Permitted and forbidden prior and concomitant therapy and non-drug therapies were similar than those of study VLA1553-301.

- **Study assessments**

Immunogenicity assessments were overall comparable than for the pivotal study VLA1553-301.

Immunogenicity assessments measuring CHIKV-specific neutralizing antibodies were performed on samples collected on Day 1, Day 8, Day 29, Day 85 and Day 180 after a single immunization. Pre-existing antibodies to CHIKV (binding antibodies), Mayaro, Dengue, and Zika viruses were also assessed.

A key difference with study VLA1553-301 is the definition of the baseline serostatus which was based on binding antibodies specific to recombinant CHIKV antigens, as measured by ELISA performed at CERBA laboratory (EUROIMMUN kit). This was justified by the Applicant by the fact a screening for CHIKV antibodies by ELISA was initially planned for enrolling participants in the study. The ELISA, which was initially assumed as logistically simple and as providing quick results, was, in the end, not suitable as screening test. Under Protocol version 3 dated 07 Apr 2021, inclusion criteria of CHIKV negative status was no longer required to pass screening. It is not fully clear however why the same approach as in study VLA1553-301 was not used (i.e. define baseline serostatus by use CHIKV-specific neutralizing antibodies).

Please refer to study VLA1553-301, and to section 2.6.2. for more details on the immunogenicity assessment and on the methods.

- **Objectives and endpoints**

Primary objective and endpoint

The primary objective was to demonstrate the Lot-to-Lot manufacturing consistency of the final dose of VLA1553 in a population aged 18 to 45 years.

The primary endpoint is the CHIKV neutralizing antibody GMT of Day 29 titre as determined by µPRNT50 in baseline CHIKV seronegative participants.

The primary analysis was based on a pair-wise comparison of GMTs corresponding to the 3 Lots.

Equivalence between lots was considered demonstrated if the 95% CIs for Day 29 neutralizing antibody GMT ratios are all between 0.67 and 1.5. The acceptance margins are deemed acceptable.

Secondary objectives and endpoints

The secondary objective and endpoints are similar than in study VLA1553-301 (with the addition of the proportion of participants reaching the threshold of µPRNT 150 at Day 29 in this study VLA1553-302, which is the primary endpoint in study VLA1553-301). As for study VLA1553-301, the main analysis population was the PP population.

Exploratory objectives and endpoints

The SAP also planned descriptive analyses of immune responses in baseline CHIKV seropositive participants.

As for study VLA-1553-301, measurement of pre-existing antibodies was planned on the baseline blood samples, including but not limited to a panel of alphaviruses (Mayaro) and Flaviviruses (Dengue and Zika).

- **Sample size**

The sample size of 402 randomized participants (i.e. 134 per Lot arm) was chosen to provide more than 90% power to demonstrate Lot-to-Lot consistency applying a margin of 0.67 and 1.5 to the pairwise GMT ratios and assuming a standard deviation of 0.32 on the log10 scale. Corresponding calculations can be followed and the resulting sample size targets are considered reasonable.

- **Randomisation and blinding (masking)**

The approximately 402 participants were block-randomised in a ratio 1:1:1 into the 3 study arms. Randomization was not stratified by site. Refer to study VLA1553-301, section *Randomisation and blinding (masking)* for justification.

The study was conducted in a double-blind manner. Investigators/sites staff, study participants, and sponsor staff were blinded. Only the DSMB voting members and the biostatistician involved in the DSMB were unblinded.

The IMP vials were provided to the sites in a blinded manner in order to ensure the blinding of study participants, site staff performing the safety assessments, and site staff performing IMP preparation and administration. Identification of the syringe was guaranteed by placing a tear-off label containing kit number, subject number, date of injection, and operator onto the label.

- **Statistical methods**

Analysis populations

The FAS contains all participants who were randomized, received the vaccination, and have evaluable immunogenicity data at the time point for the primary endpoint (i.e. Visit 3/Day 29).

The PP analysis set contains all participants who were baseline negative for CHIKV antibodies as determined by ELISA assay, had received the vaccination, had evaluable immunogenicity data at baseline and the time point for the primary endpoint, and without a major protocol deviation. This definition differs from the PP population definition of study VLA1553-301, this latter being based on µPRNT to determine the baseline CHIKV serostatus and not based on the ELISA results.

Major protocol deviations for Part A analysis leading to an exclusion from the PP are defined in a similar way as in study VAL1553-301.

Two Sensitivity Per-Protocol analysis sets (sPP1 and sPP2) were used for additional sensitivity analyses of immunogenicity data at Part B. These analyses are not predefined in the protocol.

In these sensitivity analyses, instead of defining the CHIKV baseline serostatus by ELISA, µPRNT data were used. The baseline serostatus was defined as negative if the µPRNT50 was <20 and ≤40 for sPP1 and sPP2, respectively. These sensitivity analyses are endorsed, in particular sPP1 since the definition of sPP1 is similar than the definition of PP population of study VLA1553-301.

Analyses in sPP2 population was also performed in study VLA1553-301 (referred to as sPP population in study VLA1553-301).

Planned analyses

The primary analysis was based on the PP population, which is acceptable. In principle this may not correspond to a well interpretable estimand, however, considering the number of exclusions from the

PP population was limited and immunogenicity results on the FAS are similar to the PP population results, no objections are raised.

The statistical model used to infer the between lot GMTs was an ANOVA model with lot as fixed effect and centre as covariate. This is acceptable. Considering that meeting between lot consistency criteria represent co-primary endpoints no adjustment of confidence bounds is required.

Planned sensitivity analyses and analysis for secondary endpoints, are considered acceptable.

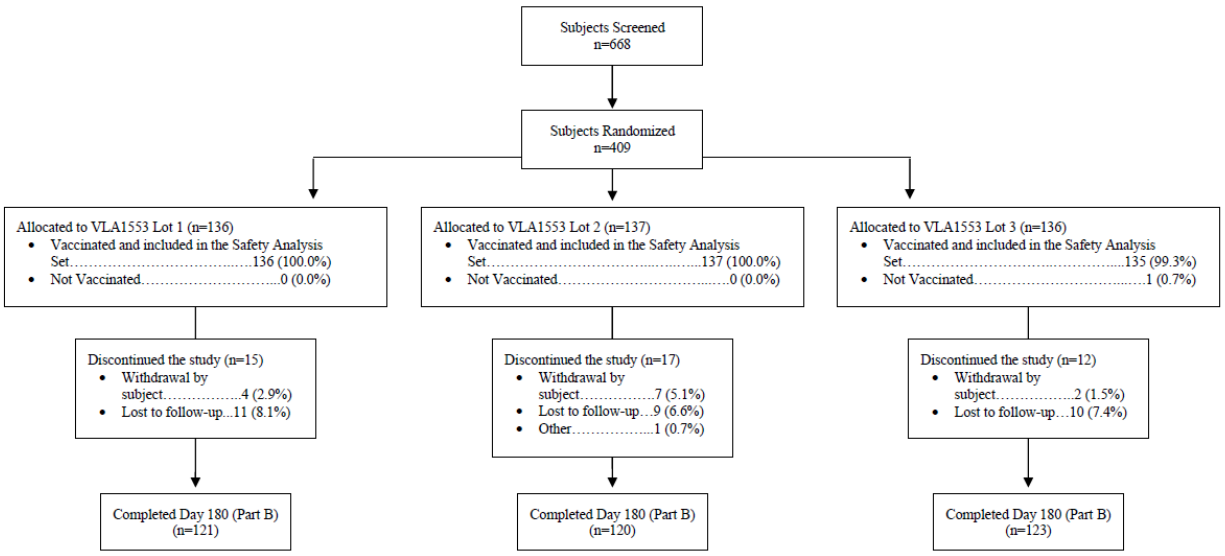
Results

- **Participant flow**

The disposition of subjects is provided in Figure 14.

A summary of the analysis sets is provided in the table below.

Figure 14. Participant disposition (Safety analysis set)



CRF=case report form; n=number of subjects.
Discontinued study includes all subjects who discontinued early, prior to Visit 5 (Day 180/Month 6).
Percentages were calculated out of subjects treated for each Lot.
Source: Table 14.1.1.1, Table 14.1.1.3 and Listing 16.2.1.2

Table 16. Participant analysis sets (all screened subjects)

Analysis Set	VLA1553 LOT 1	VLA1553 LOT 2	VLA1553 LOT 3	Total
Subjects Screened				668
Subjects Randomized [n][a]	136	137	136	409
Subjects Vaccinated [n (%)]	136 (100)	137 (100)	135 (99.3)	408 (99.8)
Subjects Randomized but Not Vaccinated [n (%)]	0	0	1 (0.7)	1 (0.2)
Safety Analysis Set (SAF) [n (%)]	136 (100)	137 (100)	135 (99.3)	408 (99.8)
Full Analysis Set (FAS) [n (%)]	132 (97.1)	128 (93.4)	131 (96.3)	391 (95.6)
Per-Protocol Analysis Set (PP) [n (%)]	122 (89.7)	118 (86.1)	122 (89.7)	362 (88.5)
Sensitivity Per-Protocol Analysis Set 1 (sPP1) [n (%)][b]	120 (88.2)	119 (86.9)	122 (89.7)	361 (88.3)
Sensitivity Per-Protocol Analysis Set 2 (sPP2) [n (%)][c]	122 (89.7)	121 (88.3)	123 (90.4)	366 (89.5)

µPRNT=micro plaque reduction neutralization test; n=number of subjects.
a Percentages are not included because subjects were grouped according to treatment actually received and not randomized treatment.
b sPP1 Analysis Set included baseline µPRNT negative subjects using $\mu PRNT_{50}$ <20.
c sPP2 Analysis Set included baseline µPRNT negative subjects using $\mu PRNT_{50}$ ≤40.
Percentages were calculated out of all randomized subjects.
Source: Table 14.1.1.1 and Listing 16.2.3.1

A total of 409 participants were randomized (136, 137 and 136 participants allocated respectively to Lot 1, Lot 2 and Lot 3). A total of 408 participants were in the Safety Analysis Set, as n=1 participant in Lot 3 arm did not received vaccination.

A total of 44 (10.8%) participants (15, 17 and 12 in Lot 1, Lot 2 and Lot 3, respectively) discontinued the study prior to Month 6. Main reasons were lost of follow-up (7.4%) and withdrawal by participants (3.2%). Out of the 409 randomized participants, 394 participants completed the Visit 3/Day 29 (primary endpoint; 134, 128, 132 respectively in the 3 Lots) and 364 completed the study (Visit 6/Day 180).

In total, 362 participants (88.5% of all randomized participants) were included in the PP population, 122 in Lot 1 arm, 118 in Lot 2 arm, and 122 in Lot 3 arm. The PP population included only participants which were ELISA seronegative at baseline, and n=12 baseline ELISA seropositive participants were excluded from the PP population. A total of n=34 participants had major protocol deviations leading to exclusion from PP population. Major protocol deviations were defined as in the VLA1553-301 trial. Overall, 4 participants attended Visit 3 but had immunogenicity sample was missing/unusable, 13 participants attended Visit 3 but were out of visit window, and 14 participants did not attend Visit 3. Major PD because of use of prohibited concomitant medication occurred in 2 participants and major PD due to an exclusion criterion occurred in 1 participant.

The FAS was composed of 391 subjects, 132 in Lot 1 arm, 128 in Lot 2 arm, and 131 in Lot 3 arm.

A total of 361 participants were included in sPP1 population, 120 in Lot 1 arm, 119 in Lot 2 arm, and 122 in Lot 3 arm. The sPP1 population included only participants which were µPRNT seronegative at baseline. Participants were excluded from the sPP1 population based on baseline µPRNT results and not baseline ELISA results. A total of n=14 baseline µPRNT seropositive ($\mu PRNT_{50}$ ≥20) participants were excluded from the sPP1 population. In total, n=34 participants were excluded from sPP1 populations for major PDs (same participants as for the PP).

A total of 366 participants were included in sPP2, 122 in Lot 1 arm, 121 in Lot 2 arm, and 123 in Lot 3 arm.

- **Recruitment**

The first study participants was enrolled on 22 Feb 2021 and the last participants last visit was on 10 Dec 2021. The study was completed on 01 Apr 2022. Participants were followed up for approximately 6 months following vaccination.

- **Conduct of the study**

Protocol amendments

The study period included the period during which the COVID-19 pandemic was occurring globally. Overall, the COVID-19 pandemic did not impact the conduct of the study.

The study was initiated under Protocol version 1.0 dated 09 Nov 2020.

Protocol version 2.0 dated 11 Feb 2021 was implemented to include feedback received from the FDA on 02 Feb 2021 regarding the definition of the PP analysis set. 'The per protocol (PP) analysis set contains all subjects who have ~~no major protocol violations that could impact the immune response.~~ received the vaccination and have evaluable immunogenicity data at baseline and the time point for the primary endpoint without a major protocol violation.'

Protocol version 3.0 dated 07 Apr 2021 was implemented due to the FDA request received on 16 Mar 2021 to include a conservative safety margin. A neutralizing antibody titre of ≥150 determined by µPRNT50 was proposed as a titre reasonably likely to predict protection in humans and was therefore an agreed surrogate endpoint to support accelerated approval. Additionally, updates were included to improve recruitment of the study and clarification:

• Inclusion criteria of CHIKV negative status no longer required to pass screening.

• Change in increased subject population reflect that we need more subjects to compensate for the possibility that 2% of our subject population are positive at baseline for CHIKV antibodies.

Protocol deviations

Refer to the assessment in study VLA1553-301 regarding the classification process of the protocol deviations.

Similarly to VLA1553-301 study, only protocol deviations until Visit 3 were assessed into major or minor protocol deviations. A total of 34 participants (8.3%) of the safety population had at least a major protocol deviations until (and including) Visit 3. A total of 220 (53.9%) participants had important minor protocol deviations and 153 (37.5%) had (not important) minor protocol deviations.

No **inspection** is mentioned in the submission.

- **Baseline data**

Demographic characteristics for the PP analysis set are provided in Table 17.

Table 17. Demographic characteristics (PP analysis set)

Characteristic	VLA1553 LOT 1 (N=122)	VLA1553 LOT 2 (N=118)	VLA1553 LOT 3 (N=122)	Total (N=362)
Sex [n (%)]				
Female	68 (55.7)	70 (59.3)	64 (52.5)	202 (55.8)
Male	54 (44.3)	48 (40.7)	58 (47.5)	160 (44.2)
Race [n (%)]				
American Indian or Alaska Native	3 (2.5)	1 (0.8)	0	4 (1.1)
Asian	6 (4.9)	5 (4.2)	6 (4.9)	17 (4.7)
Black or African American	17 (13.9)	18 (15.3)	17 (13.9)	52 (14.4)
Native Hawaiian or other Pacific Islander	0	1 (0.8)	0	1 (0.3)
White	94 (77.0)	92 (78.0)	97 (79.5)	283 (78.2)
Other	2 (1.6)	1 (0.8)	2 (1.6)	5 (1.4)
Ethnicity [n (%)]				
Hispanic or Latino	17 (13.9)	10 (8.5)	18 (14.8)	45 (12.4)
Not Hispanic or Latino	105 (86.1)	107 (90.7)	103 (84.4)	315 (87.0)
Unknown	0	1 (0.8)	1 (0.8)	2 (0.6)
Age (years)				
n	122	118	122	362
Mean (std)	33.0 (7.1)	33.4 (7.8)	33.1 (7.3)	33.2 (7.4)
Median	34.0	34.5	34.0	34.0
Q1, Q3	27.0, 38.0	27.0, 40.0	27.0, 40.0	27.0, 39.0
Min, Max	18, 45	20, 45	18, 45	18, 45
Weight (kg)				
n	122	118	122	362
Mean (std)	86.4 (24.1)	86.5 (22.6)	86.3 (22.4)	86.4 (23.0)
Median	84.1	85.1	84.3	84.6
Q1, Q3	68.5, 95.4	71.3, 97.5	72.1, 99.9	71.1, 98.3
Min, Max	49.2, 204.5	45.8, 171.4	45.5, 157.9	45.5, 204.5
Height (cm)				
n	122	118	122	362
Mean (std)	170.6 (9.1)	170.9 (9.7)	171.4 (9.8)	171.0 (9.5)
Median	170.2	170.2	172.6	170.3
Q1, Q3	164.2, 177.6	163.3, 177.8	165.0, 179.0	164.2, 177.8
Min, Max	142.2, 189.5	149.9, 197.0	148.6, 193.0	142.2, 197.0
BMI (kg/m^2)				
n	122	118	122	362
Mean (std)	29.6 (7.8)	29.7 (7.8)	29.3 (7.1)	29.5 (7.6)
Median	28.2	28.4	28.2	28.2
Q1, Q3	24.2, 34.4	25.0, 33.5	24.1, 33.3	24.6, 33.6
Min, Max	17.5, 72.8	13.7, 61.8	14.0, 49.7	13.7, 72.8

BMI=body mass index; CRF=case report form; n=number of subjects; std=standard deviation.
Note: 'Unknown' ethnicity categories was as recorded on the CRF.
Source: Table 14.1.2.2 and Listing 16.2.4.1

There were no major differences in the demographic characteristics between the safety analysis set and the PP population.

There were 54.7% and 55.8% of female participants included in the safety analysis set and in the PP population, respectively. Median (Q1-Q3) age of the participants was 34.0 years (27.0-39.0 years) in both populations. Median body mass index was 28.2 kg/m^2 for both populations with Q1-Q3 being 24.2-33.4 kg/m^2 and 24.6-33.6 kg/m^2 for the safety analysis set and the PP population, respectively.

There was a slight imbalance in terms of the percentages of female participants in the PP population (55.7%, 59.3%, 52.5%, respectively in Lot 1, 2, 3 arms). Other characteristics (age and BMI) were comparable between Lot arms.

Few study participants of the safety analysis set had a history of vaccination against yellow fever (11/408), Japanese encephalitis (2/408), and dengue (1/408). Data are not provided for the PP

population. Because of the limited number of vaccinated participants, and because no/low cross-reactivity is expected, data are not requested.

Several of the 12 sites are located in the South/South-east states of the US, where mosquitos that can transmit Dengue and CHIKV are present. There were also sites located in Florida (n=3) and Texas (n=1) where local transmission of Dengue and CHIKV is reported.

In addition to pre-existing antibodies specific to CHIKV, participants were also screened at baseline for antibodies specific to Mayaro virus, Dengue virus and Zika virus. Twelve participants of the safety analysis set were baseline antibody-CHIKV positive by ELISA, and 12 had detectable antibodies specific to Mayaro virus. How many of the participants were positive for both is not described. Respectively 5 and 18 participants had baseline antibodies specific to Zika virus and Dengue virus. The number of participants tested positive at baseline for more than 1 virus was not presented. This is acceptable as the number should be small, if not null. Refer to study VLA1553-301, corresponding section, for cross-reactivity.

Of the participants of the safety analysis set, 14 and 8 were baseline antibody-CHIKV positive by µPRNT50, with respectively the cut-off of 20 and 40.

- **Outcomes and estimation**

Primary immunogenicity endpoint results

The study met its primary immunogenicity endpoint, demonstrating Lot-to-Lot consistency based on the GMTs on Day 29 (Table 25- PP population).

Table 18. Summary and Analysis of GMTs for CHIKV-Specific Neutralizing Antibodies at Day 29 in study VLA1553-302 (PP Population)

Time Point Statistic	VLA1553 LOT 1 (N=122)	VLA1553 LOT 2 (N=118)	VLA1553 LOT 3 (N=122)
Visit 3 - Day 29			
N	122	118	122
Geometric Mean	2556.7	2767.7	2613.7
95% CI for GM	2055.63, 3179.80	2310.25, 3315.65	2128.06, 3210.17
LS Mean	2556.7	2767.7	2613.7
95% CI	2093.12, 3122.85	2258.27, 3391.98	2139.82, 3192.53

Time Point Statistic	VLA1553 LOT 1 (N=122)	VLA1553 LOT 2 (N=118)	VLA1553 LOT 3 (N=122)
Comparison	Lot 2 - Lot 1	Lot 2 - Lot 3	Lot 3 - Lot 1
GMT Ratio	1.083	1.059	1.022
95% CI	0.814, 1.440	0.796, 1.409	0.770, 1.357

CI=confidence interval; GM=geometric mean; GMT=geometric mean titre; LS=least squares; n=number of baseline ELISA negative subjects with non-missing titres at Day 29.
Note: The ANOVA model was applied to the log-transformed titres, and back-transformed results were displayed for the LS mean and difference. The difference in GMT was a ratio of the LS means.

The 3 pair-wise (Lot 2/Lot 1, Lot 3/Lot 2, Lot 3/Lot 1) 95% CIs for Day 29-GMT ratios were all between 0.67 and 1.5. These margins were pre-defined as acceptance margins for demonstrating equivalence of the 3 Lots. GMT ratios were 1.08 (Lot 2/Lot 1), 1.06 (Lot 2/ Lot 3) and 1.02 (Lot 3/Lot 1) with 95% CI of 0.81-1.44 (Lot 2/Lot 1), 0.80-1.41 (Lot 2/ Lot 3) and of 0.77-1.36 (Lot 3/Lot 1).

Day 29-GMTs were 2556.7 (95% CI: 2055.6-3179.8), 2767.7 (95% CI: 2310.3-3315.7) and 2613.7 (95% CI: 2128.1-3210.2) for Lot 1, Lot 2 and Lot 3 respectively. No significant differences were observed between lots.

Results obtained in the FAS, sPP1 and sPP2 populations were similar. These sensitivity analyses were performed in baseline seronegative participants either by using the µPRN50 threshold of 20 or 40, and consistent results were obtained. As a low background of CHIKV pre-existing immunity was expected, the consistency of the results are not unexpected (low number of seropositive participants, i.e. less chance to bias the results).

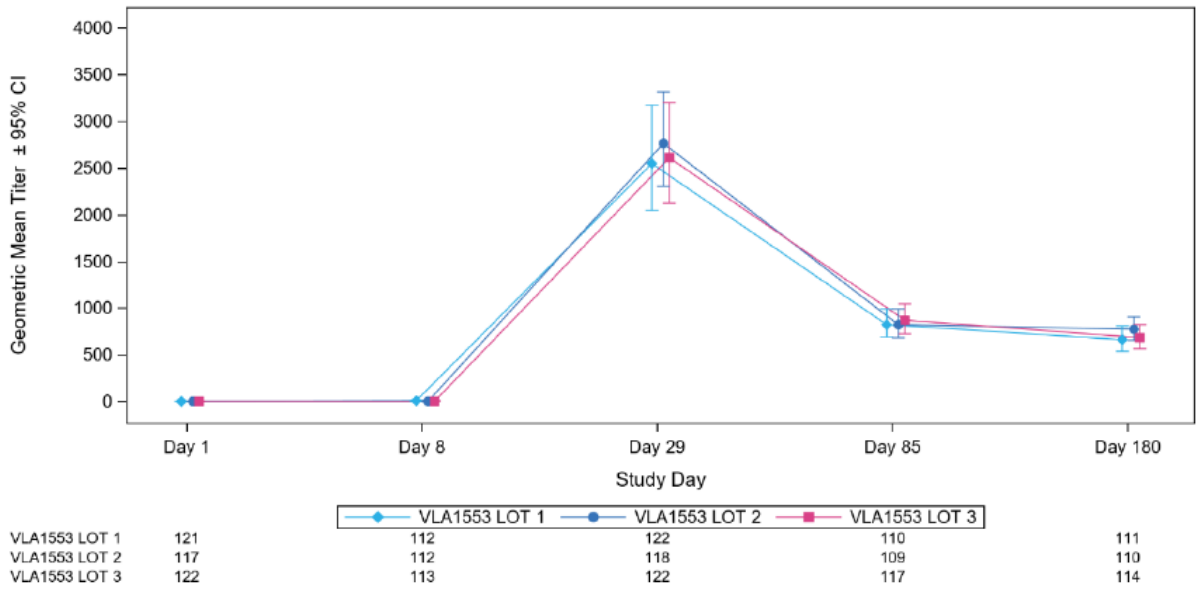
Secondary immunogenicity endpoint results

Immune response as measured by CHIKV-specific neutralizing antibody titres on Day 8, Day 29, Day 85, and Day 180 post-vaccination

A summary of GMTs for CHIKV-specific neutralizing antibodies by visit for the PP analysis set is illustrated in Figure 17 below.

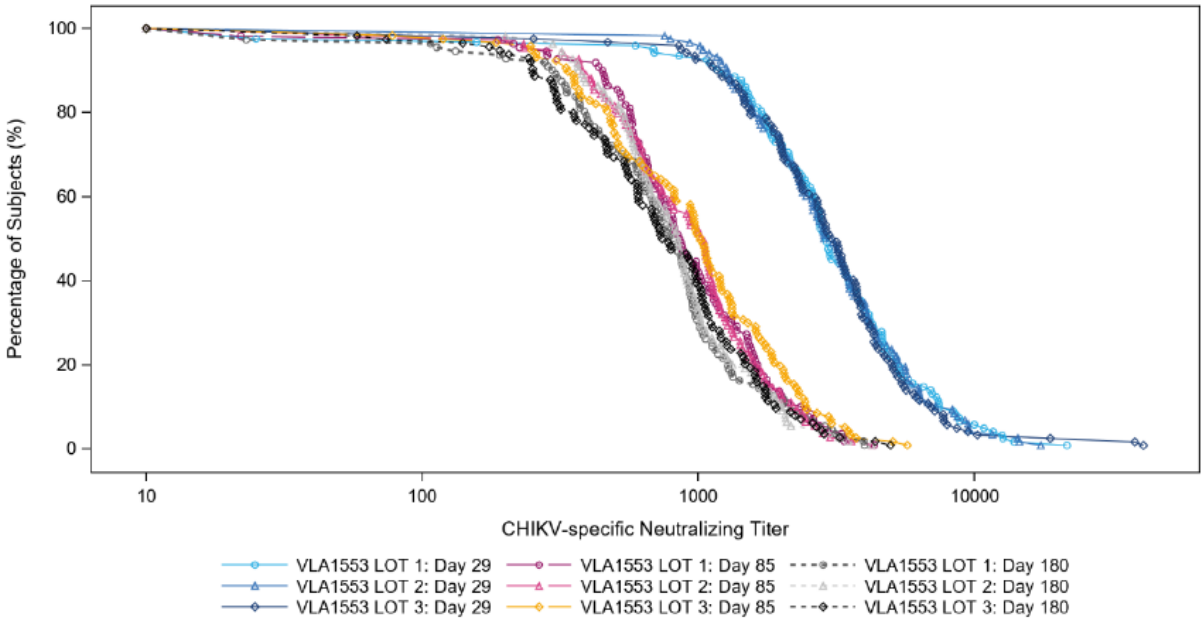
Figure 16 presents reverse cumulative distribution curves of CHIKV-specific neutralizing antibodies from Day 29 to Day 180.

Figure 15. Line plot of CHIKV-specific neutralizing antibodies (GMT) by study day and study arm (PP analysis set)



CHIKV=chikungunya virus; CI=confidence interval; GMT=geometric mean titer.
Note: Counts below the chart show the number of subjects in each study arm at the time point.
This analysis was based on the definition of seronegative at baseline being ELISA negative
Source: Figure 14.2.1.1.1 and Table 14.2.1.3

Figure 16. Reverse cumulative distribution curves of CHIKV-specific neutralizing antibodies at Day 29 to Day 180 (PP analysis set)



CHIKV=chikungunya virus.
This analysis was based on the definition of seronegative at baseline being ELISA negative
Source: Figure 14.2.1.2.1 and Table 14.2.1.3

The overall kinetics of the immune response is in line with the findings of study and VLA1553-301. The kinetics is consistent for the 3 Lots.

GMTs are similar across Lots at each timepoints. 95% CI of Day 180-GMTs were overlapping for the 3 Lots but the 95% CI on the GMT ratio of Lot 2 to Lot 1 at Day 180 was not in the defined acceptance margins of 0.67 and 1.5 (i.e. 0.91-1.53), which is however not deemed clinically relevant.

Day 8-GMTs were very low and similar to baseline (µPRNT of 12.1 versus of 10.2 at baseline for the 3 Lots combined). GMTs peaked at Day 29 as previously mentioned (2643.2 for the 3 Lot arms combined, 95% CI: 2354.0-2967.9). GMTs were decreased at Day 85 when compared to Day 29-GMTs, with a GMT for the 3 Lot arms combined of 846.1 (95% CI: 762.7-938.7). Day 180-GMTs tended to be decreased as compared to Day 85 but were still higher than GMTs at baseline (GMT of 708.8, 95% CI: 639.0-786.2 for the 3 Lots).

These observations are confirmed by reverse cumulative distribution curves of CHIKV-specific neutralizing antibodies at Day 29 to Day 180. On Day 29, most participants in the VLA1553 arm achieved antibody titres between 1,000 and 10,000 µPRNT50. On Day 180, most participants still had antibody titres of at least around 500 µPRNT50.

Results obtained in the FAS were similar. Results obtained in the sPP1 and in sPP2 were also similar.

Proportion of participants with antibody titres ≥150 µPRNT on Day 8, Day 29, Day 85, and Day 180 post-vaccination

Proportion of baseline seronegative participants with antibody titres post-vaccination above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) by visit for the PP analysis set are presented in Table 26 below. The analysis was conducted on baseline CHIKV seronegative participants defined by both ELISA and µPRNT. A total of 6 participants were defined as baseline seropositive by ELISA but as baseline seronegative by µPRNT. They were thus not included in the analysis set.

Table 19. Proportion of baseline seronegative participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) by visit (PP analysis set)

Time Point[a] [n] Statistic	VLA1553 LOT 1 (N=122)	VLA1553 LOT 2 (N=118)	VLA1553 LOT 3 (N=122)	Total (N=362)
Visit 2 - Day 8	110	110	111	331
Subjects with Seroprotection [n (%)]	0	0	0	0
95% CI for Seroprotection Rate	0.0, 3.3	0.0, 3.3	0.0, 3.3	0.0, 1.1
Difference in Seroprotection Rate[b]				
Comparison	Lot 2 - Lot 1	Lot 3 - Lot 2	Lot 3 - Lot 1	
Difference	NC	NC	NC	
95% CI	NC	NC	NC	
p-value[c]	NC	NC	NC	
Visit 3 - Day 29	120	116	120	356
Subjects with Seroprotection [n (%)]	117 (97.5)	114 (98.3)	117 (97.5)	348 (97.8)
95% CI for Seroprotection Rate	92.9, 99.5	93.9, 99.8	92.9, 99.5	95.6, 99.0
Difference in Seroprotection Rate[b]				
Comparison	Lot 2 - Lot 1	Lot 3 - Lot 2	Lot 3 - Lot 1	
Difference	0.8	-0.8	0.0	
95% CI	-2.9, 4.4	-4.4, 2.9	-4.0, 4.0	
p-value[c]	>0.9999	>0.9999	>0.9999	
Visit 4 - Day 85	108	107	115	330
Subjects with Seroprotection [n (%)]	106 (98.1)	104 (97.2)	111 (96.5)	321 (97.3)
95% CI for Seroprotection Rate	93.5, 99.8	92.0, 99.4	91.3, 99.0	94.9, 98.7
Difference in Seroprotection Rate[b]				
Comparison	Lot 2 - Lot 1	Lot 3 - Lot 2	Lot 3 - Lot 1	
Difference	-1.0	-0.7	-1.6	
95% CI	-5.0, 3.1	-5.3, 3.9	-5.8, 2.6	
p-value[c]	0.6831	>0.9999	0.6842	
Visit 5 – Day 180	109	108	112	329
Subjects with Seroprotection [n (%)]	103 (94.5)	106 (98.1)	107 (95.5)	316 (96.0)
95% CI for Seroprotection Rate	88.4, 98.0	93.5, 99.8	89.9, 98.5	93.3, 97.9
Difference in Seroprotection Rate[b]				
Comparison	Lot 2 - Lot 1	Lot 3 - Lot 2	Lot 3 - Lot 1	
Difference	3.7	-2.6	1.0	
95% CI	-1.3, 8.6	-7.2, 2.0	-4.7, 6.8	
p-value[c]	0.2799	0.4461	0.7661	

CHIKV=chikungunya virus; CI=confidence interval; µPRNT=micro plaque reduction neutralization test; $\mu PRNT_{50}$=50% plaque reduction in a micro plaque reduction neutralization test; n=number of subjects; NC=non-calculable; std=standard deviation.

a Number of baseline µPRNT negative (<20) subjects with non-missing titers at the specified time point.

b Differences, p-values, and associated CIs were presented for the difference in proportions between the Lots.

c p-value from Fisher's Exact test.

Percentages were based on the number of baseline µPRNT negative subjects with non-missing titers at the visit.

Seroprotection was defined as µPRNT50 ≥150 for baseline µPRNT negative (<20) subjects.

Two-sided 95% exact (Clopper-Pearson) CIs presented.

Source: Table 14.2.2.4 and Listing 16.2.5.1

Results are in line with results of study VLA1553-301, with 0% (95% CI: 0.0-1.1) and 97.8% (95% CI: 95.6-99.0) of participants reaching this antibody level at Day 8 and Day 29, respectively. Proportions remain high up to Day 180. No significant differences were observed between Lot arms.

Results obtained with the FAS were overall similar for this secondary endpoint. Results obtained in the sPP1 and in sPP2 populations were also similar.

Other secondary endpoints

Proportion of participants with neutralizing antibody titre thresholds of µPRNT50 ≥50 and ≥ 250 post-vaccination, proportion of participants with seroconversion post-vaccination, proportion of participants with fold increase of CHIKV-specific neutralizing titres post-vaccination, and proportion of participants reaching an at least 4-fold, 8-fold, 16-fold, or 64-fold increase in CHIKV-specific neutralizing titres were also presented by visit for the PP analysis set.

Results are overall consistent with results of study VLA1553-301, and confirmed the results observed for GMTs post-vaccination described above.

Proportions of baseline seronegative participants with antibody titres post-vaccination of at least µPRNT50 250 are similar than those found when the threshold was set at the µPRNT50 ≥150.

Findings on the proportion of participants with seroconversion post-vaccination (defined as CHIKV-specific neutralizing titre of µPRNT50 ≥20 for baseline negative µPRNT [<20] participants) were also consistent with these findings.

CHIKV-specific neutralizing antibody titres post-vaccination increased 1.4-fold 7 days after vaccination when compared to baseline and peaked at Day 29 with a 383.3-fold increase versus baseline titres. At Day 180, titres were still 97.3 higher than baseline titres. Again, no differences were observed between Lots.

Results obtained with the FAS were overall similar for all these secondary endpoints. Results obtained in the sPP1 and in sPP2 populations were also similar.

- ***Ancillary analyses***

Concomitant Therapy

In the Safety analysis set, 323/408 (79.2%) participants received concomitant medications (i.e. with a start or end date on or after date of vaccination). The most common concomitant medications were psychoanaleptics (24.5%, such as Bupropion), anti-inflammatory/anti-rheumatic products (22.3%, mainly Ibuprofen), and analgesics (21.6%, mainly Paracetamol).

Anti-inflammatory and anti-rheumatic products were recorded as concomitant medication in the VLA1553 group in 23.0% of the participants of the FAS and 22.9% of the participants of the PP population. Analgesics were recorded as concomitant medication in the VLA1553 group in 21.5% of the participants of the FAS and 22.1% of the participants of the PP population.

The proportion of participants of the safety population in study VLA1553-302 who used anti-inflammatory and anti-rheumatic products between Day 1 and Day 7 and between Day 1 to Day 29 post-vaccination was comparable to those observed in the VLA1553 group of the safety population of study VLA1553-301 (19.4% and 21.8% for the period between Day 1 and Day 7 and between Day 1 to Day 29 post-vaccination, respectively). The proportion of participants who used anti-inflammatory and anti-rheumatic products between Day 30 and Day 180 was lower as compared to the one observed in the VLA1553 group in study VLA1553-301 (8.3%). Similar proportions were observed for the FAS and for the PP population.

The proportion of participants of the safety population in study VLA1553-302 who used analgesics between Day 1 and Day 7 and between Day 1 to Day 29 post-vaccination was comparable to the VLA1553 group in VLA1553-301 (18.1% and 19.9% for the period between Day 1 and Day 7 and between Day 1 to Day 29 post-vaccination, respectively). The proportion of participants who used anti-inflammatory and anti-rheumatic products between Day 30 and Day 180 was lower as compared to the VLA1553 group in the VLA1553-301 study (11.8%). Similar proportions were observed for the FAS and for the PP population.

Analyses of immune responses stratified by use of those medications in relevant periods show that, as for study VLA1553-301, overall, the GMTs and proportion of participants with seroresponse were not influenced by concomitant use of anti-inflammatory and anti-rheumatic products or analgesics in a respective time period. No data are however provided with respect to the impact of prophylactic administration of antipyretics within 4 hours prior to and during the first 72 hours after vaccination on the immune responses (REC).

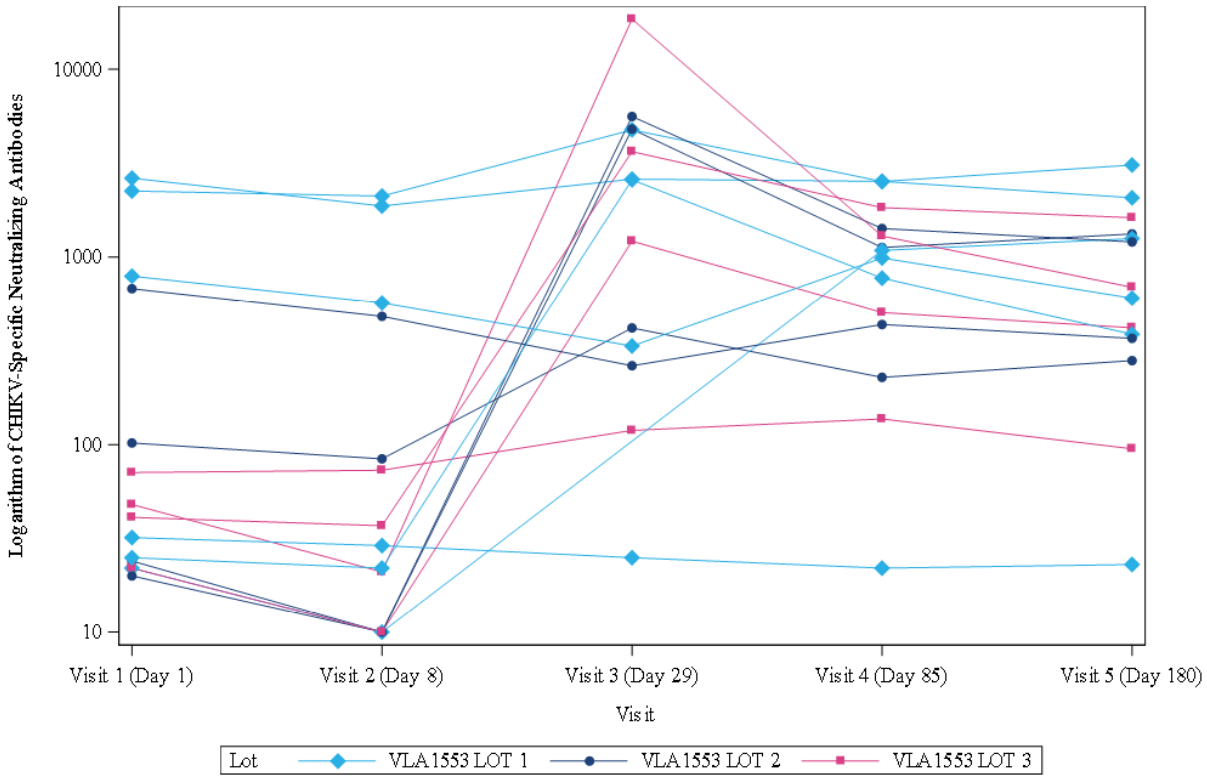
Immunogenicity results in individuals tested positive for CHIKV at baseline using the baseline threshold of µPRNT50 >40

Overall, 12, 14 and 8 participants tested positive for CHIKV at baseline using binding ELISA antibodies, µPRNT50 ≥20 or µPRNT50 >40 as definition for the seropositivity, respectively. The threshold of µPRNT50 >40 is considered more appropriate for the immunogenicity and safety analyses of CHIKV baseline seropositive participants.

The limited number of participants does not allow for a meaningful statistical analysis. Of the 8 participants, 3 seroconverted (defined as an at least a 4 fold increase in neutralizing antibody titre over baseline).

Individual CHIKV-specific neutralizing antibody curves for participants with CHIKV baseline µPRNT50 ≥20 suggest that neutralizing antibody titres can increase following VLA1553 vaccination in participants who are seropositive, especially when the baseline titre is in the lower range of values, which is illustrated in the Figure 22 below.

Figure 17. Line plot of CHIKV-specific neutralizing antibodies for subjects who are seropositive at baseline in study VLA1553-302 (Safety Analysis Set, seropositive is µPRNT50 ≥20 at baseline)



2.6.5.4. Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment.

Table 20. Summary of efficacy for trial VLA1553-301

Title: VLA1553-301 A multicentre, randomized, placebo-controlled, double-blinded pivotal study to evaluate safety and immunogenicity of a live-attenuated chikungunya virus vaccine candidate (VLA1553) in adults aged 18 years and above			
Study identifier	IND NUMBER: 17854 Clinical Trial No.: NCT04546724		
Design	prospective, randomized, double-blinded, placebo-controlled, multicentre pivotal clinical study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		17 Sep 2020 - 15 October 2021 not applicable not applicable
Hypothesis	Proportion of baseline CHIV seronegative subjects administered VLA1553 with a μPRNT50⩾150 on Day 29, with a non-acceptance threshold of 70% for the lower bound of the 95% CI required. The primary endpoint was considered met if the lower bound of the 95% confidence interval (CI) around the proportion is >70%.		
Treatments groups	VLA1553		VLA1553 (1x10E4 TCID50 / 0.5 mL) single intramuscular immunization on Day 1 number randomized: 4,128 number in the immunogenicity subset: 375
	placebo		placebo single intramuscular immunization on Day 1 number randomized: 1,035 number in the immunogenicity subset: 126
Endpoints and definitions	Primary endpoint	Immunogenicity (Day 29) – proportion above threshold	proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 29 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150 (in the PP population)
	Secondary	Immunogenicity (Day 8) - proportion above threshold	proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 8 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150 (in the PP population)
	Secondary	Immunogenicity (Day 180) - proportion above threshold	proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 180 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150 (in the PP population)
Database lock	20 Jan 2022 (soft lock) / 27 Jul 2022 (hard lock)		

Title: VLA1553-301 A multicentre, randomized, placebo-controlled, double-blinded pivotal study to evaluate safety and immunogenicity of a live-attenuated chikungunya virus vaccine candidate (VLA1553) in adults aged 18 years and above			
Study identifier	IND NUMBER: 17854 Clinical Trial No.: NCT04546724		
Results and Analysis			
Analysis description	**Primary Analysis** proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 29 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150		
Analysis population and time point description	The Per protocol (PP) population contained all randomized and vaccinated participants of the immunogenicity subset, who were CHIKV seronegative at baseline (defined as μPRNT50 <20), who had at least one evaluable post-baseline titre measurement, and who had no major protocol deviations that could impact the measurement of immune responses. The PP population for immunogenicity data was composed of a total of 266 subjects (respectively 207 and 59 adults of 18-64 years and ⩾65 years). Day29		
Descriptive statistics and estimate variability	Treatment group	VLA1553	placebo
	Number of subject	266	96
	Immunogenicity (Day 29) – proportion above threshold [%, n]	98.9% (263)	0% (0)
	95% CI on proportion above threshold	96.7%; 99.8%	0.0%, 3.8%
Notes	The lower bound of the 95% CI around the proportion exceeds the non-acceptance threshold of 70%. Similar proportions of VLA1553 vaccinated participants above the defined threshold were estimated for subjects aged 18-64 years (98.6% [204/207], 95% CI: 95.8-99.7) and for subjects aged ⩾65 years (100% [59/59], 95% CI: 93.9-100.0). None of the placebo participants reached the threshold.		
Analysis description	**Secondary analysis** proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 8 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150		

Title: VLA1553-301

A multicentre, randomized, placebo-controlled, double-blinded pivotal study to evaluate safety and immunogenicity of a live-attenuated chikungunya virus vaccine candidate (VLA1553) in adults aged 18 years and above

Study identifier	IND NUMBER: 17854 Clinical Trial No.: NCT04546724		
Analysis population and time point description	The Per protocol (PP) population contained all randomized and vaccinated participants of the immunogenicity subset, who were CHIKV seronegative at baseline (defined as μPRNT50 <20), who had at least one evaluable post-baseline titre measurement, and who had no major protocol deviations that could impact the measurement of immune responses. The PP population for immunogenicity data was composed of a total of 266 subjects (respectively 207 and 59 adults of 18-64 years and ⩾65 years). Day8		
Descriptive statistics and estimate variability	Treatment group	VLA1553	placebo
	Number of subject	251	93
	Immunogenicity (Day 8) – proportion above threshold [%, n]	1.6% (4)	0% (0)
	95% CI on proportion above threshold	0.4%, 4.0%	0.0%, 3.9%
Analysis description	**Secondary analysis** proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 180 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150		
Analysis population and time point description	The Per protocol (PP) population contained all randomized and vaccinated participants of the immunogenicity subset, who were CHIKV seronegative at baseline (defined as μPRNT50 <20), who had at least one evaluable post-baseline titre measurement, and who had no major protocol deviations that could impact the measurement of immune responses. The PP population for immunogenicity data was composed of a total of 266 subjects (respectively 207 and 59 adults of 18-64 years and ⩾65 years). Day180		
Descriptive statistics and estimate variability	Treatment group	VLA1553	placebo
	Number of subject	242	91
	Immunogenicity (Day 180) – proportion above threshold [%, n]	96.3% (233)	0% (0)
	95% CI on proportion above threshold	93.1%, 98.3%	0.0%, 4.0%

Title: VLA1553-301 A multicentre, randomized, placebo-controlled, double-blinded pivotal study to evaluate safety and immunogenicity of a live-attenuated chikungunya virus vaccine candidate (VLA1553) in adults aged 18 years and above	
Study identifier	IND NUMBER: 17854 Clinical Trial No.: NCT04546724
Notes	Immunogenicity results up to Year 2 are available from study VLA1553-303, which is an ongoing open-label, single arm, study evaluating antibody persistence (up to 5 years post-vaccination) in a subset of participants of study VLA1553-301 (n=363). The primary immunogenicity endpoint is the proportion of participants with seroresponse defined as CHIKV antibody level μPRNT50 ⩾150 at Year 1, Year 2, Year 3, Year 4, and Year 5 post-vaccination. The PP population for the Year 1 immunogenicity data is composed of a total of 184 subjects (respectively 157 and 27 adults of 18-64 years and ⩾65 years). The PP population for the Year 2 immunogenicity data is composed of a total of 339 subjects (respectively 290 and 49 adults of 18-64 years and ⩾65 years). On Year 1, 183/184 (99.5%, 95% CI: 97.0% to 100.0%) of participants have CHIKV antibody level μPRNT50 ⩾150. No difference in proportion above the threshold is noted when results are stratified by age, for subjects aged 18-64 years, 156/157 participants are above the threshold (99.4% (95% CI: 96.5% to 100.0%)) and for subjects aged ⩾65 years, 27/27 participants are above the threshold (100.0%, (95% CI: 87.2% to 100.0%)). On Year 2, 268/276 (97.1%, 95% CI: 94.4% to 98.7%) of participants have CHIKV antibody level μPRNT50 ⩾150. No difference in proportion above the threshold is noted when results are stratified by age, for subjects aged 18-64 years, 227/234 participants are above the threshold (97.0% (95% CI: 93.9% to 98.8%)) and for subjects aged ⩾65 years, 41/42 participants are above the threshold (97.6%, (95% CI: 87.4% to 99.9%)).

Table 21: Summary of efficacy for trial VLA1553-302

Title: VLA1553-302 A Randomized, Double-Blinded Phase 3 Study to Demonstrate Lot-to-Lot Consistency of Three Lots of a Live-Attenuated Chikungunya Virus Vaccine Candidate (VLA1553) in Healthy Adults Aged 18 to 45 Years			
Study identifier	IND NUMBER: 17854 Clinical Trial No.: NCT04786444		
Design	prospective, randomized, double-blinded, multicentre lot-to-lot consistency clinical study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		22 Feb 2021 – 10 Dec 2021 not applicable not applicable
Hypothesis	Demonstration of Lot-to-Lot consistency based on the primary endpoint, comparison of GMTs between Lots as determined by microneutralization (μPRNT) assay on Day 29 post-vaccination, by 3 pair-wise comparisons and acceptance margins of 0.67 and 1.5 for the GMT ratios.		
Treatments groups	VLA1553 (lot 1)		VLA1553 (1x10E4 TCID50 / 0.5 mL) single intramuscular immunization on Day 1 number randomized: 136
	VLA1553 (lot 2)		VLA1553 (1x10E4 TCID50 / 0.5 mL) single intramuscular immunization on Day 1 number randomized: 137
	VLA1553 (lot 3)		VLA1553 (1x10E4 TCID50 / 0.5 mL) single intramuscular immunization on Day 1 number randomized: 136
Endpoints and definitions	Secondary	Immunogenicity (Day 8) – proportion above threshold	proportion of baseline CHIKV seronegative participants in the VLA1553 arms achieving a Day 8 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150 (PP population / lots combined)
	Secondary	Immunogenicity (Day 29) - proportion above threshold	proportion of baseline CHIKV seronegative participants in the VLA1553 arms achieving a Day 29 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150 (PP population / lots combined)
	Secondary	Immunogenicity (Day 180) - proportion above threshold	proportion of baseline CHIKV seronegative participants in the VLA1553 arms achieving a Day 180 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150 (PP population / lots combined)
Database lock	01 Apr 2022 (Part B Data Hard Lock)		
Results and Analysis			

Title: VLA1553-302 A Randomized, Double-Blinded Phase 3 Study to Demonstrate Lot-to-Lot Consistency of Three Lots of a Live-Attenuated Chikungunya Virus Vaccine Candidate (VLA1553) in Healthy Adults Aged 18 to 45 Years		
Study identifier	IND NUMBER: 17854 Clinical Trial No.: NCT04786444	
Note	The primary objective of VLA1553-302 was to demonstrate Lot-to-Lot manufacturing consistency of a live-attenuated CHIKV vaccine candidate (VLA1553) in terms of GMT 28 days following vaccination in a healthy population aged 18 to 45 years after a single immunization. Only secondary analyses related to proportions of baseline CHIKV seronegative participants achieving a Day 8, Day 29 and Day 180 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150 are presented in this summary table (combined by lots). Primary analysis results are not presented in this summary table. In the PP population, the CIs on the GMT ratios of all Lots were in the defined acceptance margins of 0.67 and 1.5. The primary endpoint of Lot-to-Lot consistency was met.	
Analysis description	**Secondary Analysis** proportion of baseline CHIKV seronegative participants in the pooled VLA1553 arms achieving a Day 8 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150	
Analysis population and time point description	The PP analysis set contained all subjects who were baseline negative for CHIKV antibodies as determined by ELISA assay, had received the vaccination and had evaluable immunogenicity data at baseline and the time point for the primary endpoint without a major Protocol deviation. The PP analysis set was composed of 362 subjects (VLA1553 Lot 1: 122, Lot 2: 118, Lot 3: 122). Day8	
Descriptive statistics and estimate variability	Treatment group	VLA1553
	Number of subject	331
	Immunogenicity (Day 8) – proportion above threshold [%, n]	0% (0)
	95% CI on proportion above threshold	0.0%, 1.1%
Analysis description	**Secondary analysis** proportion of baseline CHIKV seronegative participants in the pooled VLA1553 arms achieving a Day 29 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150	
Analysis population and time point description	The PP analysis set contained all subjects who were baseline negative for CHIKV antibodies as determined by ELISA assay, had received the vaccination and had evaluable immunogenicity data at baseline and the time point for the primary endpoint without a major Protocol deviation The PP analysis set was composed of 362 subjects (VLA1553 Lot 1: 122, Lot 2: 118, Lot 3: 122). Day29	

Title: VLA1553-302 A Randomized, Double-Blinded Phase 3 Study to Demonstrate Lot-to-Lot Consistency of Three Lots of a Live-Attenuated Chikungunya Virus Vaccine Candidate (VLA1553) in Healthy Adults Aged 18 to 45 Years		
Study identifier	IND NUMBER: 17854 Clinical Trial No.: NCT04786444	
Descriptive statistics and estimate variability	Treatment group	VLA1553
	Number of subject	356
	Immunogenicity (Day 29) – proportion above threshold [%, n]	97.8% (348)
	95% CI on proportion above threshold	95.6%, 99.0%
Analysis description	**Secondary analysis** proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 180 CHIKV neutralizing antibody titre μPRNT50 ⩾ 150	
Analysis population and time point description	The PP analysis set contained all subjects who were baseline negative for CHIKV antibodies as determined by ELISA assay, had received the vaccination and had evaluable immunogenicity data at baseline and the time point for the primary endpoint without a major Protocol deviation The PP analysis set was composed of 362 subjects (VLA1553 Lot 1: 122, Lot 2: 118, Lot 3: 122). Day180	
Descriptive statistics and estimate variability	Treatment group	VLA1553
	Number of subject	329
	Immunogenicity (Day 180) – proportion above threshold [%, n]	96.0% (316)
	95% CI on proportion above threshold	93.3%, 97.9%

2.6.5.5. Clinical studies in special populations

No specific clinical study was conducted in older people. Refer to main study VLA1553-301 which enrolled younger (18-64 years) and older (≥65 years) adults.

2.6.5.6. Analysis performed across trials (pooled analyses and meta-analysis)

Cross-neutralization with other lineages and strains

Objective

Since the primary objective of the pivotal trial VLA1553-301 relates to the demonstration of the ability of VLA1553 to raise CHIKV neutralizing antibody responses (above a defined threshold) against the 181/clone 25 strain which is an attenuated strain, there are uncertainties around the capacity of vaccine-induced antibodies to neutralize virulent wild-type CHIKV.

CHIKV strains of different genotypes are considered to constitute a single serotype and antibodies specific to one genotype are thought to have the ability to also cross-neutralize strains from any other

genotype. However, results from the limited literature available suggest that neutralizing antibodies specific to a given genotype (induced either after natural infection or vaccination) might specifically cross-neutralize an heterologous genotype at levels that are not always equivalent in terms of magnitude and kinetic to the one measured against the homologous genotype (Chusri et al. ; Am J Trop Med Hyg 2014; doi: 10.4269/ajtmh.12-0681, Chua et al. ; PLoS Negl Trop Dis 2016 ; doi : 10.1371/journal.pntd.0004960, Auerswald et al. ; Emerg Microbes Infect 2018 ; doi : 10.1038/s41426-017-0010-0, Goo et al. ; J Infect Dis 2006 ; doi : 10.1093/infdis/jiw431).

Adequate demonstration of neutralization of several wild-type CHIKV strains representative of circulating strains is thus considered of utmost importance. The importance to generate sufficient data to provide strong support for claims that the vaccine will provide protection against a broad range of circulating CHIKV strains was already mentioned in the Scientific Advices of 2020 and 2021 (EMEA/H/SA/4412/1/2020/III and EMA/SA/0000063772). CHMP specified that data on cross-neutralisation against a broad range of heterologous CHIKV strains including the major lineages Asian Urban, Indian Ocean, East/Central/South African (ECSA) and West African (WA) was required for marketing authorisation.

A panel of samples from participants of studies VLA1553-101, VLA1553-301 and VLA1553-303 were tested for cross-neutralization of different wild-type CHIKV strains by classical PRNT assays at UTMB and OHSU. A limited number of samples from convalescent patients were also tested in both laboratories.

Cross-neutralization analyses at UTMB laboratory

The neutralization capacity of vaccine-induced antibodies were tested against 3 wild-type CHIKV strains on a limited panel of samples from studies VLA1553-101 and VLA1553-301. Tested strains included strains representing the IOL/ECSA lineage (LR2006_OPY1 strain [also referred as 7000 CHIKV-LR], La Reunion 2006), the West African lineage (37997 strain, Senegal 1983) and the Asian lineage (Caribbean M109 strain, Martinique 2014). Testing were performed with developed (VLA1553-101) or qualified (VLA1553-301) PRNT assays (refer to section 2.6.2.).

A total of 119 samples from 44 participants of study VLA1553-101 (n=47 from 12 participants randomized to different dose levels) and of study VLA1553-301 (n=72 from 32 participants) were analysed in the PRNT assays at UTMB. There were at least 2 samples per participant (not always including the baseline sample). A complete longitudinal sample set (at least 4 timepoints) was tested for 9 participants of VLA1553-101, but for none of the VLA1553-301 participants (maximum of 3 longitudinal samples). How the samples were selected was not described. A limitation of the testing performed at UTMB is that samples were not tested in assays specific to the 181/clone 25 or the vaccine strain, which would have facilitated comparisons of results with those generated with the µPRNT assay (for samples from VLA1553-301 and VLA1553-302) or with those generated with the µNT assay (for samples from VLA1553-101). Refer to additional PRNT testing performed at OHSU against the 181/clone 25 described below.

In the Scientific Advice of 2021 (EMA/SA/0000063772), it was strongly encouraged to increase the number of participants tested (with longitudinal samples) in the different neutralization assays. The advice was partially followed by the Applicant since samples from the Phase 3 VLA1553-301 study were tested (only samples from study VLA1553-101 were available at the time of the Scientific Advice). However, the number of samples with a complete longitudinal follow-up tested at UTMB is considered limited (refer to additional PRNT testing of longitudinal samples at OHSU below).

Samples from convalescent participants (n=6) were also previously tested (historical PRNT values) using the same CHIKV neutralization assays at UTMB. Six of the convalescent sera were collected in January 2015 from an unspecified location. The date of symptom onset, and therefore the time elapsed up to the collection date is neither described. The 3 additional sera were from Columbia and were collected in February and May 2016, approximately 1 month post-symptom onset.

Results at UTMB on samples from study VLA1553-101

Neutralizing antibody titres (PRNT50) obtained for the 47 samples of study VLA1553-101 against the 3 wild-type CHIKV isolates tested are summarized in Table 22. Results obtained with the µPRNT (specific to the 181/clone 25) are also provided. In the Phase 1 VLA1553-101 trial, samples were also tested against the vaccine strain in a µNT assay, but results were not submitted.

Table 22. PRNT50 and µPRNT50 titre from VLA1553-101 sera tested against different CHIKV strains (Table 3, UTMB report VIE-DR-0181 [01])

UTMB No.	Days post vaccination	$PRNT_{50}$			$\mu PRNT_{50}$
		LaReunion 7000 LR	W-African 37997	Caribbean Clone M109	181/clone 25
UTMB #1	0	<10	<10	<20	<20
UTMB #2	14	20	640	80	1897
UTMB #3	84	640	640	80	316
UTMB #4	180	320	160	40	280
UTMB #5	0	<10	<10	<20	<20
UTMB #6	7	<10	<10	<20	29
UTMB #7	14	160	80	160	2375
UTMB #8	84	1280	40	80	1309
UTMB #9	180	320	640	160	358
UTMB #10	0	<10	<10	20	<20
UTMB #11	7	<10	20	<20	<20
UTMB #12	14	20	320	320	1278
UTMB #13	84	160	640	20	592
UTMB #14	180	160	640	40	228

UTMB No.	Days post vaccination	PRNT50			µPRNT50
		LaReunion 7000 LR	W-African 37997	Caribbean Clone M109	181/clone 25
UTMB #15	0	<10	<10	<20	<20
UTMB #16	84	320	640	20	1032
UTMB #18	180	640	320	20	415
UTMB #17	210	640	320	40	495
UTMB #19	0	<10	<10	<20	<20
UTMB #20	180	>5120	1280	20	1784
UTMB #21	0	<10	<10	<20	<20
UTMB #22	84	>5120	640	40	1472
UTMB #24	180	1280	2560	20	1242
UTMB #23	210	2560	1280	40	1341
UTMB #25	0	<10	<10	<20	<20
UTMB #26	84	20	1280	40	2123
UTMB #28	180	2560	1280	40	698
UTMB #27	210	640	1280	80	539
UTMB #29	0	<10	<10	<20	<20
UTMB #30	84	80	1280	40	1701
UTMB #31	180	5120	2560	320	1458
UTMB #32	0	<10	<10	<20	<10
UTMB #33	160	2560	1280	160	1004
UTMB #34	0	<10	<10	<20	<20
UTMB #35	7	<10	<10	<20	<20
UTMB #36	84	1280	640	40	589
UTMB #37	180	1280	320	80	240
UTMB #38	0	<10	<10	<20	<20
UTMB #39	7	<10	<10	<20	45
UTMB #40	14	20	320	40	3122
UTMB #41	84	640	160	40	576
UTMB #42	180	320	160	20	263
UTMB #43	0	<10	<10	<20	31
UTMB #44	7	<10	<10	<20	42
UTMB #45	14	2560	160	80	2916
UTMB #46	84	640	160	80	530
UTMB #47	180	640	160	40	382

Titer values lower than the smallest dilutions (1:10 for the La Reunion and W-African strains and 1:20 for the Caribbean and the attenuated CHIK strain 181/clone 25) were reported as <10 and <20, respectively.

Titer values greater than the highest dilutions (1:5120) were reported as >5120.

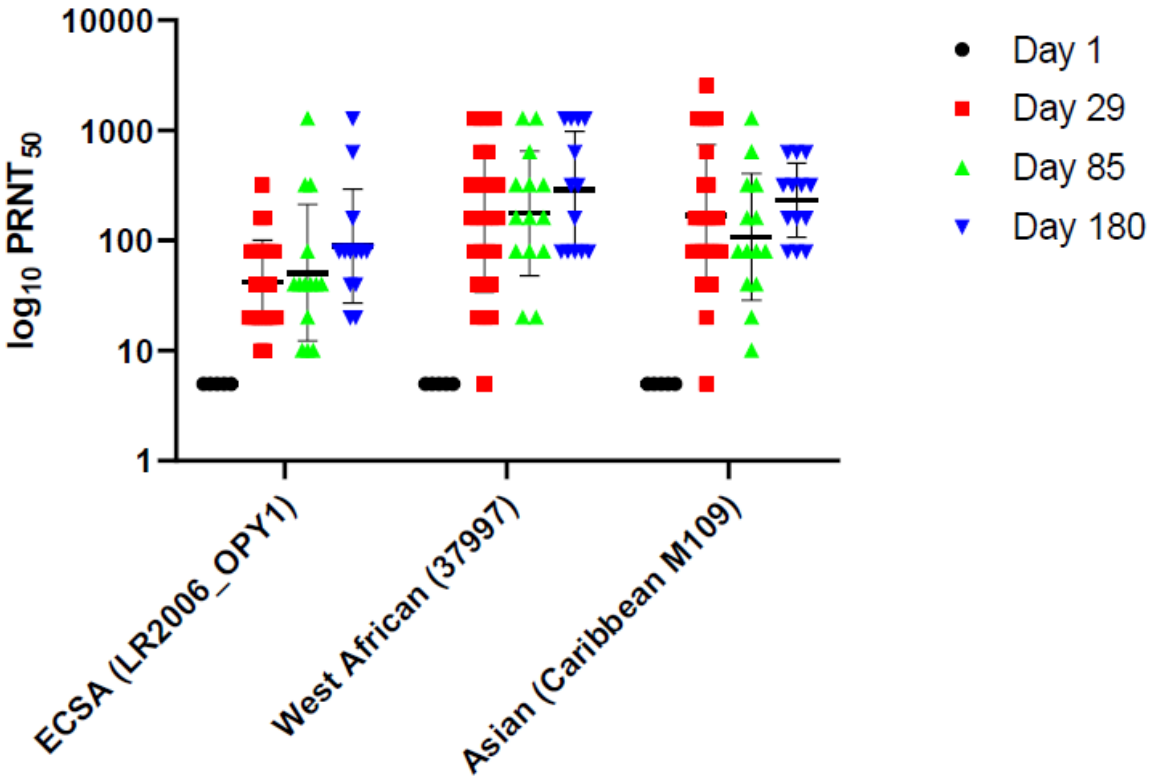
Samples tested ranged from <20 to 3122 µPRNT50 which is deemed appropriate as covering a large range of antibody titres, and also corresponding to the range observed post-vaccination in the pivotal study VLA1553-301. It is expected that higher antibody titres in µPRNT50 would also results in higher titres in any of the 3 PRNT assays.

Although quantitative comparison is not possible because of the use of different neutralization assay set-ups (PRNT versus µPRNT), results were overall consistent, except for the Caribbean strain. Negative samples in the CHIKV µPRNT assay were also negative in the 3 different wild-type CHIKV PRNT assays. All samples with detectable neutralizing antibody in the CHIKV µPRNT assay also had detectable neutralizing antibody in the La Réunion strain-PRNT and in the West African strain-PRNT. This was not the case for the Caribbean strain-PRNT as some samples resulted negative in this assay while positive in the CHIKV µPRNT assay. Results show overall no/low induction of antibody against the Caribbean strain following vaccination with VLA1553. Neutralizing antibodies against La Reunion and West African strains are induced but kinetics seems to differ. These findings are not in line with findings of study VLA1553-301 (see below).

Results at UTMB on samples from study VLA1553-301

Neutralizing antibody titres (PRNT50) obtained for the 72 samples of study VLA1553-301 against the 3 wild-type CHIKV isolates tested are presented in Figure 20, which represent PRNT50 titre values for all samples excluding those from participants with measurable neutralizing antibodies (PRNT50 ≥10) at Day 1, by day of sample collection (n=5).

Figure 18. PRNT50 titres by day of sampling, excluding samples from subjects with detectable neutralizing antibody at Day 1



Overall, in contrast to findings with the Phase 1 samples (study VLA1553-101), titres against Asian lineage Caribbean strain clone M109 are roughly similar to West African lineage and not clearly lower. This contradicting observation was attributed to assay variability. Antibody titres against La Reunion strain tend to be lower than for both other strains.

Baseline seronegative samples as defined by µPRNT50 were also negative for the 3 other strains tested. Antibodies were induced following vaccination in all except 1 sample at Day 29 which had no detectable antibody specific to both the West African strain and the Asian strain.

One participant had no/low antibody specific for the 3 strains tested at baseline although being baseline seropositive as defined by µPRNT50. Antibodies were detectable in the samples of the 4 other participants at baseline and in the post-vaccination samples but with no increase in antibody titres.

Samples from participants with pre-existing immunity were overall in the same range than the samples from convalescent participants. Among the 5 VLA1553 participants seronegative at baseline, 3 of them

had antibody titres post-vaccination in the same range than the antibody titres detected in the convalescent sera.

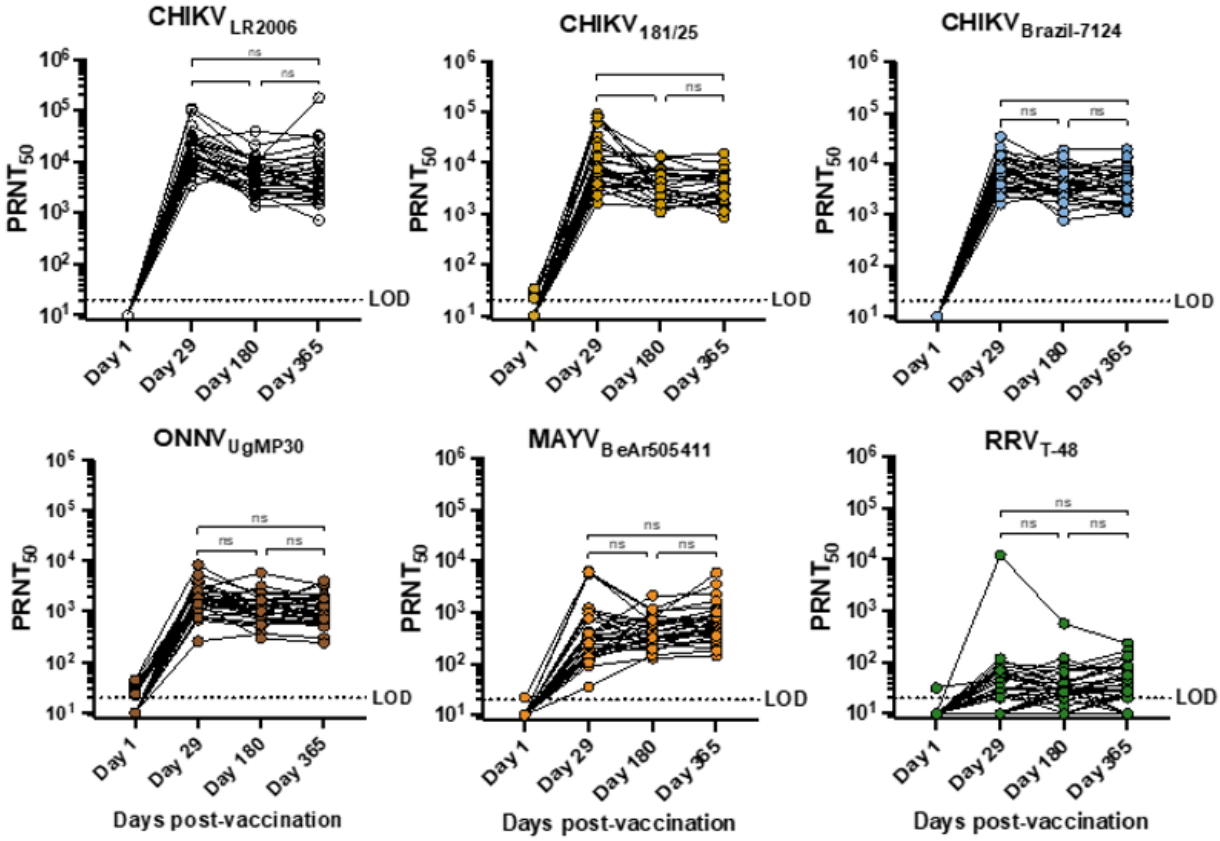
Cross-neutralization analyses at OHSU laboratory

The neutralization capacity of vaccine-induced antibodies were tested against 2 wild-type CHIKV strains (La Reunion strain LR2006 of ECSA lineage and a recent ECSA lineage Brazilian isolate 7124), the attenuated CHIKV 181/25 strain (corresponding to the strain used in the validated µPRNT50 assay) and against 3 other arthritogenic alphaviruses (ONNV strain UgMP30, MAYV strain BeAr505411 and RRV strain T-48). Testing were performed with classical PRNT assays of developed status on sera from 30 participants of VLA1553-301/VLA1553-303 collected at Day 1, Day 29, Day 180 and 1 year after vaccination.

Overall, submitted data indicate that up to 1-year post-vaccination VLA1553 induced high neutralising antibody titres against the 3 CHIKV strains tested, with overall comparable GMTs at each timepoints. Peak of GMTs was observed at 28 days post-vaccination, followed by a decrease in antibody titres observed at 6 months post-vaccination. GMTs at month 6 were comparable to those at year 1 (Figure 21). Additional testing of samples isolated from VLA1553 vaccinated subjects in PRNT assays specific to West African and Asian/Caribbean CHIKV strains are planned at OHSU (REC).

Neutralizing antibodies specific to ONNV and MAYV were also detected in sera from VLA1553 vaccinated subjects, but PRNT50 titres were higher against CHIKV (e.g. at Day 29, CHIKV 181/25 PRNT50 10879.0 (95% CI: 6917;17109)) as compared to PRNT50 titres against ONVV (e.g. at Day 29, ONNV PRNT50 1676.0 (95% CI: 1289;2180)) and as compared to PRNT50 titres against MAYV (e.g. at Day 29, MAYV – PRNT50 373.8 (95% CI: 234;598)). No neutralizing antibodies specific to RRV were detected in sera from VLA1553 vaccinated subjects.

*Figure 19. : Antibodies in VLA1553 human immune sera cross-neutralize different CHIKV strains and related arthritogenic alphaviruses. Individual data per participant over time is displayed by virus strain. Neutralizing antibody titres are compared by one-way ANOVA with multiple comparisons (Friedman test) where * p < 0.05, ** p < 0.01. The LOD is shown with a dotted line and refers to the minimum dilution of 1:20 tested (source figure 26 of AtQ 150)*



The OHSU laboratory also recently published PRNT assay results obtained in samples (n=12) from patients previously infected by CHIKV (confirmed or suspected cases), which were tested in the same assay set-up against a panel of alphaviruses including CHIKV (clone 181/25), ONNV (UgMP30), MAYV (BeAr505411) and RRV (T-48). Levels of antibody specific to CHIKV 181/25 observed at 28 days post-vaccination with VLA1553 are in the same range as levels reported > 1 year post-infection (time post-infection ranging from 1.1 year to 24.3 years) in Powers 2023. However, levels measured 6 months post-vaccination are overall lower than those observed after infection and reported in Powers 2023.

Additional testing of samples isolated from CHIKV convalescent subjects in PRNT assays specific to different CHIKV strains is also planned at OHSU. Preliminary data obtained on n=9 samples isolated from convalescent subjects approximately 8 years after natural CHIKV infection indicate that higher neutralizing responses are detected following natural infection as compared to responses detected 1 year after VLA1553 vaccination. Additional analyses are planned on samples isolated from baseline seropositive subjects of study VLA1553-321 and on samples isolated at different time-points after natural infection from children/adolescents of Nicaragua (REC).

Overall conclusion on data related to the cross-neutralization with other lineages and strains

Data generated at UTMB were considered limited in terms of number of tested samples, of number of viral strains analysed (lack of testing against more recently circulating CHIKV isolates and against related arthritogenic alphaviruses), and in terms of the comparability of UTMB's data with data generated by µPRNT or µNT assays on the same set of samples isolated from VLA1553-vaccinated volunteers.

Limitations of the analyses performed at UTMB are partially addressed by data generated at OHSU. Indeed, additional longitudinal samples isolated from VLA1553-vaccinated subjects (up to year 1) have been tested in PRNT assays against different wild-type CHIKV strains of ECSA lineage (including a more recent ECSA isolate from Brazil), against the attenuated CHIKV 181/25 strain (allowing comparison with data generated with the µPRNT assay) and against related alphaviruses (generating data on cross-reactivity against MAYV, ONNV and RRV of VLA1553-induced responses). Further analyses at OHSU in assays specific to other wild-type CHIKV lineages are needed.

Collectively, data generated at UTMB and OHSU indicate that vaccination with VLA1553 induces antibody responses able to neutralize wild-type CHIKV strains from different CHIKV genotypes. Data from UTMB indicate however variable magnitude of titres against the different wild-type CHIKV strains tested. At this stage, this cannot be confirmed by data generated at OHSU as only two wild-type CHIKV strains of ECSA lineage were tested. Additional testing in PRNT assays specific to a West African and an Asian/Caribbean strain is planned at OHSU (REC). Although the level of VLA1553-induced antibodies needed to (cross-)protect against wild-type CHIKV of different lineages is not known, results suggest that a (similar) protective effect against homotypic and heterotypic CHIKV strains/isolates might be expected. Still the protective effect of VLA1553 remains to be demonstrated.

In addition, data generated at OHSU, also indicate that VLA1553-induced responses are able to neutralize *in vitro* also ONNV and MAYV but not RVV. Clinical relevance of these findings in terms of potential protective efficacy of VLA1553 against ONNV and MAYV is not known. Results indeed suggest that some protective effect against ONNV and MAYV might be expected, but this still remains to be demonstrated.

Only limited data are available on acute/convalescent samples from naturally infected subjects tested in comparable assays for neutralizing antibodies against different wild-type CHIKV strains and related alphaviruses. Limited available data indicate that overall, antibody titres induced by VLA1553 are either in the same range or lower than levels induced after natural infection, which sometimes occurred years before sampling. Additional analyses on increased number of well-characterized samples are still needed to enable more solid comparisons of antibody responses elicited after VLA1553 vaccination versus those induced by natural infection (REC).

2.6.5.7. Supportive study(ies)

Study VLA1553-303

Methods

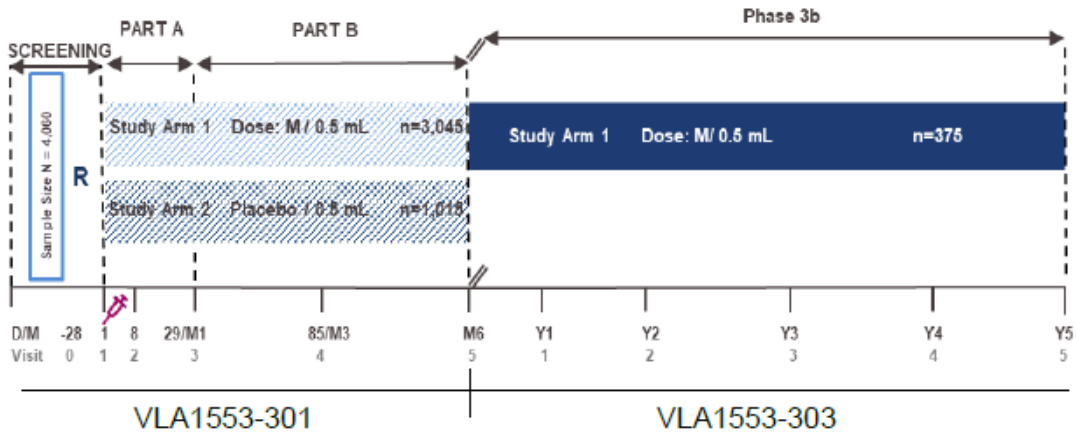
The assessment of the methods is based on on Protocol Version 3.0 dated 23 Dec 2021.

Overall study design

VLA1553-303 is an open-label Phase 3b, single arm clinical study evaluating antibody persistence (up to 5 years post-vaccination) and long-term safety (up to 2 years post-vaccination). This study is the follow-up of a subset of participants included in study VLA1553-301.

The overall study design is displayed in the Figure 20 below.

Figure 20. Open-label Phase 3b study design



In study VLA1553-301, 4,128 participants aged 18 years and above were randomized in a ratio 3:1 to either the final dose of VLA1553 or placebo, and 4,117 were vaccinated. The first enrolled and randomized approximately 500 participants constituted the Immunogenicity subset.

A total of approximately 375 baseline CHIKV seronegative participants of study VLA1553-301 were targeted for enrolment into this study VLA1553-303. Participants from the Immunogenicity subset were first approached to join VLA1553-303. Thereafter, additional participants were approached in a pre-defined manner (under protocol version 2) to achieve the number of participants in study VLA1553-303.

Target participants were of the VLA1553 arm of study VLA1553-301. However, inclusion could occur before unblinding of study VLA1553-301 and placebo participants were included in study VLA1553-303 (n=12). Once study VLA1553-301 was unblinded, the placebo participants of study VLA1553-301 were withdrawn from study VLA1553-303 (prior the Year 1 visit in VLA1553-303).

The CSR specifies that participants of the Immunogenicity subset of study VLA1553-301 recruited at 10 pre-selected sites across the US constituted the initial group of participants to be approached. The Immunogenicity subset of study VLA1553-301 was recruited over 12 pre-selected sites but 2 sites did not participate to study VLA1553-303.

The additional participants to be approached were those recruited by the same 10 sites, according to a pre-defined process. This was possible because immunogenicity samples were collected for all the participants of study VLA1553-301. Participants aged 65 years of age or older were prioritized to be invited to join study VLA1553-303 by way of ascending participant identifiers, followed by participants aged 18 to 64 years of age who were invited to participate in study VLA1553-303, also in ascending participant identifiers order on a study site level. As the recruitment of the Immunogenicity subset was modified during the course of study VLA1553-301, resulting in more younger adults than planned, elderly participants might have been under-represented in study VLA1553-303. Therefore, the process to recruit the additional elderly participants first to ensure more adequate representation of this age category among the study participants is appropriate.

All participants were asked to return to the study site at Month 6 of VLA1553-301 (Visit 0 of VLA1553-303), followed by Year 1, Year 2, Year 3, Year 4 and Year 5 post-vaccination for immunogenicity

sampling. Timepoints Year 1 to Year 5 correspond to years after vaccination administered in study VLA1553-301. Similarly, when referring to baseline, it should be understood baseline in study VLA1553-301. The CSR specified that, for a large subset of the Whole Sample (WS) population (i.e. participants included in study VLA1553-303, as per inclusion/exclusion criteria, and had the 1 year visit [Visit 1]), Visit 0 could be performed as a visit combined with Visit 5 (Day 180) of study VLA1553-301. Participants who had not joined the VLA1553-303 study at Day 180 of study VLA1553-301 had their Visit 0 at a later date, performed as a separate study visit.

It was initially specified in the protocol that, if participants fall below titres <150 µPRNT50 during immunogenicity sampling, they will have an end of study visit at the next scheduled visit. Therefore, participants with antibody titres <150 µPRNT50 at the Year 1 visit were excluded, based on the protocol version that was in effect at the time. This was modified in protocol version 4.0., and participants are to be excluded if the antibody titre fall below the threshold of µPRNT50 40 instead of 150. As the clinical relevance of the threshold of 150 µPRNT50 is not known, it is valuable to follow the kinetics and magnitude of the immune response over time, even if below 150 µPRNT50.

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 enrolment to study completion unless prematurely discontinued.

- The study was split into 5 parts which were planned to be reported separately: Part A: Visit 0 to Visit 1 (Year 1); the results are presented in this current Part A Clinical Study Report.
- Part B (Visit 2, Year 2) to Part E (Visit 5, Year 5); the results will be presented as annual updates of the current Clinical Study Report.
- The final report including all data from preceding parts will be prepared after completion of the study.

Study Participants

Participants had to be baseline CHIKV seronegative negative in study VLA1553-301 (≤40 µPRNT50) to be included in this study. As discussed above, the serostatus of participants within the 20-40 µPRNT50 stratum is uncertain, and to ensure the most correct classification of the baseline CHIKV seronegative participants, the threshold of 20 µPRNT50 should be used. The PP population of VLA1553-303 included all participants with baseline CHIKV antibodies ≤40 µPRNT50 as per inclusion criteria. As VLA1553-303 is conducted in the US, a low number of baseline CHIKV false seropositive participants are expected, hence results are not expected to be biased due to misclassification of the participants by baseline serostatus. Only 5 participants had baseline antibody titres between 20 and 40 µPRNT50. In addition, sensitivity analyses were performed by using the threshold of 20 µPRNT50 (sPP1 population). Results are consistent with those obtained in the PP population (see below).

Participants had to have been sampled for immunogenicity at baseline and at one other timepoint post-baseline in VLA1553-301. A complete follow-up of the immune response would have been more valuable, i.e. selection of participants with samples at each timepoint of study VLA1553-301. This criterion was included in Protocol version 1, but was amended during the study course, probably due difficulties in recruiting participants.

Treatments

Participants of VLA1553-303 study were vaccinated with VLA1553 at Day 1 in the VLA1553-301 study.

Refer to section 2.6.5.3.

Concomitant therapies

The following medications were 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.

Study assessments

Assessment of immunogenicity

Neutralizing antibodies are measured by the same µPRNT as used in VLA1553-301 study. Measurements have to be performed on samples collected after Year 1, 2, 3, 4 and 5.

The (seroresponse) threshold likely to predict protection was defined as previously, i.e. achieving a CHIKV antibody level of µPRNT50 ≥150.

In contrast to the secondary analyses performed on the PP population of study VLA1553-301, seroconversion was similar for both baseline negative participants and baseline positive participants (defined as µPRNT50 >40) and defined as >4-fold increase of µPRNT50 compared to baseline. This seroconversion definition is thus justified. For part B, baseline values between 20 and 40 were not imputed and were reported as measured. Data below the µPRNT50 of 20 (LLOQ) were imputed with 10. Of note, for part A, data below the µPRNT50 of 40 were imputed with 10. No rationale was provided for this difference in approach.

Objectives and endpoints

Immunogenicity

The primary objective is to assess the persistence of VLA1553-induced antibodies up to 5 years post-vaccination. The primary endpoint is the proportion of participants with antibody titres equal or above the threshold likely to predict protection (µPRNT50 ≥150).

There is no secondary objective defined for immunogenicity but secondary endpoints are defined. Immune responses are to be characterized in terms of GMT, seroconversion, fold increase compared to baseline, as in study VLA1553-301.

Sample size

No formal sample size calculation was performed. However, with a sample size of 375, two-sided exact (Clopper-Pearson) 95%-confidence intervals for an actual seroresponse rate (SRR) of 60% will have a width of 10.2%. Confidence intervals for an SRR of 80% will have a width of 8.3%.

Randomisation and blinding (masking)

Not applicable

Statistical methods

Analysis populations

All analyses of immunogenicity data will be performed primarily on the Per Protocol population and secondarily on the Whole Sample population.

The **whole sample (WS) population** contains participants included in study VLA1553-303, as per inclusion/exclusion criteria, and had the 1 year visit (Visit 1).

The **Per Protocol (PP) population** contains participants included in WS population, have a yearly visit antibody titre measurement available for the timepoint analysed and have no major protocol deviations during the study period being analysed that could impact the immune response.

Examples of such protocol deviations that could lead from removal from the PP population are provided in the protocol (medications not permitted) and/or SAP. These are overall comparable to those of study VLA1553-301. Protocol deviations can apply for one/several parts of the study only.

Participants could be excluded from the PP analysis of immunogenicity if blood samples were not taken within pre-defined window visit. Window visits are approximately 3 months around Year 1, and 1 ½ month for the subsequent yearly timepoint. Three months (84 days) is deemed wide. Visit window length was increased during the course of the study, linked to the difficulties in recruiting participants and keep the Visit 1 within the pre-defined visit window. Analyses stratified by time window will be performed (sPP3, see below), which is acknowledged. Windows for subsequent visits are deemed appropriate.

Three sensitivity PP (sPP) analysis sets were also defined for additional sensitivity analyses of immunogenicity data. **sPP1 population** is defined similarly than the PP population but with the baseline serostatus defined as negative if the µPRNT50 is <20 for sPP1 population instead of ≤40 for PP population, as in the primary analyses of studies VLA1553-301 and VLA1553-302. The **sPP2 population** is also defined using the same approach as the PP population for the baseline serostatus defined (negative if the µPRNT50 is ≤40) but also includes participants with out-of-window Visit 1 if no other major protocol deviations. The applicant explained that, because of delay in unblinding of study VLA1553-301, a high number of participants were not able to attend the Visit 1 within the pre-defined visit window. This population will then be stratified according to their time window around vaccination (i.e. ± 4 weeks, ± 8 weeks, ± 12 weeks, and ± 16 weeks). **sPP3 population** includes participants (n=20) for which there was temperature excursion for the IMP (instead as being excluded because of this major protocol deviation).

Methods of analysis

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

Immunogenicity analyses

No formal hypothesis testing was planned, and no adjustments were made for multiplicity in this study.

The primary immunogenicity analysis will analyse the observed proportion of participants with a CHIKV antibody level µPRNT50 ≥ 150 for baseline negative subjects over time. The denominator for the percentage will be the number of baseline CHIKV antibody negative subjects with non-missing neutralizing titre values at each timepoint. Two-sided exact (Clopper-Pearson) 95% CIs for the seroresponse rate (SRR) will be presented. An exact binomial test for the null-hypothesis H0: SPR ≤ 70% against the alternative H1: SPR > 70% with a one-sided significance level of 2.5% will also be presented. This will be presented for the PP population (SAP section 14.5.2.1).

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 titres will be evaluated by summary statistics of the GMTs. Annual decline rate of antibody titres will be estimated using log-linear regression.

All immunogenicity analyses will be repeated by age stratum.

Results

Participant flow

A total of 393 participants from study VLA1553-301 were invited to participate into the study, including 12 placebo participants who were invited prior to unblinding of that study and subsequently removed from the study after unblinding of study VLA1553-301.

Of the remaining 381 invited participants, 363 agreed and constituted the WS population. Main reasons for non-inclusion in study VLA1553-303 were lost to follow-up (13 participants), meeting non-inclusion criteria (5 participants), and withdrawal by participant (1 participant).

All 363 participants completed the Year 1 Visit (Part A of the study) while 317 (87.3%) completed the Year 2 Visit (Part B of the trial) and 316 (87.1%) were ongoing after Visit 2 Year 2. Main reasons for trial discontinuation were lost to follow-up (27/47 participants, 57.4%) and withdrawal by participant (14/47 participants, 29.8%).

Of note, 4 participants were identified as seronegative (i.e., with titres below µPRNT50 ≤40), 2 participants from the beginning of the trial who should have been discontinued from the trial, and 2 participants at Year 2 who will be discontinued in Parts C and D.

Table 23. A summary of the populations analyzed is provided in the Table below.

Populations of analysis	18-64 yoa	≥65 yoa	Total
Total participants invited			393
WS population	310	53	363
PP population – Part A (Year 1)	157	27	184
PP population – Part B (Year 2)	290	49	339
sPP1 population	153	26	179
sPP2 population	289	49	338
sPP3 population	162	30	192

The initial target (Protocol version 1) of 260 younger adults and 115 older adults was not reached for the elderly participants. These targets were removed from Protocol version 3. The number of elderly participants actually included in the PP population is limited. It would be difficult to interpret the results over time, i.e. distinguish a trend for a different kinetics between age category versus inherent variability due to the limited sample size. It might be that waning of neutralizing antibody is more rapid for elderly participants versus younger adults.

The percentage of participants excluded from the PP population Part A is extremely high (approximately half of the participants from the WS population were excluded to constitute the PP population Part A). All exclusion (n=179) were related to major protocol deviations. There were 169 major protocol deviations classified as 'Visit window', representing 94% (169/179) of the major protocol deviations leading to exclusion from the PP population. The allowed time window for the Year 1 visit was +/- 12 weeks. There were 19 major protocol deviations 'investigational product'. These are temperature excursion of IMP storage captured during study VLA1553-301. One participant had major deviation captured during the VLA1553-301 study for temperature excursion of IMP (storage). It was classified as major protocol deviation with missing importance for a temperature excursion. There were 2 major protocol deviations 'lab/endpoint data' and 1 'exclusion criteria'.

A total of 20 participants of study VLA1553-303 have been vaccinated with a vaccine that had temperature excursion before being administered in study VLA1553-301. However, only 8 additional participants were included in sPP3 population versus PP population, as other major protocol deviations were identified for the remaining participants.

Difference between number included in the PP population Part A and sPP2 population is high, reflecting the high number of participants who had a visit out of the pre-defined window, i.e. 169/363 of the WS population.

The number of participants included in sPP2 population is 338, thus including 154 additional participants with out-of-window Visit 1 when compared to the PP population. This is lower than the 169 participants of the WS population presenting time window deviations, as other participants were not included in the sPP2 population because of other major protocol deviations.

For Part B, although the PP population consisted in 339 participants according to the CSR, there were 42 missing samples or missing titre and 21 samples out of window. It is not clear why these events were not classified as major protocol deviations leading to exclusion from the PP population. In the end, Year 2 data were obtained on 276 participants, 234 aged 18-64 years and 42 aged 65 years and above.

Recruitment

The study began on 02 April 2021 (first participant enrolled). The study is ongoing. Part A data cut-off date is 13 May 2022. Part B data cut-off date is 12 October 2023.

Conduct of the study

Protocol amendments

There were 2 protocol amendments to the study up to now with updates mainly related to participant recruitment.

The Protocol version 1 dated 05 Jan 2021 was amended. Protocol version 2 dated 03 Sept 2021. Under this protocol, recruitment of participants from study VLA1553-301 not included in the Immunogenicity subset could be done. Inclusion of participants without a complete immunogenicity sample follow-up in study VLA1553-301 could be done if sampling at baseline and at another timepoint post-baseline was performed. Visit windows were extended (from ± 28 days to 84 [Year 1] or 42 days [Year 2 to Year 5]). In addition, the defined threshold likely to predict protection was changed from µPRNT50 ≥50 to µPRNT50 ≥150.

Protocol version 3, dated 23 Dec 2021, was implemented to allow approaching additional participants in a pre-defined manner and ensure more adequate representation of elderly participants, as explained in the section on the study design. The total number of participants targeted did not change but target numbers by age category was removed. Visit 0 of study VLA1553-303 may be combined with Visit 5 of study VLA1553-301, or alternatively Visit 0 and Visit 1 of study VLA1553-303 may be combined. Baseline seronegative status was defined as µPRNT ≤40 instead of ≤20. Seroconversion definition was defined as >4-fold increase of µPRNT50 compared to baseline, for both baseline seronegative and seropositive participants (instead of 2 different definitions). No baseline seropositive participants were to be included in VLA1553-303, as per inclusion criteria.

Protocol deviations

The same approach regarding definitions of important/not-important and major/minor PDs was used as in VLA1553-301.

A total of 188/363 (51.8%) participants with major important protocol deviations were identified. Out of the 363 major protocol deviations 181 (49.9%) participants presented time window deviations and 19 (5.2%) participants presented IMP deviations (temperature excursion before administration in study VLA1553-301).

One of the 363 (0.3%) participants presented important minor protocol deviation ("Assessment Safety"), while not important minor protocol deviations were observed in 8/363 (2.2%) participants, all into the category "Informed Consent".

Refer above for the reasons for exclusions from the PP population.

Baseline data

Demographic characteristics were provided for the WS and PP populations but not for sPP1, SPP2 and sPP3 populations. This is deemed acceptable since sPP1 and sPP3 populations include very similar numbers of participants than the PP population, and sPP2 population includes similar number of participants than the WS population (for both age category).

Baseline demographic characteristics are those at VLA1553-301 study entry.

WS population versus PP populations Part A and Part B (study VLA1553-303)

There were no major differences in the demographic characteristics between the WS population and the PP population Part A and Part B, except for the sex distribution in WS population versus PP population Part A.

There were 57.0 % and 51.1% of female participants included in the WS population and in the PP population Part A, respectively. In the PP population Part B, 57.2% of the participants were females. Median (Q1-Q3) age of the participants were 49.0 years (37.0-59.0 years), 46.0 years (35.5-57.5 years) and 48.0 (36.0-58.0) in the WS population, in the PP population Part A and in the PP population Part B, respectively. Percentages of elderly participants were similar in all 3 analysis populations, i.e.

14.6%, 14.7% and 14.5% for the WS population, PP population Part A and PP population Part B, respectively. Median body mass index (Q1-Q3) were 29.3 kg/m^2 (25.4-34.6 kg/m2), 28.8 kg/m^2 (25.5-34.9 kg/m^2) and 29.05 (25.20-34.50) in the WS population, in the PP population Part A and in the PP population Part B, respectively.

PP populations of study VLA1553-303 versus PP population of study VLA1553-301

There were no major differences in the demographic characteristics between the PP population Part A and Part B of study VLA1553-303 and the PP population of study VLA1553-301, except for the age distribution (18-64 years and ≥65 years).

There were 59.1% female participants included in the PP population of study VLA153-301. Median age for the PP population in study VLA1553-301 was 49.5 years (Q1-Q3: 36.0-64.0). There was 22.7% of elderly participants in the PP population of study VLA1553-301, i.e. a higher percentage than in the PP populations of study VLA1553-303. Median body mass index (Q1-Q3) was 29.5 kg/m^2 (Q1-Q3: 25.6-34.8) for the PP population in study VLA1553-301.

There were no differences in terms of neutralizing antibody responses observed between age category in study VLA1553-301. The difference in proportion of elderly participants is thus not expected to impact result analyses. The limited number of elderly participants in study VLA1553-303 might however influence the results over time (> 6 months post-vaccination).

PP population Part A and Part B of study VLA1553-303

There was an imbalance in terms of the percentages of female participants in the PP population Part A between age category (49.7% and 59.3%, respectively in the 18-64 years and ≥65 years categories). Proportions were comparable between age categories in the PP population Part B (57.12% and 57.1% of female participants, respectively in the 18-64 years and ≥65 years categories).

Median (Q1-Q3) was 44.0 years (34.0-54.0) and 68.0 years (66.0-69.0) in the 18-64 years and ≥65 years categories respectively in the PP population Part A. In the PP population Part B, median (Q1-Q3) was 45.0 years (34.0-54.0) and 68.0 years (66.0-70.0) in the 18-64 years and ≥65 years categories respectively.

BMIs were comparable between age categories, for both PP populations.

Outcomes and estimation

Primary immunogenicity endpoint results

Proportion of participants with antibody titres ≥150 µPRNT50 up to Year 2

A summary of the proportion of participants with antibody titres ≥150 µPRNT50 up to Year 2 for the PP population is provided in Table 24.

Table 24. Proportion of baseline seronegative participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) by visit and age strata (PP population)

Time Point [n] Statistic	18 to 64 Years (Stratum A) (N=290)	≥65 Years (Stratum B) (N=49)	Total (N=339)
VLA1553-301 Visit 1 - Day 1	290	49	339
Participants with Seroresponse [n (%)][a]	0	0	0
VLA1553-301 Visit 3 - Day 29	240	39	279
Participants with Seroresponse [n (%)][a]	238 (99.2)	39 (100)	277 (99.3)
95% CI[b]	97.0, 99.9	91.0, 100.0	97.4, 99.9
VLA1553-301 Visit 5 - Day 180	279	48	327
Participants with Seroresponse [n (%)][a]	274 (98.2)	47 (97.9)	321 (98.2)
95% CI[b]	95.9, 99.4	88.9, 99.9	96.0, 99.3
VLA1553-303 Visit 1 - Year 1	157	27	184
Participants with Seroresponse [n (%)][a]	156 (99.4)	27 (100)	183 (99.5)
95% CI[b]	96.5, 100.0	87.2, 100.0	97.0, 100.0
VLA1553-303 Visit 2 - Year 2	234	42	276
Participants with Seroresponse [n (%)][a]	227 (97.0)	41 (97.6)	268 (97.1)
95% CI[b]	93.9, 98.8	87.4, 99.9	94.4, 98.7

CHIKV=chikungunya virus; CI=confidence interval; µPRNT$_{50}$=50% plaque reduction in a micro plaque reduction neutralization test; n=number of participants; SAP=statistical analysis plan.

a Number of participants and percentages were based on the number of baseline seronegative participants with non-missing titers at the specified time point.

b Two-sided 95% exact (Clopper-Pearson) CIs presented.

Note: Seroresponse was defined as µPRNT$_{50}$ ≥150 for baseline negative participants. Participants in the Per Protocol population were CHIKV-seronegative at baseline (µPRNT$_{50}$ titer ≤40), had no major protocol deviation, and had at

Table 25 shows a summary of the proportion of participants with antibody titres ≥150 µPRNT50 at Year 1 for the sPP1 population. The applicant did not perform analyses in the sPP1 population for Year 2 since Year 1 analyses did not show any impact on results. This also applies to analysis for sPP2 and sPP3 populations. Since only 5 participants had CHIKV baseline antibody titres between 20 and 40 µPRNT50, since most of the participants presenting time window deviations was observed at Year 1, and since only 8 additional participants were included in sPP3 population versus PP population Part A, this is deemed acceptable.

Table 25. Proportion of baseline seronegative participants with antibody titres post-vaccination equal or above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) by visit and age strata (sPP1 population) (Table 12, CSR)

Time Point [n] Statistic	18 to 64 Years (Stratum A) (N=153)	≥65 Years (Stratum B) (N=26)	Total (N=179)
VLA1553-301 Visit 3 - Day 29	140	23	163
Participants with Seroresponse [n (%)][a]	138 (98.6)	23 (100)	161 (98.8)
95% CI[b]	94.9, 99.8	85.2, 100.0	95.6, 99.9
p-value[c]	<0.0001	0.0003	<0.0001
VLA1553-301 Visit 5 - Day 180	146	26	172
Participants with Seroresponse [n (%)][a]	146 (100)	25 (96.2)	171 (99.4)
95% CI[b]	97.5, 100.0	80.4, 99.9	96.8, 100.0
p-value[c]	<0.0001	0.0011	<0.0001
VLA1553-303 Visit 1 - Year 1	153	26	179
Participants with Seroresponse [n (%)][a]	152 (99.3)	26 (100)	178 (99.4)
95% CI[b]	96.4, 100.0	86.8, 100.0	96.9, 100.0
p-value[c]	<0.0001	<0.0001	<0.0001

CHIKV=chikungunya virus; CI=confidence interval; µPRNT$_{50}$=50% plaque reduction in a micro plaque reduction neutralization test; n=number of participants; SRR=seroresponse rate.
a Number of participants and percentages were based on the number of baseline seronegative participants with non-missing titers at the specified time point.
b Two-sided 95% exact (Clopper-Pearson) CIs presented.
c P-value from an exact binomial test for the null-hypothesis H_0: SRR ≤70% against the alternative H_1: SRR >70% with a one-sided significance level of 2.5%.
Note: Seroresponse was defined as µPRNT$_{50}$ ≥150 for baseline negative participants. Baseline positive participants were not included in this summary. This sensitivity analysis was based on the definition of serostatus being negative if µPRNT$_{50}$ <20 and positive if µPRNT$_{50}$ ≥20. Samples taken on or prior to Day 1 were included in VLA1553-301 Visit 1 per the baseline definition.
Source: Table 14.2.6.1.1 and Listing 16.2.5.1

Proportion of participants of the PP population with µPRNT50 ≥150 at Day 29 and Day 180 post-vaccination for the participants of study VLA1553-303 were of 99.3% (277/279) (95% CI: 97.4-99.9) and 98.2% (321/327) (95% CI:9.60-99.3), respectively. Results are consistent with those of study VLA1553-301 (PP population), which were of 98.9% (263/266) (95% CI: 96.7-99.8) and 96.3% (233/242) (95% CI: 93.1-98.3) at Day 29 and Day 180, respectively.

At 1 year post-vaccination, 183/184 (99.5%) participants of the PP population had a µPRNT50 ≥150 (95% CI: 97.0-100.0). Proportions were comparable between age categories, i.e. 99.4% (156/157) (95% CI: 96.5-100.0) and 100% (27/27) (95% CI: 87.2-100.0) for the younger and older adults, respectively. At year 2 post-vaccination, 268/276 (97.1%) participants of the PP population had a µPRNT50 ≥150 (95% CI: 94.4-98.7). Proportions were comparable between age categories, i.e. 97.0%

(227/234) (95% CI: 93.9-98.8) and 97.6% (41/42) (95% CI: 87.4-99.9) for the younger and older adults, respectively.

Year 1 results obtained in the sPP1 population, i.e. when baseline negative participants were defined as per µPRNT50 ≤20, as in study VLA1553-301, were similar with a proportion of participants with µPRNT50 ≥150 of 99.4% (95% CI: 96.9-100.0). Consistent results were expected since numbers of participants include in both analysis populations are closed (184 and 179 in PP population Part A and sPP1 population, respectively). It is also deemed acceptable to include the results obtained in the PP populations in the SmPC, despite the use of the cut-off of <40 µPRNT50 for defining CHIKV seronegative.

Year 1 results obtained in the SPP2 population were also similar and no difference was observed when the analysis was stratified by the length of out-of-window (ranging from 96.3% to 100%). Number of elderly participants by out-of-window lengths category were too small to be assessed.

Results obtained in sPP3 population and WS population were also consistent with those obtained in the PP population, i.e. with a proportion of participants with µPRNT50 ≥150 at Year 1 of 99.5% (95% CI: 97.1-100.0) for the sPP3 population and of 98.9% (95% CI: 97.2-99.7) for the WS population, and at Year 2 of 96.8% (95% CI: 94.3-98.5) for the WS population.

Secondary immunogenicity endpoint results

Immune response as measured by CHIKV-specific neutralizing antibody titres up to Year 2 post-vaccination

A summary of GMTs for CHIKV-specific neutralizing antibody by visit and age category for the PP analysis set is provided in Table 33 and illustrated in Figure 23 below.

Figure 24 presents reverse cumulative distribution curves of CHIKV-specific neutralizing antibodies from Day 29 to Year 2.

Table 26. GMTs and annual rate of change in CHIKV-specific neutralizing antibody titre by visit and age strata (PP population)

Time Point [n] Statistic	18 to 64 Years (Stratum A) (N=290)	≥65 Years (Stratum B) (N=49)	Total (N=339)
VLA1553-301 Visit 1 - Day 1			
n*	290	49	339
Geometric Mean	10.3	10.3	10.3
95% CI	10.09, 10.42	9.88, 10.73	10.11, 10.41
Geometric std	1.15	1.15	1.15
Median	10.0	10.0	10.0
Q1, Q3	10.0, 10.0	10.0, 10.0	10.0, 10.0
VLA1553-301 Visit 3 - Day 29			

n[a]	240	39	279
Geometric Mean	3750.1	3836.1	3762.0
95% CI	3339.08, 4211.62	2859.51, 5146.23	3379.26, 4188.03
Geometric std	2.49	2.48	2.49
Median	3926.5	4020.0	3940.0
Q1, Q3	2336.0, 6119.5	2401.0, 6862.0	2345.0, 6258.0
VLA1553-301 Visit 5 - Day 180			
n[a]	279	48	327
Geometric Mean	1039.1	910.7	1019.1
95% CI	921.89, 1171.11	738.89, 1122.41	916.31, 1133.50
Geometric std	2.76	2.05	2.66
Median	1115.0	972.0	1091.0
Q1, Q3	558.0, 2148.0	622.0, 1402.0	577.0, 1961.0
VLA1553-303 Visit 1 - Year 1			
n[a]	157	27	184
Geometric Mean	1056.6	1153.2	1070.3
95% CI	912.02, 1224.17	808.90, 1643.92	935.35, 1224.64
Geometric std	2.54	2.45	2.53
Median	1061.0	1053.0	1056.5
Q1, Q3	585.0, 1912.0	465.0, 2906.0	576.5, 1956.0
VLA1553-303 Visit 2 - Year 2			
n[a]	234	42	276
Geometric Mean	782.3	1061.6	819.5
95% CI	690.69, 886.07	812.75, 1386.66	731.74, 917.81
Geometric std	2.63	2.36	2.60
Median	845.0	1072.0	900.5
Q1, Q3	399.0, 1543.0	588.0, 1738.0	424.5, 1606.5

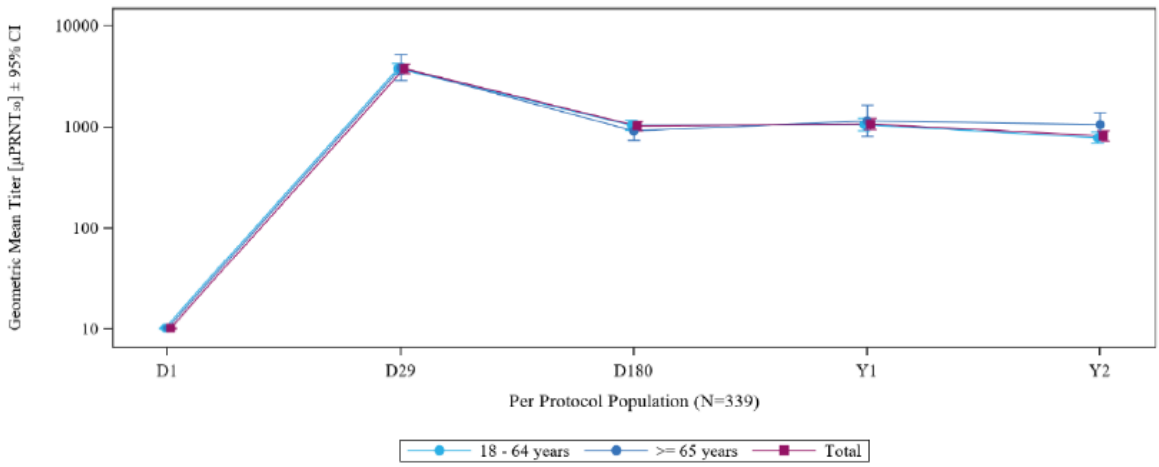
CHIKV=chikungunya virus; CI=confidence interval; GMT=geometric mean titer; $\mu PRNT_{50}$=50% plaque reduction in a micro plaque reduction neutralization test; n=number of participants; Q=quartile; SAP=statistical analysis plan; std=standard deviation.

a Number of participants with non-missing titers at the specified time point.

Note: Participants in the Per Protocol population were CHIKV-seronegative at baseline ($\mu PRNT_{50} \leq 40$), had no major protocol deviation, and had at least one evaluable post-baseline titer measurements of either Day 29, Day 85, or Day 180 (within SAP visit windows for trial VLA1553-301; i.e. within per protocol trial day as defined in Section 11.2 of the SAP). Samples taken on or prior to Day 1 were included in VLA1553-301

Visit 1 per the baseline definition. Any titer falling under the limit of quantification was imputed to 10. If participants discontinued from the trial because their titers had fallen below $\mu PRNT_{50} \leq 40$, their titers were imputed to 10 at each of the scheduled visits post-discontinuation.

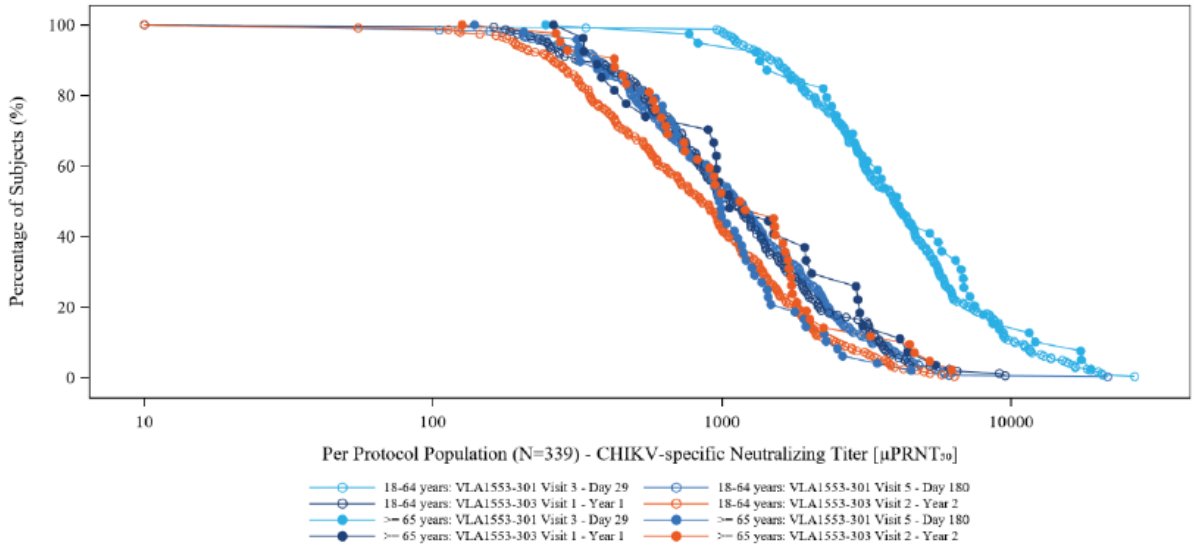
Source: Table 14.2.2.1 and Listing 16.2.5.1



Age Strata	D1	D29	D180	Y1	Y2
18 - 64 years	290	240	279	157	234
>= 65 years	49	39	48	27	42
Total	339	279	327	184	276

CHIKV=chikungunya virus; CI=confidence interval; D1=VLA1553-301 Visit 1 - Day 1; D29=VLA1553-301 Visit 3 - Day 29; D180=VLA1553-301 Visit 5 - Day 180; GMT=geometric mean titers; SAP=statistical analysis plan; Y1=VLA1553-303 Visit 1 - Year 1; Y2=VLA1553-303 Visit 2 - Year 2.
Note: Counts below the chart show the number of participants in each age strata and overall at the time point. Samples taken outside of SAP visit windows for trial VLA1553-301 (i.e. within per protocol trial day as defined in Section 11.2 of the SAP) or associated with events likely to interfere with immune response were not included in the analysis. If participants discontinued from the trial because their titers had fallen below µPRNT$_{50}$ ≤40, their titers were imputed to 10 at each of the scheduled visits post-discontinuation.
Source: Table 14.2.2.1

Figure 21. Line plot of CHIKV-specific neutralizing antibody titres (GMT) by visit and age strata, using logarithmic scale (PP population)



CHIKV=chikungunya virus; µPRNT$_{50}$=50% plaque reduction in a micro plaque reduction neutralization test.
Note: Samples taken outside of visit window or associated with events likely to interfere with immune response were not included in the analysis. If participants discontinued from the trial because their titers had fallen below µPRNT$_{50}$ ≤40, their titers were imputed to 10 at each of the scheduled visits post-discontinuation.
Source: Listing 16.2.5.1

Figure 22. Reverse cumulative distribution curves of CHIKV-specific neutralizing antibodies by visit and aga strata (PP population)

Baseline GMT was 10.3 (95% CI: 10.11-10.41).

GMTs at Day 29 and Day 180 post-vaccination were of 3762.0 (95% CI: 3379.26-4188.03) and 1019.1 (95% CI:916.31-1133.50), respectively. Day 29 results are consistent with those of study VLA1553-301 (PP population), with GMT of 3361.6 (95% CI: 2993.8-3774.4). Day 180 results were higher than in study VLA1553-301 (GMT of 752.1 [95% CI: 665.9-849.5]). 95% CIs do not overlap. The higher GMT value observed at Day 180 in the PP population of study VLA1553-303 versus in the PP population of study VLA1553-301 might be due to inter-study variability and to the variability of the antibody measurement. The clinical relevance of this observation is not known.

Antibody titres were sustained up to Year 2 post-vaccination with GMT of 1070.3 (95% CI: 935.35-1224.64) at Year 1 and of 819.5 (95% CI: 731.74-917.81) at Year 2.

At Year 1 and Year 2, results were similar for younger and older adults as illustrated with the reverse cumulative distribution curves.

However at Year 2, a trend for decreased GMT was observed in the 18-64 years-of-age category, as compared to Year 1, with Year 2-GMT of 782.3 (95% CI: 690.69-886.07). This is consistent with the slightly lower number of participants with antibody titre ≥150 µPRNT50, i.e. 227/234, observed ta Year 2 as compared to previous timepoints. Year 2-GMT in the >65 years age category were of 1061.6 (95% CI: 812.75-1386.66), i.e. comparable to Year 1-GMT.

Results were similar for sPP1 from baseline to Year 1 post-vaccination. GMT at baseline was 10 (95% CI: non-calculable, the maximum value was 10). Day 29 GMT was 3844.2 (95% CI: 3293.39-4487.12) and Day 180-GMT 1264.4 (1116.05-1432.53). Year 1-GMT was of 1059.2 (95% CI: 922.80-1215.71).

Results up to Year 1 were similar for sPP2 and no differences were observed when the analysis was stratified by length of out-of-windows (Table 27)

Table 27. GMTs by visit and out-of-window Visit 1 (SPP2 population)

Out-of-window length*	N participants included	Day 29-GMT (95% CI)	Day 180-GMT (95% CI)	Year 1-GMT (95% CI)
±4 weeks	N= 40	3394.4 (2740.21-4205.49)	731.9 (554.69-965.76)	1049.6 (831.64-1324.80)
±8 weeks	N= 60	4116.3 (3369.79-5028.17)	778.3 (614.98-985.05)	1121.0 (929.56-1351.79)
±12 weeks	N= 10	3412.1 (2568.38-4533.00)	1234.0 (705.65-2158.00)	958.2 (425.31-2158.65)
±16 weeks	N=2	-	-	-

Results were similar for sPP3 with Year 1-GMT of 1035.0 (95% CI: 906.90-1181.20). Year 1- GMT was of 1055.5 (95% CI: 958.98-1161.71) for the WS population.

Altogether, results indicate a sustained humoral immune response up to 2 year post-vaccination. A trend for a decline is observed in the 18-64 years age category. Antibody results observed in study VLA1553-303 confirm the findings of study VLA1553-101 and indicate that the single dose regimen

induces a sustained antibody response up to 1 year post-vaccination. However, whether a booster dose would be needed at a certain time is not known. Based on the results of the ongoing study VLA1553-303, the Applicant would either extend the follow-up beyond 5 years post-first vaccination or consider the conduct of a booster study (if a drop in the seroresponse rate is observed).

Study VLA1553-321

Methods

This assessment is based on Protocol Version 6.0 (08-Nov-2021).

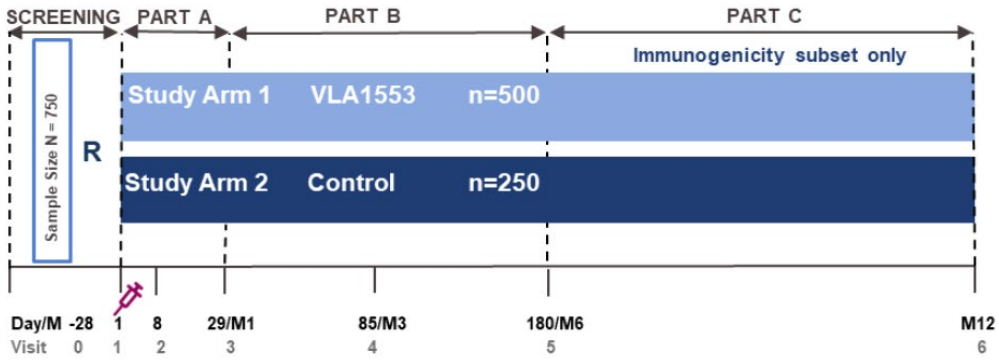
Overall study design

VLA1553-321 is an ongoing randomized, double-blinded, multicentre Phase 3 clinical study evaluating the safety and immunogenicity of the live-attenuated chikungunya virus vaccine candidate VLA1553 at the final selected dose (target 1x10E4 TCID50 per 0.5 mL) in adolescents aged 12 years to <18 years. The study was to be conducted at approximately 5-10 study sites in Brazil, which is an endemic country for CHIKV.

All participants received a single intramuscular administration of VLA1553 or of placebo (PBS) in the deltoid region of the arm.

The overall study design is displayed in the figure below.

Figure 23. Study design



In addition, as specified in the CSR, this study, conducted in an endemic setting, aimed to evaluate the effect of VLA1553 vaccination according to pre-existing antibodies against other alpha- and arboviruses.

Day 29 results from study VLA1553-321 were submitted in support of the MAA for adult indication. This was because adult studies were conducted in the US (a non-endemic area for CHIKV), while VLA1553-321 is conducted in an endemic area (Brazil) and therefore provides safety and immunogenicity results not only for CHIKV baseline negative participants but also for CHIKV baseline seropositive participants. The extension of indication to adolescents could be submitted after initial authorisation of VLA1553. Some concerns were identified during the assessment and raised as REC. It is expected that the Applicant addresses these RECs if the extension of indication to adolescents is sought or at the submission of the final CSR.

Approximately 750 participants were to be randomized in a 2:1 ratio to VLA1553 (n=500) or control group (n=250). The randomisation to study arm was stratified by CHIKV ELISA baseline serostatus, with 20% seropositive and 80% seronegative for CHIKV as targets for enrolment. The serostatus at baseline was to be determined at screening visit in a central diagnostic laboratory using a qualitative ELISA assay. The ELISA was to be used in order to drive the enrolment of 20% seropositive subjects.

Approximately 385 participants were to be randomized to the immunogenicity subset. Thereof, approximately 75 participants constituted the viraemia subset. It is specified that all sentinel participants of Cohort I (constituted of adolescents from 15-17 years, n=30) and Cohort II (constituted of adolescents from 12-14 years, n=30) were to be allocated to the immunogenicity subset.

Participants were followed up for approximately 6 months following vaccination, and approximately 1 year in the immunogenicity subset.

The study design is outlined in Table 28.

Table 28. Subject groups and study subsets

Study Groups	Treatment	Number of subjects (n)	Immunogenicity (Viremia) Subset (n)
Study Arm 1	**VLA1553[a]**	**500**	**335 (50)**
Seropositive by ELISA		*100*	*67 (10)*
Seronegative by ELISA		*400*	*268 (40)*
Study Arm 2	**Control**	**250**	**50 (25)**
Seropositive by ELISA		*50*	*10 (5)*
Seronegative by ELISA		*200*	*40 (20)*
Total N		**750**	**385 (75)**

Immunogenicity blood samples were taken from all the participants at baseline (Day 1), 7 days (Day 8), 28 days (Day 29), Month 3 (Day 85) and Month 6 (Day 180) post-vaccination.

Baseline µPRNT serostatus (Day 1) was assessed for all participants for stratification. For the other timepoints, testings were done in the immunogenicity subset only. An immunogenicity sample at Month 12 (Day 365) was also planned for participants from the immunogenicity subset.

Participants in the viraemia subset had viraemia samples collected at Day 1, Day 8 and Day 29. The sample collected at Day 29 was to be analysed only if sample of Day 8 is positive.

If VLA1553 is proven to be safe and effective, it will be offered to the control group at the end of the study if approved by Brazilian regulatory authorities.

Study Participants

A total of approximately 750 generally healthy participants, 12-17 years of age, were targeted for enrolment into this study. Adolescents were recruited in 10 sites in Brazil.

Participants with well-controlled (defined as stable and on therapy for the past 6 months) chronic conditions such as hypertension, type 2 diabetes mellitus, or hyperlipidemia were allowed to be enrolled.

Participants immunocompromised due to medical condition or due to immunosuppressive treatments were to be excluded. History of immune-mediated or clinically relevant arthritis or arthralgia were also part of the key exclusion criteria, as well as administration of an investigational CHIKV vaccine, of any inactivated vaccine within 2 weeks before vaccination, or of any live vaccine within 4 weeks before vaccination.

Participants could have been previously infected by CHIKV or not. Participant with unresolved symptoms attributed to a previous CHIKV infection or with ongoing or recent CHIKV infection (as defined by IgM+/IgG- ELISA) were to be excluded.

Treatments

All participants received a single intramuscular vaccination in the deltoid region of the arm of VLA1553 or placebo (2:1 ratio).

Two batches of VLA1553 were used. Batch number 2006300061 had an actual potency of 1.9 x 10E4 TCID50/0.5 mL (4.3 log 10 TCID50/0.5 mL). Batch number 2109170078 had an actual potency of 1.3 x 10E4 TCID50/0.5 mL (4.1 log 10 TCID50/0.5 mL).

The potency of the two batches are in the commercial release specification range (4.0x10E3 – 4.0x10E4), as the dose used in the pivotal VLA1553-301 study. All clinical lots are representative of (and very similar to) commercial lots.

Concomitant therapies

Permitted and forbidden prior and concomitant therapy and non-drug therapies were similar than those of study VLA1553-301.

Study assessments

Assessment of immunogenicity

Immunogenicity assessments are overall comparable than for the pivotal study VLA1553-301.

Neutralizing antibodies are measured by using the validated µPRNT assay (Nexelis), as for the adult VLA1553-301 and VLA1553-302 studies.

Specific CHIKV IgM and IgG were measured by ELISA in screening samples from all participants (testing for stratification and exclusion of recent CHIV infections).

Assessment of antibodies specific for Mayaro virus, Dengue virus and Zika virus are also planned in Day 1 samples from all participants. Pre-existing antibodies specific for additional alphaviruses could also be performed.

Please refer to assessment of study VLA1553-301 for more details.

Assessment of efficacy

Incidence of natural CHIKV infections occurring during the course of the study is to be assessed as part of the exploratory objectives by comparing incidence of CHIKV infection between VLA1553 and placebo arms 14 days after vaccination. The efficacy assessment is not further discussed in this procedureas no efficacy data were available.

Objectives and endpoints

Objectives and endpoints are overall endorsed.

The primary endpoint is the proportion of participants with a CHIKV antibody level ≥150 µPRNT50, for µPRNT baseline negative participants 28 days post-vaccination.

Seronegativity was defined as µPRNT50 ≤40, instead of <20, which is not consistent with the definition used in the pivotal VLA1553-301 trial. The serostatus of participants within the 20-40 µPRNT50 stratum is uncertain. To ensure the most correct classification of the participants by baseline CHIKV serostatus, the threshold of 20 µPRNT50 should be used to identify seronegative participants and the threshold of 40 µPRNT50 should be used to identify the seropositive participants (REC).

Analyses stratified by baseline serostatus were performed. Immune responses were characterized in participants positive for CHIKV antibodies at baseline.

As part of the secondary objectives, immune responses were characterized in terms of GMT, seroconversion, fold increase compared to baseline, as in study VLA1553-301. Immune responses will be characterized up to 1 year post-vaccination.

Efficacy will be explored by comparing incidence of CHIKV infections between VLA1553 and placebo arms 14 days after vaccination.

Sample size

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

The immunogenicity subset of 268 VLA1553-vaccinated ELISA baseline seronegative subjects will allow for sufficient statistical power when applying a one-sided exact binomial test with a significance level of 2.5% against a non-acceptance threshold of 70% on the proportion of subjects with a seroprotective level (defined as µPRNT50 ≥150 for µPRNT baseline seronegative subjects) at Day 29. A seroresponse rate (SRR) of 80% is assumed, and 200 VLA1553-vaccinated subjects would thus be necessary for a statistical power of 90%. With an expected drop-out/major protocol deviations rate of approximately 10%, at least 223 seronegative subjects vaccinated with VLA1553 need to be allocated to the immunogenicity subset.

Statistical analyses will be based on µPRNT serostatus. ELISA CHIKV results will be used for enrollment at the sites only.

Sample size considerations can be followed and appear plausible.

Randomisation and blinding (masking)

The approximately 750 participants were randomised in a ratio 2:1 to VLA1553 or placebo arm.

The randomization to study arm was stratified according to CHIKV ELISA baseline status (ELISA: 20% seropositive and 80% seronegative for CHIKV). In order to meet predefined stratum sizes, inclusion was to be driven by baseline ELISA. It is not clear whether randomization was stratified by study subset (Immunogenicity/Viraemia) or any other factors (e.g. centre).

The study was conducted in a double-blind manner. Investigators/sites staff (apart from those designated to randomize participants and handle the IMP), study participants, and sponsor staff were

blinded. Study staff who randomized the participants to study arms and are concerned with IMP handling, the DSMB voting members and the biostatistician involved in the DSMB were unblinded.

Also refer to VLA1553-301 methods for preparation and administration of IMP.

No emergency unblinding occurred during the study. At the end of the part A, the study was unblinded but sites and participants remained unblinded until the end of the study.

Statistical methods

Datasets and analysis cohorts

The **safety analysis population** contains all subjects who entered into the study and received one vaccination. Subjects will be analysed as treated. The Safety population was the primary analysis set for all safety endpoints.

The **immunogenicity subset** was defined as all participants who were initially randomized into the immunogenicity evaluation group or the viraemia subset of the immunogenicity group, regardless of any other factors.

The **viraemia subset** was defined as participants from the immunogenicity subset with viraemia samples analysed from Visit 1 and Visit 2, (and Visit 3, if Visit 2 sample was positive) who were initially randomized into the viraemia evaluation group.

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

The **per protocol analysis population (PP)** contains all IMM subjects who have no major protocol violations that could impact immune response (according to the Applicant). Subjects will be analysed in the PP according to their actual treatment.

The PP population was the primary analysis set for all immunogenicity analyses.

Both the IMM and PP populations included participants defined as seronegative (µPRNT ≤40) and seropositive (µPRNT >40) at baseline but the primary immunogenicity endpoint analysis was limited to baseline µPRNT seronegative participants of the PP population.

Protocol deviations that could lead from removal from the PP population are either pre-defined in the protocol and/or the SAP, or could be defined during the course of the study. As for the adult studies, it is expected that the Applicant provides complete and detailed reasons for exclusion of the PP population for each study part.

The list of evaluable PP participants and exclusionary protocol deviations were finalized at the Blind Data Review Meeting prior to unblinding at Part A.

A **modified PP population** was used for a supplemental analysis to the primary endpoint. This modified PP population excluded participants borderline ELISA at screening. These were to be considered as seropositive at baseline, and would have thus not been included in the study. However, these were considered as seronegative by the investigators because there were no evidence of CHIKV circulation in the regions where the study was conducted at the time of enrolment.

Immunogenicity Analysis

Immunogenicity analysis were limited to the PP population portion of the immunogenicity subset and performed separately for seropositive and seronegative populations. Limiting the primary analysis to PP population is considered appropriate for vaccine trials.

The primary hypothesis was tested using an exact binomial test comparing the observed proportion of subjects reaching seroresponse levels to a fixed lower bound of 70%. This is in principle acceptable, however, a comparison of seroresponse rates between groups would have been preferred. According analyses were provided based on Fisher's exact test, which is acceptable.

Missing data were only planned to be imputed in case of more than 5% missing values. Based on presented results this threshold was not reached and analyses are based on observed values only. Regarding the limited rated of missing values substantial impact of potential informative missingness can be excluded and no concern is raised.

Overall, statistical methods planned to evaluate secondary objectives are considered acceptable.

Planned Data Analysis of the Study

The following data analyses was/will be performed:

➢ Part A includes safety and immunogenicity data after all subjects have completed Visit 3 (Day 29).

➢ Part B includes safety and immunogenicity data after all subjects have completed Visit 5 (Month 6).

➢ Part C includes safety and immunogenicity data after all subjects in the immunogenicity subset have completed Visit 6 (Month 12).

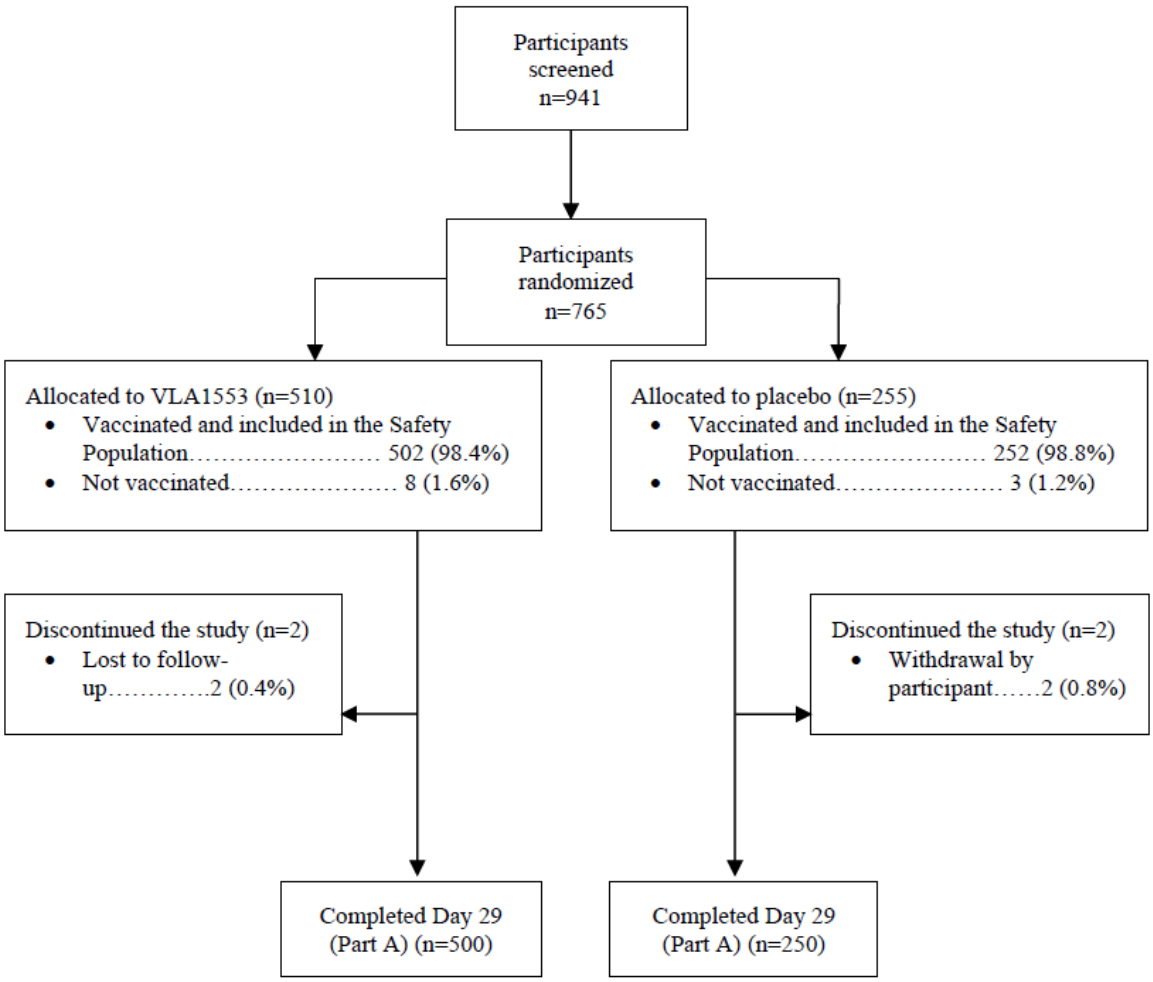
Results

Participant flow

Of the 941 screened participants, 765 were randomized 2:1 to VLA1553 or placebo (510 participants to VLA1553 and 255 participants to placebo). Eleven participants were randomized but not vaccinated; thus, 754 participants (502 to VLA1553 and 252 to placebo) were vaccinated and included in the Safety population. A total of 753 participants were stratified by their µPRNT baseline serostatus: 614 participants to the seronegative stratum and 139 participants to the seropositive stratum. One participant was vaccinated with placebo and included in the Safety population, but did not have a µPRNT baseline serostatus result. Within the seronegative stratum, 408 participants received VLA1553 and 206 participants received placebo. In the seropositive stratum, 94 participants received VLA1553 and 45 participants received placebo.

Four (0.5%) of the vaccinated participants discontinued the study before Day 29, only one participant (in the placebo arm of the seronegative stratum) was part of the IMM population.

The disposition of subjects is provided in Figure 24 below.



n=number of participants
Sources: Table 14.1.1.1, Table 14.1.1.3

Figure 24. Participant disposition (Safety population) (Figure 2, CSR)

The different participants analysis sets stratified by µPRNT status are summarized in the Table below.

Populations of analysis	**VLA1553**	**Placebo**	**Total**
Randomized participants	510	255	765
Safety population	502	252	754
Baseline seronegative	*408*	*206*	*614*
Baseline seropositive	*94*	*45*	*139*
Immunogenicity subset	335	57	392
Immunogenicity population	328	56	384
Baseline seronegative	*268*	*48*	*316*
Baseline seropositive	*60*	*8*	*68*
PP population	303	48	351
Baseline seronegative	*251*	*42*	*293*
Baseline seropositive	*52*	*6*	*58*
Modified PP population	297	47	344
Baseline seronegative	*245*	*41*	*286*
Baseline seropositive	*52*	*6*	*58*
Viraemia subset (and vaccinated)	53 (53)	25 (24)	78 (76)
Baseline seronegative	*43*	*20*	*63*
Baseline seropositive	*9*	*4*	*13*

A total of 385 participants were to be randomized to the immunogenicity subset. A total of 392 participants, including 335 in the VLA1553 arm and 57 in the placebo arm, were actually enrolled in the immunogenicity subset.

A total of 351 participants were included in the PP population, corresponding to 85.9% of the immunogenicity subset (90.5% for the VLA1553 arm and 84.2% for the placebo arm). Although, percentage of participants excluded from the PP population is acceptable, reasons for exclusion from the PP population should be further detailed (REC). Among the 351 participants of the PP population, 293 and 58 were baseline seronegative (µPRNT≤40) and seropositive (µPRNT >40), respectively. The percentage of baseline seropositive participants (16.5%) is thus lower than the 20% expected. In the safety population, there were 139 out of 754 participants with a µPRNT > 40, corresponding to 18.4%.

Of the 10 centres involved in the study, 4 centres enrolled 100 or more participants, and all other centres enrolled 12 or more participants. Of these centres, 4 enrolled baseline seronegative participants only. Four enrolled more than 15 baseline seropositive participants or more (for a total of participants enrolled of 56 or more participants), and two enrolled only 2 and 4 baseline seropositive participants (out of 112 and 36 participants, respectively). The unequal distribution of baseline seropositive volunteer recruitment most probably reflects the various epidemiological contexts across Brazil (REC).

Up to Day 29, 73 and 47 out of the 754 participants underwent for an acute visit or convalescent visit, respectively. It is not known how many of these participants were initially included in the immunogenicity subset and if they were excluded from the PP population.

Unscheduled visits occurred for 41 out of 754 participants.

Recruitment

The study began on 14 Feb 2022 (first participant enrolled) and Part A ended on 14 Mar 2023 (last participant last visit, Day 29). For the submitted CSR, the last data included in analysis is 14 Mar 2023.

Conduct of the study

Protocol deviations

In the Safety population, any important protocol deviations were reported for 389/754 (51.6%) participants. Important major protocol deviations were identified for 33/754 (4.4%) participants, among which 30 participants had a deviation in the category "visit window" and 3 participants had a deviation in the category "inclusion criteria."

Important minor protocol deviations were identified for 380/754 (50.4%) participants.

In the IMM population any important protocol deviations were reported for 210/384 (54.7%) participants. Important major protocol deviations were identified for 33/384 (8.6%) participants, among which 30 participants had a deviation in the category "visit window" and 3 participants had a deviation in the category "inclusion criteria." Important minor protocol deviations were identified for 201/384 (52.3%) participants.

No EMA inspection was performed.

Baseline data

PP population versus safety population

There were no major differences in the demographic characteristics between the safety population and the PP population.

Proportion of female participants was similar in the safety and PP population, i.e. 53.8% and 52.1%, respectively.

Median age of the participants was 15.0 years (Q1-Q3: 13.0-16.0) in both populations. There were 373 (49.5%) and 381 (50.5) adolescents of, respectively, 12 - <15 years and 15 - <18 years in the safety population. Same distribution was observed in the PP population with 167 (47.6%) and 184 (52.4%) adolescents of, respectively, 12 - <15 years and 15 - <18 years.

Median body mass index was 20.57 kg/m^2 (Q1-Q3: 18.33-24.02) and 20.66 kg/m^2 (Q1-Q3: 18.48-23.82) in the safety and PP populations, respectively.

A total of 81.4% and 83.5% of the participants in, respectively, the safety and the PP populations were seronegative at baseline according to the µPRNT results. As mentioned earlier, the cut-off of µPRNT50 <20 would have been preferable over the cut-off of µPRNT50 ≤ 40 for the seronegative determination. 18.4% and 16.5% of the participants in, respectively, the safety and the PP populations were seropositive at baseline according to the µPRNT results (µPRNT50 > 40).

In the safety population, out of the 614 and 139 baseline seronegative (µPRNT≤40) and seropositive (µPRNT >40) participants, 578 were CHIKV seronegative defined as µPRNT50 <20 (385 VLA1553 recipients and 193 placebo recipients), 36 were in the µPRNT50 20-40 stratum (23 VLA553 recipients and 13 placebo recipients), and 139 were CHIKV seropositive (defined as µPRNT50 >40) (94 VLA553 recipients and 45 placebo recipients).

Out of the 293 and 58 baseline seronegative (µPRNT≤40) and seropositive (µPRNT >40) participants in the PP population, 269 were CHIKV seronegative defined as µPRNT50 <20 (234 VLA1553 recipients and 35 placebo recipients), 24 were in the µPRNT50 20-40 stratum (17 VLA553 recipients and 7 placebo recipients), and 58 were CHIKV seropositive (defined as µPRNT50 >40) (52 VLA553 recipients and 6 placebo recipients).

These numbers of baseline seropositive or seronegative participants slightly vary when the ELISA is used to determine the serostatus (either as interpreted at randomization or post-randomization [laboratory results]).

In the safety population, 414 out of 754 participants (54.9%) received concomitant medications (i.e. with a start or end date on or after date of vaccination). The most common concomitant medications were analgesics (36.7%, mainly Paracetamol) and anti-inflammatory/anti-rheumatic products (8.4%, mainly Ibuprofen). Eight of 754 participants (1.1%) received concomitant systemic steroids with suspected immunosuppressive activity. Numbers were not given for the PP population.

In the safety population, 60 out 754 (8.0%) participants had a history of yellow fever vaccination.

Participants were to be tested at baseline at least for antibodies to Mayaro virus, Dengue virus and Zika virus. Results are not yet provided neither for the safety population, nor for the PP population.

PP population

Overall, participants of the PP population were more frequently female (52.1%), and there was a slightly higher proportion of adolescents of 15 -< 18 years (52.4%) compared to the proportion of adolescents 12 - < 15 years (47.6%).

Characteristics were roughly comparable between arms in the PP population. Small imbalances between arms were however noted for the proportion of baseline seronegative and seropositive participants (defined based on µPRNT results). There were 82.8% versus 87.5% of baseline seronegative participants in the VLA1553 and placebo arm, respectively. There were 17.2% and 12.5% baseline seropositive participants in the VLA1553 and placebo arm, respectively. Imbalances between baseline seropositive and seronegative participants within each arm for the age group distribution and differences between the VLA1553 and the placebo baseline seropositive participants (including for the sex and age group distribution) were also noted.

Out of the 293 baseline seronegative participants as per µPRNT results, 282 were seronegative by ELISA (IgM-/IgG-). Ten participants were baseline seropositive by ELISA, including 3 with antibody status IgM-/IgG+ and 7 IgM+/IgG-. Whether these 7 participants were IgM borderline is not known, as the IgM+/IgG- should be excluded from the study. Out of the 58 baseline seropositive participants as determined by µPRNT, 3 were classified as seronegative by ELISA. 29 had recent CHIKV infection as they were IgM+/IgG+ and 25 more remote infection (IgM-/IgG+).

Outcomes and estimation

Primary immunogenicity endpoint results

A summary of the seroresponse rates (SRR) for CHIKV-specific neutralizing antibodies at Day 29 for baseline seronegative participants (µPRNT50 ≤40) of the PP population is provided in Table 29.

A summary of the SRR for CHIKV-specific neutralizing antibodies at Day 29 by µPRNT baseline serostatus for the PP population is provided in Table 30.

Table 29. Seroresponse rate for CHIKV-specific neutralising antibodies at Day 29 (PP population, CHIKV baseline seronegative participants)

	Stratum: Seronegative by µPRNT	
Statistic	VLA1553 (N=251)	Placebo (N=42)
Total[a] [n]	250	42
Participants with Seroresponse [n (%)]	247 (98.8)	2 (4.8)
95% CI for Seroresponse Rate	96.5, 99.8	0.6, 16.2
p-value[b]	<0.0001	>0.9999
Difference in Seroresponse Rate[c] (%)		
Difference	94.0	
95% CI	87.5, 100.0	
p-value[d]	<0.0001	

CI=confidence interval; n=number of participants; SRR=Seroresponse rate.
a Number of baseline negative participants with non-missing titers at Day 29.
b p-value from an exact binomial test for the null-hypothesis H_0: SRR ≤70% against the alternative H_1: SRR >70% with a one-sided significance level of 2.5%.
c Difference, p-value, and associated CI are presented for the VLA1553 arm minus placebo treatment arm.
d. p-value from Fisher's Exact test.
Percentages are based on the number of baseline µPRNT negative participants with non-missing titers at the visit.
Baseline seropositive participants (defined as $\mu PRNT_{50}$ >40) are not included in this summary.
Seroresponse is defined as $\mu PRNT_{50}$ ≥150.
Two-sided 95% exact (Clopper-Pearson) CI is presented for the SRR and Chan-Zhang exact 95% CI is presented for the difference in SRR.
Source: Table 14.2.1.1, Listing 16.2.5.1

Table 30. Seroresponse rate for CHIKV-specific neutralising antibodies at Day 29 by baseline µPRNT serostatus (PP population)

Statistic	Stratum: Seronegative by µPRNT		Stratum: Seropositive by µPRNT		Stratum: Total	
	VLA1553 (N=251)	Placebo (N=42)	VLA1553 (N=52)	Placebo (N=6)	VLA1553 (N=303)	Placebo (N=48)
Total[a] [n]	250	42	52	6	302	48
Participants with Seroresponse [n (%)]	247 (98.8)	2 (4.8)	52 (100)	6 (100)	299 (99.0)	8 (16.7)
95% CI for Seroresponse Rate	96.5, 99.8	0.6, 16.2	93.2, 100.0	54.1, 100.0	97.1, 99.8	7.5, 30.2
p-value[b]	<0.0001	>0.9999	<0.0001	0.1176	<0.0001	>0.9999
Difference in Seroresponse Rate[c] (%)						
Difference	94.0		0		82.3	
95% CI	87.5, 100.0		NC		71.7, 92.9	
p-value[d]	<0.0001		NC		<0.0001	

CI=confidence interval;; µPRNT=Micro Plaque Reduction Neutralization Test; n=number of participants; NC=non-calculable SRR=Seroresponse rate.
a Number of participants with non-missing titers at Day 29.
b p-value from an exact binomial test for the null-hypothesis H_0: SRR ≤70% against the alternative H_1: SRR >70% with a one-sided significance level of 2.5%.
c Differences, p-values, and associated CIs are presented for the VLA1553 arm minus placebo treatment arm.
d p-value from Fisher's Exact test.
Percentages are based on the number of participants with non-missing titers at the visit. Baseline seropositive and seronegative participants are included in this summary.
Seroresponse is defined as $\mu PRNT_{50}$ ≥150. Baseline seronegative participants are those with baseline $\mu PRNT_{50}$ ≤40, while baseline seropositive participants are those with baseline $\mu PRNT_{50}$ >40.
Two-sided 95% exact (Clopper-Pearson) CI is presented for the SRR and Chan-Zhang exact 95% CI is presented for the difference in SRR.
Source: Table 14.2.1.4, Listing 16.2.5.1

The primary endpoint was met. At 28 days post-vaccination, 98.8% (247/250) of the **baseline CHIKV seronegative participants** had an antibody titre of at least 150 µPRNT50 (95% CI of 96.5-99.8) in the VLA1553 arm (PP population).

In total, 3 baseline seronegative participants did not reach the threshold of 150 µPRNT50 in the VLA1553 arm. At least one of these 3 vaccinees did not respond at all to the vaccination (see below, GMT results).

Two of the baseline seronegative placebo participants (out of 42) reached the threshold of 150 µPRNT50 at Day 29 post-vaccination. Both placebo participants were CHIKV seronegative at baseline and at Day 8 post-vaccination, as measured by µPRNT.

Results obtained in the IMM population were similar. The results were aligned when the serostatus at baseline was determined by ELISA (baseline stratification), instead of µPRNT and when participants with borderline ELISA results at baseline were excluded from the analysis population (modified PP population).

There were a total of 58 participants of the PP population who were **baseline seropositive** (defined as µPRNT50 >40), 52 in the VLA1553 arm and 6 in the placebo arm. Out of the 52 VLA1553 participants, 50 had antibody titres above the threshold of 150 µPRNT50. All the 6 placebo participants had antibody titres above the threshold of 150 µPRNT50. The 2 participants who did not have antibody titres of at least 150 µPRNT50 at baseline have antibody titres of at least 71 µPRNT50 (see below, GMT results). All participants (52) had antibody titres above the threshold of 150 µPRNT50 at 28 days post-vaccination.

Post-hoc analyses using the cut-off for defining respectively seropositive (µPRNT50 >40) and seronegative (µPRNT50 <20) status at baseline were performed.

At Day 29, 98.7% (95% CI: 96.3-99.7, n=231/234) of seronegative VLA1553 participants (µPRNT50 <20) had seroresponse and 5.7% (95% CI: 0.7-19.2, n=2/35) of placebo participants.

In the stratum µPRNT50 20-40, 100% (95% CI: 79.4-100.0, n=16/16) VLA1553 participants had a seroresponse at Day 29 and none of the placebo participants (n=0/7).

In the seropositive VLA1553 recipients (µPRNT50 >40), 96.2% (50 of 52) of the VLA1553 participants were seroresponders at baseline and 100% (6 of 6) of the placebo participants. At Day 29, all participants were seroresponders.

Results of the IMM population are similar than those observed for the PP population.

Secondary Immunogenicity Endpoint Results

Immune Response as Measured by CHIKV-Specific Neutralizing Antibodies on Day 8 and Day 29 Post-Vaccination

A summary of GMTs for CHIKV-specific neutralizing antibodies by visit and baseline µPRNT serostatus for the PP population is provided in Table 31.

Table 31. GMTs for CHIKV-specific neutralising antibodies by visit and baseline µPRNT serostatus (PP population)

	Stratum: Seronegative by µPRNT		Stratum: Seropositive by µPRNT		Stratum: Total	
Time point Statistic	VLA1553 (N=251)	Placebo (N=42)	VLA1553 (N=52)	Placebo (N=6)	VLA1553 (N=303)	Placebo (N=48)
Visit 1 – Day 1						
n	250	42	52	6	302	48
Geometric Mean	10.6	11.7	3097.1	3409.0	28.2	23.8
95% CI for GM	10.31, 10.89	10.46, 13.17	2324.90, 4125.89	2244.36, 5178.13	21.96, 36.12	13.61, 41.78
Geometric std	1.24	1.45	2.80	1.49	9.00	6.90
Mean (std)	10.9 (3.58)	12.8 (6.56)	4579.4 (4200.62)	3632.2 (1367.04)	797.6 (2444.28)	465.2 (1289.25)
Median	10.0	10.0	2872.5	3811.5	10.0	10.0
Q1, Q3	10.0, 10.0	10.0, 10.0	1797.5, 5810.5	2288.0, 4203.0	10.0, 10.0	10.0, 21.0
Min, Max	10, 37	10, 34	71, 21882	1990, 5689	10, 21882	10, 5689
Visit 2 - Day 8						
n	245	42	51	5	296	47
Geometric Mean	17.6	10.6	3251.2	3993.3	43.2	19.9
95% CI for GM	15.41, 20.06	9.75, 11.44	2458.75, 4298.98	1507.71, 10576.49	33.48, 55.77	11.43, 34.48
Geometric std	2.85	1.29	2.70	2.19	9.30	6.55
Mean (std)	138.5 (633.93)	11.1 (5.43)	4412.0 (3667.26)	5211.6 (4544.17)	869.2 (2278.83)	564.3 (2103.00)
Median	10.0	10.0	3277.0	2602.0	10.0	10.0
Q1, Q3	10.0, 26.0	10.0, 10.0	2405.0, 5484.0	2570.0, 6212.0	10.0, 69.0	10.0, 10.0
Min, Max	10, 5439	10, 43	10, 19634	1916, 12758	10, 19634	10, 12758
n[a]	245	42	51	5	296	47

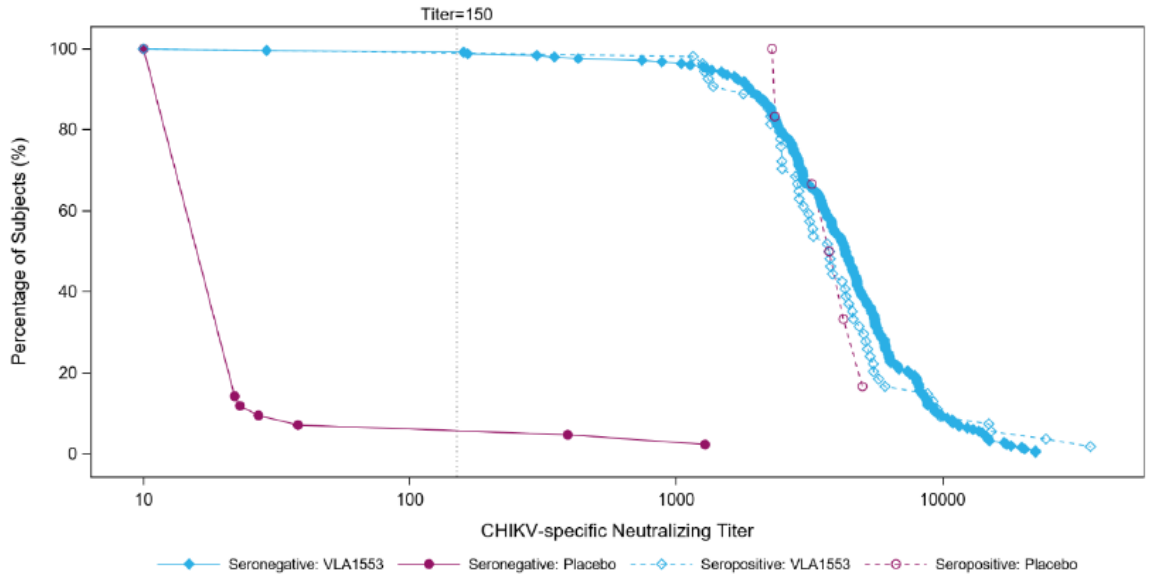
	Stratum: Seronegative by µPRNT		Stratum: Seropositive by µPRNT		Stratum: Total	
Time point	VLA1553	Placebo	VLA1553	Placebo	VLA1553	Placebo
Statistic	(N=251)	(N=42)	(N=52)	(N=6)	(N=303)	(N=48)
LS Mean (SE)[b]	17.58 (1.06)	10.56 (1.16)	3251.18 (1.15)	3993.28 (1.55)	244.49 (1.08)	159.21 (1.17)
95% Confidence Interval[b]	15.55, 19.87	7.86, 14.20	2469.66, 4280.00	1659.53, 9608.90	211.53, 282.58	117.79, 215.19
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	1.66 (1.18)		0.81 (1.58)		1.54 (1.17)	
95% Confidence Interval[c]	1.21, 2.29		0.32, 2.04		1.14, 2.08	
p-value[c]	0.0019		0.6560		0.0055	
Visit 3 - Day 29						
n	250	42	52	6	302	48
Geometric Mean	3889.7	13.5	3886.5	3339.2	3889.2	26.8
95% CI for GM	3460.86, 4371.69	9.99, 18.12	3063.40, 4930.87	2394.37, 4656.76	3503.31, 4317.51	14.78, 48.62
Geometric std	2.55	2.60	2.35	1.37	2.52	7.77
Mean (std)	5170.7 (3751.50)	51.1 (203.55)	5879.5 (6476.31)	3747.1 (1208.52)	5292.3 (4336.45)	579.1 (1387.65)
Median	4291.5	10.0	3797.0	3751.0	4255.0	10.0
Q1, Q3	2760.0, 6104.5	10.0, 10.0	2476.0, 5503.0	2347.0, 5006.0	2730.0, 6083.0	10.0, 22.0
Min, Max	10, 22339	10, 1284	165, 35919	2291, 5356	10, 35919	10, 5356
n[a]	250	42	52	6	302	48
LS Mean (SE)[b]	3889.71 (1.06)	13.46 (1.16)	3886.55 (1.12)	3339.16 (1.40)	4732.46 (1.09)	33.56 (1.19)
95% Confidence Interval[b]	3460.08, 4372.68	10.11, 17.90	3093.70, 4882.58	1705.84, 6536.37	4016.11, 5576.57	23.89, 47.14
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	289.04 (1.17)		1.16 (1.42)		141.03 (1.19)	
95% Confidence Interval[c]	212.29, 393.54		0.57, 2.37		100.07, 198.75	
p-value[c]	<0.0001		0.6698		<0.0001	

	Stratum: Seronegative by µPRNT		Stratum: Seropositive by µPRNT		Stratum: Total	
Time point	VLA1553	Placebo	VLA1553	Placebo	VLA1553	Placebo
Statistic	(N=251)	(N=42)	(N=52)	(N=6)	(N=303)	(N=48)

CI=confidence interval; GM=geometric mean; GMT=geometric mean titer; LS=least squares; µPRNT=Micro Plaque Reduction Neutralization Test; max=maximum; min=minimum; n=number of participants with available result NC=non-calculable; Q=quartile; SE=standard error; std=standard deviation.
a n is the number of participants that contribute data at least once in the primary analysis model.
b LS means, standard errors, CIs, and p-values are from an analysis of variance (ANOVA) model with fixed factors for study arm and baseline µPRNT serostatus strata. The models for the data within serostatus categories do not account for the baseline serostatus.
c p-values, LS mean differences, and associated CIs are presented for the VLA1553 arm minus placebo treatment arm.
Note: The ANOVA model is applied to the log-transformed titers, and back-transformed results are displayed for the LS mean and difference. The difference in GMT is a ratio of the LS means.
Baseline seronegative participants are those with baseline $\mu PRNT_{50} \leq 40$, while baseline seropositive participants are those with baseline $\mu PRNT_{50} > 40$. Note that any $\mu PRNT_{50}$ value <20 is imputed as 10 for analysis.
Source: Table 14.2.2.1, Listing 16.2.5.1

Reverse cumulative distribution curves for CHIKV-specific neutralizing antibodies at Day 29 by study arm and baseline µPRNT serostatus strata for the PP population are presented Figure 25.

Figure 25. Reverse cumulative distribution curve of CHIKV-specific neutralising antibodies at Day 29 by study arm and baseline µPRNT serostatus strata (PP population



µPRNT=Micro Plaque Reduction Neutralization Test.
Source: Figure 14.2.1.4, Table 14.2.2.1

Baseline GMTs (Day 1) were 10.6 (95% CI: 10.31-10.89) and 11.7 (95% CI: 10.46-13.17) in **baseline seronegative participants** of the VLA1553 and placebo arm, respectively. The maximum value was 37 µPRNT50, which is considered borderline (> 20 but < 40 µPRNT50).

At Day 8, GMTs slightly increase but remained low, i.e. 17.6 (95% CI: 15.41-20.06) and 10.6 (95% CI: 9.75-11.44) in baseline seronegative participants of the VLA1553 and placebo arm, respectively.

At Day 29, GMT was high in the VLA1553 arm, with GMT value of 3,889.7 (95% CI: 3,460.86-4,371.69). The minimum GMT was 10, meaning that at least one of the 3 vaccinees who did not reach the threshold of 150 µPRNT50 did not respond at all to the vaccination. Day 29-GMT of the placebo participants remained low, i.e. 13.5 (95% CI: 9.99-18.12), with a Q3 of 10.0 µPRNT50 and a maximum value of 1,284 µPRNT50 (as described above, 2 placebo participants have antibody titres of at least 150 µPRNT50, one with a Day 29 µPRNT50 of 1284 and the second with a Day 29 µPRNT50 of 391).

Results were not presented by age category. Results were not presented by sex.

Baseline GMTs (Day 1) were 3,097.1 (95% CI: 2324.90-4125.89) and 3,409.0 (95% CI: 2244.36-5178.13) in **baseline seropositive participants** of the VLA1553 and placebo arm, respectively. The minimum value was 71 (meaning that the 2 participants who did not have antibody titres of at least 150 µPRNT50 at baseline have antibody titres of at least 71 µPRNT50) and the maximum value was 21,882.

At Day 8, GMT was similar than at Day 1 for the VLA1553 arm. Difference in GMT is not fully interpretable for the placebo arm as only 6 participants were included in the seropositive stratum. For the VLA1553 arm, GMT was 3,251.2 (95% CI: 2,458.75-4,298.98).

At Day 29, GMT in the VLA1553 arm was of 3,886.5 (95% CI: 3,063.40-4,930.87), thus similar than at Day 1 and Day 8.

Results presented overall and not individually or by µPRNT50 strata at baseline, do not allow to assess if VLA1553 boosted the antibody response in some seropositive participants (for example those with lower antibody titres at baseline) (REC).

Post-hoc analyses using the cut-off for defining respectively seropositive (µPRNT50 >40) and seronegative (µPRNT50 <20) status at baseline were further performed.

The Day-29 GMT were of 3916.0 (95% CI: 3461.80-4429.86) in VLA1553 baseline seronegative participants and of 13.7 (95% CI: 9.66-19.55) in placebo baseline seronegative participants.

In the stratum µPRNT50 20-40, the VLA1553 and placebo recipients had Day-29 GMTs of 3524.3 (95% CI: 2566.13-4840.32) in VLA1553 recipients and of 12.1 (95% CI: 7.59-19.30) in placebo recipients.

Baseline GMTs of the baseline seropositive participants were of 3097.1 (95% CI: 2324.90-4125.89) and of 3409.0 (95% CI: 2244.36-5178.13), respectively, in the VLA1553 and in the placebo participants. Day-29 GMTs were of 3886.5 (95% CI: 3063.40-4930.87) and of 3339.2 (95% CI: 2394.37-4656.76), respectively, in the VLA1553 and in the placebo participants.

The Day 29 GMT in baseline CHIKV seronegative participants is similar to the baseline GMT of baseline CHIKV seropositive participants (in the PP population and in the post-hoc analysis, i.e. when using the cut-off of 40 or 20).

Results of the IMM population are similar than those observed for the PP population.

Together with the SRR results, these results suggest that either the participants with a baseline between 20-40 µPRNT50 are not true seropositive participants, or that low neutralising antibody titres (≤40 or maybe even up to 100 µPRNT50) are not able to neutralise the vaccine virus, and might not be able to prevent from (re-infection).

In **conclusion**, the findings observed in CHIKV baseline seronegative participants are consistent with results of both VLA1553-301 and VLA1553-302 studies, conducted in adults leaving in non-endemic areas. At 28 days post-vaccination, 98.8% (247/250) of the baseline CHIKV seronegative participants had an antibody titre of at least 150 µPRNT50 (95% CI of 96.5-99.8) in the VLA1553 arm (PP population). At Day 29, GMT was 3,889.7 (95% CI: 3,460.86-4,371.69), i.e. in the same range than Day 29-GMT of studies VLA1553-301 and VLA1553-302. The Day 29-GMT observed in the baseline CHIKV seronegative participants are comparable to the baseline GMT of baseline CHIKV seropositive participants, i.e. to the titres elicited by the natural infection. The time elapsed between the infection and the sampling is unknown (but on average likely much longer than one month), and thus the antibody level reached shortly after natural infection is unknown, but likely much higher than the level induced by the vaccine. Data in CHIKV seropositive participants suggest the absence of booster effect, as Day 29 GMT in the VLA1553 arm are similar as Day 1 and Day 8 GMTs. As stratified analyses are not provided, it is not known if a booster effect would be observed in subjects with lower antibody titres.

2.6.5.8. Planned post-authorisation efficacy/effectiveness studies

Introduction

Although the immunogenicity results of the VLA1553-301 are compelling, they are based on a neutralizing antibody titre threshold reasonably likely to predict protection (supported by animal and sero-epidemiological data) and not on an established immune correlate of protection. Uncertainties remain around how this threshold actually translates into protection against disease and/or infection. Therefore, the actual protection of VLA1553 remains to be confirmed.

Two post-approval studies are planned to be conducted to gather effectiveness data:

- Study VLA1553-402 which is a test-negative (TN) case-control (CC) study to estimate the VE of VLA1553 in Brazil which will be conducted following its licensure in the country.
- Study VLA1553-404 which is a randomized, controlled trial with pragmatic elements to estimate the VE and safety of VLA1553.

Whereas study VLA1553-402 will be conducted in Brazil only, study VLA1553-404 would gather effectiveness data in several endemic countries (still to be determined).

Concept protocols were submitted but no feasibility evaluation is completed yet.

For both studies, estimated timelines for preparedness activities and study conduct were submitted and a list of the activities that will be included in the feasibility assessment was provided. It is acknowledged that timelines are provisional. It is expected that the Applicant regularly inform EMA about the advancement and outcomes of the different preparedness activities (REC).

Study VLA1553-402

VLA1553-402 is an observational effectiveness study to be conducted in endemic areas of Brazil.

In 2022-23, after a period of relatively low activity, chikungunya struck back in parts of Brazil. Both the Asian lineage and the East/Central/South African (ECSA) lineage circulate, and the latter lineage became the dominant variant in the country (Iasmim Ferreira de Almeida et al.; Lancet Reg Health Am 2023; doi: 10.1016/j.lana.2023.100571).

The study will use a test-negative case-control (TNCC) design. The primary objective of VLA1553-402 is to estimate the vaccine effectiveness (VE) of VLA1553 in the prevention of RT-PCR confirmed Chikungunya in individuals 12 yoa or above. There is a secondary objective to estimate VE by age category. Exploratory objectives encompass the estimation of VE according to presence of chronic conditions as well as VE over time since vaccination and for each of the CHIKV seasons covered by the study.

To be eligible, participants must be aged 12 years of age or older, reside in the selected municipalities, and have performed RT-PCR testing to investigate CHIKV infection. Data from the Laboratory Information System (GAL) will be used to identify potential participants (i.e. individuals undergoing RT-PCR testing for CHIKV). After verification of the inclusion/exclusion criteria, participants will be classified into cases or controls based on CHIKV RT-PCR testing results. The target sample size consists in 446 cases and 892 controls. Vaccinated participants are defined as those who received VLA1553 ≥14 days before the onset of symptoms. The protocol mentions health centre, calendar time of symptoms onset, age, sex, and number of chronic conditions as potential confounders. For the data collection (exposure, outcome and covariates), the study will rely on routine databases. It is not clear if patients phone interviews will be used as well, as statements of the Applicant in this regard are not consistent. Conditional logistic regression modelling will be used to estimate crude and adjusted Odds ratios (OR)s. VE estimates will be generated based on the ORs, with their 95% confidence interval (CI).

Study protocol

This assessment is based on the VLA1553-402 Protocol Version 4.3 submitted (dated 8 November 2023). The version 4.4 was later provided in the submission, the only change is the definition of the AESI.

The protocol provided in the submission is not considered mature yet. A proper assessment could not be made due to the many unclarities and key information missing. Some comments are however provided below.

In principle, a TNCC design could be appropriate, provided that it is feasible, and that the Applicant can demonstrate that it can lead to reasonably unbiased estimates.

Chronic chikungunya, which greatly contributes to the burden of disease, is not part of the study endpoints. It is agreed that this TNCC study does not allow for an estimation of the effectiveness in preventing chronic chikungunya (which would require another syndrome for eligibility). It is however considered a limitation that the effect of the vaccine on the most clinically relevant endpoint, i.e. chronic arthritis will remain unknown. The evaluation of VE against occurrence of chronic CHIKV disease and CHIKV disease leading to hospitalization in confirmed and suspected cases of CHIKV disease is part of the secondary objectives of study VLA1553-404.

The study inclusion process may need to be revised. A TNCC design requires that participants are identified based on a given syndrome (and then classified into cases vs. controls based on a test result). With the current proposal, potential eligible participants will be identified based on the presence of a CHIKV RT-PCR test result in the GAL database. The study design requirement can thus only be met at the condition that the use RT-PCR testing by HCP is standardized and systematic in all individuals meeting a given syndrome (suspected CHIK-like cases) corresponding to a case definition. However, in actual facts, there is variability with regards to the use of RT-PCR (see also below). In most instances, RT-PCR is not used in case of Chik-like symptoms. In addition, when used, it is likely not followed in a standardized way as clinical judgment enters into consideration. Variability in the approach is expected to be high (physicians may have different thresholds for using RT-PCR, which may also vary according to the epidemiological context). The lack of standardization is of concern as it will generate biases, for example, since the physician will be aware of the vaccination status. The applicant is proposing to work with the laboratories and MoH to ensure the use of RT-PCR as per MoH recommendation. It is not clear however whether this will suffice, as the MoH case definition may not be appropriate to meet the study requirements. This definition is very broad and incorporates an exclusion based on the presence of another cause (see also below). The focus of this definition is patient's appropriate medical care, but it is not clear whether this will be adequate as inclusion criteria for a TNCC study. So, a specific case definition and guidance may be needed for the purpose of the study. (REC).

A definition is provided for 'CHIKV-Like Case', which correspond to the Brazilian MoH definition. It is based on a very broad list of non-specific symptoms and signs (such as fever, headache, arthralgia, rash, …), of which at least one symptom or sign must be present to meet the definition. In contrast to what the Applicant is trying to convey, using a wide range of clinical signs and symptoms as clinical criteria for testing does not help to mitigate the bias due to differences in healthcare-seeking behaviour. It might be more appropriate to restrict the case definition to more severe cases. In addition, based on the MoH definition, it is understood that RT-PCR testing is recommended to be used only if symptoms of the patients cannot be explained by other conditions. Suspected cases explained by other conditions should not be excluded as these will correspond to the test negative controls.

Pre-existing immunity to CHIKV is an important potential confounder. Individuals with a history of chikungunya before vaccination will be excluded from the study. However, individual data on CHIKV serostatus will not be available via this TNCC design (no pre-vaccination sample can be obtained). This is deemed as a limitation of the study, given the importance of obtaining vaccine effectiveness data stratified by pre-exiting CHIKV immunity. Incidence is expected to be negligible/null in the seropositive individuals, and differences across serostatus could bias the estimates if vaccination uptake varies across serostatus. Serosurvey data (which the Applicant plans to use) are not expected to allow for

controlling this bias. Although participants with a history of prior infection will be excluded, this source of bias thus remains of concern.

The applicant did not include an in-depth analysis of the sources of biases nor a description of risk mitigation approaches. This should be part of the feasibility assessment. In particular, variability in practices could be a source for biases which needs to be addressed. In addition, the list of potential confounders seems incomplete (for ex. use of protective measures to avoid mosquito bites is missing). Furthermore, some variables (such as age or chronic conditions) can also constitute potential effect modifier. Effect modifiers are not presented.

Feasibility aspects

At the moment, it is not known whether the conduct of the study, with a TNCC design, is realistic. The applicant only provided a preliminary concept protocol, in which many practical aspects are missing, and a feasibility evaluation is lacking.

The pilot vaccination campaign, planned in 5 to 10 municipalities, is a prerequisite for study VLA1553-402 and is due to start by 01-Oct-2025, provided that the vaccine has been approved by ANVISA. Target municipalities with potential enhanced CHIKV transmission have been pre-selected based on a prediction model using data on mosquito incidence rates and seroprevalence of chikungunya due to previous outbreak(s) (municipalities with a limited seroprevalence would be selected). In view of the start of the pilot vaccination campaign by 01-Oct-2025, the Applicant intends to confirm the selection of municipalities at the latest in March 2025. However, the final selection will not be done before December 2024 to increase the chances of selecting municipalities with chikungunya transmission by using the most recent chikungunya data.

Two scenarios for the pilot vaccination program are investigated, i.e. a pre-emptive scenario (vaccination campaign in a large number of municipalities) and a reactive scenario (vaccination campaign early after detection of cases). The approach is acknowledged.

If deemed feasible, the study would be conducted when an increase in CHIKV transmission is observed and if the vaccination coverage is at least 15%.

The applicant provided a short and non-exhaustive description of activities that will be included in the study's feasibility assessment, reflecting their current thinking. The feasibility assessments will cover 5 aspects for both studies: I) epidemiology, II) operational, III) ethical, IV) regulatory, and V) community engagement. Timelines for reporting have been provided.

The study will rely on the routine health care and on the chikungunya surveillance system. Several uncertainties with respect to the feasibility of the study are described below (not exhaustive).

Use of the RT-PCR testing

No information is provided on the actual use of the RT-PCR testing in Brazil for the diagnosis of Chikungunya. It is stated in the protocol that the MoH of Brazil recommends the use of RT-PCR within the first 6 days of CHIK-like symptoms onset (CHIKV serologic testing based on IgM and IgG is being used for the diagnostic thereafter). However, during epidemics, diagnostic confirmation is not recommended by the MoH for clinically typical cases, unless severe. A recent paper indicates that RT-PCR is not commonly used in routine practice in Brazil (most cases in Brazil are defined by clinical/epidemiological criteria with no laboratory tests [Iasmim Ferreira de Almeida et al.; Lancet Reg Health Am 2023; doi: 10.1016/j.lana.2023.100571]). So, a strong reinforcement of the use of RT-PCR testing in needed before study initiation.

Data collection

For the data collection, the study will rely on routine databases including the laboratory information system (GAL) that will register the results of the RT-PCR testing, the notifiable diseases information system (SINAN) database which should record the symptom(s) and the national immunization program information system (SI-PNI) which will record the vaccination status. The national identification number will be used to link data sources. Reliable and complete demographic data, medical data (such as data on the chikungunya event including CHIK-like signs and symptoms, date of onset of symptoms, hospitalization, and acute complications and medical history), RT-PCR testing information, and vaccination data will thus have to be retrieved from these routine databases. Preliminary evidence however already indicates that the routine databases do not include all the critical information needed. For example, only very limited medical data seems to be available from these sources. In the submitted version of the protocol, it appeared that the Applicant was aware of these deficiencies and planned to rely on patients interviews for most/all the key clinical data items, as well as for the vaccination status and date (although it was not clear whether obtaining such data via interviews is realistic and reliable). In addition, it is unclear how important covariate data such as for example knowledge about prevention measures for chikungunya will be obtained. During the assement, it is noticed that the Applicant will rely entirely on the routine databases instead of collection of data through interviews. This approach can be supported, at the condition that (i) evidence in relation to the feasibility of collecting the key data is provided, (ii) the recording of information in databases of surveillance system is reinforced as necessary to meet the study goals.

Verification of the eligibility status

Potentially eligible participants of study VLA1553-402 will be identified based on the presence of an RT-PCR result in the GAL routine database (laboratory information system). Both the GAL database and the SINAN database (national notifiable diseases information system) will be consulted to confirm eligibility. For that purpose, age and acute symptomatic CHIKV-like disease i.e. symptom(s) that prompted the RT-PCR testing (inclusion criteria) will need to be collected from the databases. The applicant states that it is not planned anymore to obtain the data retrospectively via interviews, which is acknowledged considering the limitations of such approach. The text of the Applicant is however ambiguous as it is still stated (although the wording ‘interview’ is not used anymore) that the symptoms will be 'verified during consent procedure . These inconsistencies reflect the difficulties the Applicant is facing to determine the best approach for the identification of study participants. As said above, preliminary evidence suggests that the needed medical data (including the symptom or set of symptoms that prompted the testing) are currently not recorded in the databases, and that the surveillance system will need to be reinforced to meet the study goals.

Sample size

The target sample size consists in 446 cases and 892 controls. The assumptions for the sample size calculation are not justified. Additional sample size calculations, exploring different case control ratios aligned with epidemiological data on the incidence of diseases that may lead to symptoms required for study inclusion should be provided.

Planned feasibility assessment

The operational feasibility assessment includes, a.o. the completion of assessment of data representativeness, the data relevance and the data quality of GAL and SINAN (following approval for access) is intended for end of 2024. The approval for access to the databases is still pending. An assessment of the quality and process of linkage between databases (GAL, SINAN and SI-NPI) is recommended to be included in the feasibility assessment. The overall process of data collection should be reviewed based on an in-depth feasibility evaluation.Reinforcement activities

The applicant stated that regular interactions with the MoH of Brazil have started since mid-January to discuss operational aspects which are key for the success of the study execution. A formal agreement with the MoH detailing the collaboration activities is targeted for September 2024.

The applicant previously indicated that they are working with the MoH at the federal, state, and local level to enhance the 3 pillars (immunization, surveillance, and laboratorial capabilities). Overall support of the MoH of Brazil and close collaboration with the MoH and local health centres are key for the success of the study execution and validity of the data.

The activities planned under the wording 'enhancement of the system' are only briefly outlined. For example, the Applicant indicated that they will work with the laboratories and MoH to enhance the use of the RT-PCR testing. Discussions with the MoH also include identification of prerequisites to support the continuous testing in an outbreak setting. A key component will be to assess how many additional PCR kits will be needed and provide them to the Lacens (central labs in each state) so that PCRs are performed for all suspected CHIK cases even during an outbreak. In addition, communication outreach to HCP's / local health centres to reinforce the importance of testing will be planned. These activities are key to ensure the success of the study execution. Training of health care providers on when to order a RT-PCR testing could also be planned. It is not clear at this stage whether enhancement of the system will be sufficient or whether profound adaptations will be needed to make the study feasible. These reinforcement/adaptations of the system are essential to the success of this study and will require strong and close collaboration with the MoH and local health centers.

In **conclusion**, an in-depth feasibility assessment, including with respect to the recruitment of the target population, identification of cases and controls, and collection of complete and accurate data, is crucial for the success of the study. The complete feasibility assessment is planned for end of the year. Strong reinforcement and adaptation of the routine care practices and of the surveillance system will be needed. Overall, although the reasons for planning the study in Brazil are acknowledged, and the approach of the Applicant is strongly encouraged, the feasibility of study VLA1553-402 in Brazil remains uncertain. The applicant is strongly recommended to engage into discussions with countries where MAA are planned, in order to explore other options for doing a TNCC (REC).

Study VLA1553-404

Study VLA1553-404 is entitled 'Trial of the Effectiveness and Safety of Ixchiq Against Chikungunya Virus Disease in an Endemic Country: A Pragmatic Randomized Controlled Trial.'

Study protocol

A concept protocol was submitted. Final full protocol is expected in September 2024.

This is an individual-level randomized, observer-blind, controlled trial with pragmatic elements, conducted across multiple centres. Participants will be assigned at random to either the VLA1553 vaccine or a placebo/active control in a 1:1 ratio. The applicant currently targets active control.

The primary objective of the study is to assess vaccine effectiveness (VE) in preventing symptomatic virologically-confirmed CHIKV disease among adults, in VLA1553 vaccinees compared to control participants, both overall and by age groups. Secondary objectives include: (i) The evaluation of VE against symptomatic probable or suspected CHIKV disease among adults, in VLA1553 vaccinees compared to control participants during the same trial period, both overall and by age groups; (ii) The evaluation of VE against occurrence of chronic CHIKV disease and CHIKV disease leading to hospitalization in confirmed and suspected cases of CHIKV disease; (iii) The assessment of VLA1553 safety in VLA1553 vaccinees compared to control participants during the same trial period. Exploratory

objectives are, a.o., to examine the vaccine's effectiveness across the initial and subsequent transmission (rainy) seasons.

Participants will be sampled at baseline to allow for testing of CHIKV serological status, and randomized to receive either the VLA1553 vaccine or control (i.e., placebo or active control).

After vaccination, participants will be passively followed during all the trial period, with instructions to come back to site in case of chikungunya-like symptoms. Reminders about the symptoms of CHIKV disease will be sent to participants via app notifications or phone calls at the beginning and end of each rainy season, encouraging them to seek medical attention if they display symptoms. The study duration is currently estimated to be at least 3 years. A dried blood spot (DBS) will be collected for virological confirmation of CHIKV infection in participants attending the trial health clinics with chikungunya-like symptoms. Participants with confirmed or suspected CHIKV infections will undergo an extended monitoring period. Twelve weeks following their initial CHIKV confirmation or suspicion, they will be evaluated for chronic CHIKV disease or CHIKV-related hospitalization.

DBS will be processed at a centralized, FDA-validated laboratory. The DBS should be stored and sent to the central lab as per the sponsor's guidelines. Testing at the central lab will employ PCR or other nucleic acid amplification tests, with specific procedures detailed in the complete protocol.

There will be no immunogenicity assessments in this study. No samples will be taken beside the baseline sample and samples taken in the context of a visit for chikungunya-like symptoms.

Pragmatic element of this trial design is the routine aspect of data collection, that is, collecting data of participants that attend at the point-of-care presenting CHIKV symptoms. This aspect is currently not sufficiently described in the concept protocol, in particular, it is unclear at this stage how the Applicant will ensure the robustness of the data in this ‘pragmatic’ context. It is assumed that specific data collection tools will be developed for the purpose of the study, but this is not described at the moment, and it is very unclear what is meant by ‘routine aspect of data collection’ (REC). Inexpensive sample collection and storage methods will be provided to the selected clinics, such as obtaining blood samples via contrived dried blood spots.

Potential disadvantages of the design are a greater risk of misclassification and loss to follow-up, leading to dilution effects. To mitigate these risks the Applicant will develop a mobile application and trial the opportunity of using follow-up calls to remind participants of CHIKV symptomatology and to attend health clinics if they experience symptoms.

The primary endpoint is symptomatic virologically-confirmed CHIKV disease occurring ≥ 2 weeks after vaccination. The success criterion (LB of the 95%CI around VE estimate) is still under discussion. Secondary endpoints are suspected acute CHIK cases, Chronic CHIK (persistence >12 weeks) and CHIK related hospitalizations. A formal definition of "suspected CHIKV disease" still needs to be included in the protocol.

Severe chikungunya-like adverse reactions and prolonged arthralgia will be monitored in at least 10,000 individuals vaccinated with VLA1553.

A sample size of approximately 20,000 participants is targeted. The sample size calculation should be confirmed.

Feasibility aspects

The trial will take place in settings with no or low-incidence CHIKV transmission but at high risk of future outbreaks of CHIKV (based on previous outbreaks). The study initiation is planned for 01-Oct-2025.

In the concept protocol V6.0 (dated 8 Nov 2023), the trial is planned to be run in a single CHIKV-endemic country. However, the Applicant clarified during the assessment that the trial is now planned to be multi-country/region. This would allow, a.o. to estimate the VE against different circulating CHIKV strains which is supported. Initial interactions with different countries in South/Latin-America, Africa and Southeast-Asia are taking place. The applicant is in the process of concluding Confidentiality Disclosure Agreement (CDAs) with potential countries for sharing of data in support of an epidemiological analysis. CDAs were signed already with Panama, Senegal, and Brazil. Epidemiological feasibility analyses similar to the ones performed for VLA1553-402 are ongoing.

Pre-selection of countries (based on historic/current age-specific chikungunya epidemiological data, as well as operational considerations such as preliminary trial capabilities/capacities assessment) is planned for 30-Jun-2024. The presence of Chikungunya vectors and the area's proximity to past outbreaks will be key indicators. Predictive modelling will be used to assess the risk of future outbreaks in the area. Using epidemiological data on dengue as a proxy for chikungunya transmission is being explored. Countries will be selected, and all sites identified by December 2024.

The current plans include thus a multi-country approach with a possible combination of pre-emptive vaccinations completed prior to (increased) CHIKV transmission and reactive vaccinations in an outbreak setting strategies (as for study VLA1553-402). The feasibility of such approach will be assessed. Regulatory and logistical preparations need to be in place to facilitate immediate activation in case of an outbreak.

In terms of operational feasibility considerations, the Applicant plans to carry out the standard activities to be performed when planning a RCT.

The overall feasibility assessment will be completed by end of 2024.

Key potential feasibility issues identified:

Efficacy trials were deemed not feasible prelicensure due to short-lived and unpredictable nature of chikungunya outbreaks, and it is not clear whether it is feasible at the present time in the post-licensure setting, although pragmatic trials present advantages in terms of operational feasibility.

In addition, this trial poses questions in terms of ethical feasibility, certainly in an outbreak situation, since for the duration of the study, the participants of the control group will be denied the intervention.

The applicant believes that running VLA1553-404 may be feasible if the study is conducted in countries where no chikungunya vaccine has been licensed (trial would likely be run as phase 3b), since in this case, participants are expected to potentially benefit from trial participation. However, it is not known whether randomizing vs. placebo/active control will be approved by local Ethics Committee, even if Ixchiq has not be licensed in the country (considering the data available on Ixchiq and knowing that the vaccine has been approved by EMA and FDA). The applicant considers to offer VLA1553 after the study as a potential mitigation, but whether this will be considered sufficient is not known. The feedback from local Ethics Committee is currently lacking on these topics. It is also unclear at this stage how the trial design will be affected in case Ixchiq is becoming licensed in study country(ies) during the trial (as it may not be ethically feasible anymore to randomise to a control group and Ethics Committees may consider that all control participants must be cross-vaccinated).

Ethical considerations of randomising vs. placebo/active control is cited by the Applicant as one of the operational aspects to assess. Unfortunately, no information has been provided yet on the views of the local Ethics Committees on that specific point (equipoise), which may be a major obstacle to the study implementation. Design adaptation might be needed. (REC).

2.6.6. Discussion on clinical efficacy

The current application includes data from 3 completed clinical studies conducted in generally healthy adults in the US: a Phase 1 dose finding study (VLA1553-101), a Phase 3 pivotal study (VLA1553-301), and a Phase 3 Lot-to-Lot consistency study (VLA1553-302). Data from study (VLA1553-303), an ongoing long-term follow-up of a subset of participants of study VLA1553-301, are also provided. It has been previously agreed by CHMP that efficacy trials are currently not feasible pre-authorization due to unpredictable and short-lived outbreaks. Therefore, the approach to rely on a threshold value of neutralizing antibodies to infer efficacy has been accepted. This approach is in alignment with the Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1).

Dose selection

The selection of the dose was based on the results obtained in the Phase 1 VLA1553-101 study which investigated a single immunization of VLA1553 in 120 CHIKV seronegative adults (18 to 45 years). The dose levels tested were of 3.2x103 TCID50/dose (low dose, 0.5mL), 3.2x104 TCID50/dose (medium dose, 1mL) and 3.2x105 TCID50/dose (high dose, 1mL). The neutralizing antibody titres at the peak of the response (28 days post-vaccination) were comparable between dose levels. A trend for lower response at 14 days post-vaccination was observed for the low dose when compared to both other dose levels. Considering also the reactogenicity and viraemia profile, the medium dose was selected for further clinical development.

Main trials

Methods

VLA1553-301 is a randomised, placebo-controlled, double-blind, multicenter, Phase 3 trial designed to assess the immunogenicity and safety of the final dose of VLA1553 (1x10E4 TCID50 per 0.5 mL) in generally healthy adults (≥18 years). The study was conducted at 43 sites in the US. Approximately 4,000 adult participants (including approximately 400 participants ≥65 years) were planned to be randomly allocated in a 3:1 ratio to VLA1553 or placebo. The sample size was driven by the need to accumulate a sufficient number of participants in the safety database. The inclusion and exclusion criteria were acceptable. Participants with well-controlled chronic conditions (defined as stable and on therapy for the past 6 months) were allowed to be enrolled. Participants immunocompromised due to medical condition or due to immunosuppressive treatments were excluded. History of (suspected) CHIKV infection and history of immune-mediated or clinically relevant arthritis or arthralgia were also part of the key exclusion criteria, as well as administration of any inactivated vaccine within 2 weeks before vaccination, or any live vaccine within 4 weeks before vaccination.

The first approximately 500 participants (including approximately 150 participants ≥65 years) enrolled at 12 pre-selected study sites were to be part of the Immunogenicity subset. The size of the Immunogenicity subset is based on the primary endpoint.

Participants received a single intramuscular administration of either VLA1553 or placebo in the deltoid region of the arm on Day 1. Vaccinated participants had immunogenicity blood samples taken at pre-vaccination baseline (Day 1), 7 days (Day 8), 28 days (Day 29), 84 days (Day 85), and 179 days (Day 180) after vaccination. Samples were taken from all participants at each timepoint. However, immunogenicity evaluations were performed only in the Immunogenicity subset. Participants were followed up for approximately 6 months following vaccination. Data up to 2 years post-vaccination are available from the ongoing follow up study VLA1553-303.

Following difficulties in recruiting elderly participants (≥65 years), several changes were implemented with Version 6.0 of the study protocol, resulting in a number of elderly participants in the Immunogenicity subset lower than initially planned. Still, the target number of elderly participants in

the safety population (n=463) reaches (and even exceeds) the initially planned number (n=407). The randomization was stratified by age using two age strata, i.e. Stratum A (18–64 years) and Stratum B (≥65 years) and by inclusion in the Immunogenicity subset. The process for blinding is overall deemed appropriate.

Overall, the design of the study is acceptable.

The primary endpoint of study VLA1553-301 is the proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 29 CHIKV neutralizing antibody titre μPRNT50 ≥ 150.

The threshold of CHIKV μPRNT50 antibody titre ≥150 has been discussed in several Scientific Advices. It was agreed that it can be considered as reasonably likely to predict protection and might be used to support MA even though it does not correspond to an established ICP.

Even if the exact mechanisms of protection are unknown, neutralizing antibodies have a major role in protecting against CHIKV infection and/or disease. The applicant proposed the threshold of 150 μPRNT50 based on data from a NHP passive transfer study using human samples from VLA1553-101. Non-human primates with human (transferred) VLA1553-antibody titres above the threshold of 150 μPRNT50 were shown to be protected from viraemia after a challenge with the wild-type La Reunion strain. This threshold is also in agreement with available sero-epidemiological data. In a prospective study in the Philippines (Yoon et al.), it was observed that individuals with PRNT80 titre ≥10 - reflecting a prior natural infection - experienced a lower frequency of symptomatic PCR confirmed CHIKV infections for the 2-year study period. Data also suggest they were protected from subclinical/asymptomatic infection (based on a rise in neutralizing antibody titres from baseline). A PRNT80 of 10 in the sero-epidemiological study of Yoon corresponds to a μPRNT50 of approximately 50 in the Applicant's μPRNT assay (based on upper limit of the 99% CI around the Geometric Mean ratio μPRNT50/PRNT80 using sera from 39 participants of the Yoon study tested with both assays). Despite several limitations for both the animal and the human data, the use of a threshold of 150 μPRNT50 is considered conservative.

Uncertainties remain around how this threshold actually translates into protection against CHIKV disease (including chronic arthritis) and/or infection, and therefore around the actual protection offered by VLA1553. Since VLA1553 is a live-attenuated vaccine, mechanisms of protection might resemble those resulting from natural infection. Immune responses after vaccination should be more broadly characterized and compared to those induced after natural infection. This would also support the likely protective effect of the VLA1553.

The primary immunogenicity assay used to measure CHIKV-specific neutralizing antibodies was a validated micro Plaque Reduction Neutralization Test (μPRNT). The μPRNT was performed at a central laboratory (Nexelis Laboratories, Canada). The strain used in the μPRNT assay is an attenuated strain from the Asian genotype (181/clone 25). The primary immunogenicity μPRNT assay is thus based on an heterologous strain, which is the same as the strain used in the Yoon et al. study. This approach is supported.

The Immunogenicity (IMM) population includes all vaccinated participants from the Immunogenicity subset who were CHIKV seronegative at baseline and had at least one evaluable post-baseline titre measurement. The cut-off to define CHIKV seronegativity at baseline was μPRNT50 titre <20, which corresponds to the lower limit of quantitation (LLOQ) of the assay. The per protocol (PP) population is based on the IMM population from which participants who had major protocol violations that could affect the assessment of immune responses were excluded. To compensate for the elderly participants lacking in the Immunogenicity subset compared to the initial plan, elderly participants were randomly selected from the safety analysis population and added up to the IMM population in order to constitute the Immunogenicity Elderly (eIMM) population. This approach is considered acceptable.

All analyses of immunogenicity were performed primarily on the PP population and secondarily on the IMM and eIMM populations. Two sensitivity analyses were also performed in populations defined in a similar way as the PP population but using respectively a higher threshold to define CHIKV seronegative at baseline (µPRNT50 ≤40) and an alternative definition of visit windows.

The primary analysis compared the proportion of participants achieving a Day 29 CHIKV µPRNT50 ≥150 (also referred to as seroresponse rate) against a non-acceptance threshold of 70%. The primary endpoint was evaluated based on the VLA1553 vaccinated participants only. In general, in the absence of an established ICP, the immunogenicity conclusion should be based on the difference between treatment groups. However, due to the absence of control group subjects with seroresponse in the present study, results obtained in the treatment group only can be expected to closely resemble corresponding between group comparisons. Furthermore, the Applicant provides corresponding between group comparisons as a secondary endpoints which support the immunogenicity conclusions based on the primary endpoint. The primary endpoint was evaluated using an exact binomial test and corresponding exact confidence interval to demonstrate statistical significance in terms of the proportion of participants above the threshold in excess of 70%. The corresponding analysis does not appear to be stratified by age cohort (as would be required by the Guideline on adjustment for baseline covariates in clinical trials EMA/CHMP/295050/2013). However, results across individual strata are highly consistent, such that no substantial difference in estimates between adjusted and unadjusted analyses is to be expected.

VLA1553-302 is a randomized, double-blinded, multicenter Phase 3 clinical study investigating 3 Lots of VLA1553 at the final selected dose (target 1x10E4 TCID50 per 0.5 mL). The study was conducted at 12 study sites in the US. It was designed to demonstrate manufacturing consistency of 3 Lots and expand the safety and immunogenicity database of the final dose of VLA1553. A total of 409 participants, 18-45 years of age, were randomized in a 1:1:1 ratio to each of the 3 Lot arms. Participants received a single intramuscular administration of 1 of the 3 Lots of VLA1553 in the deltoid region of the arm. Apart from the age range, eligibility criteria are comparable to those of the pivotal study VLA1553-301. Study duration, timepoints, procedures, and immunogenicity assessments were overall similar to study VLA1553-301. The primary endpoint was Day 29 CHIKV neutralizing antibody GMT as determined by µPRNT50 in baseline CHIKV seronegative participants. Consistency between Lots was considered demonstrated if the 95% CIs for Day 29 neutralizing antibody GMT ratios are all between the acceptance margins of 0.67-1.50. The primary immunogenicity assay was the same as in VLA1553-301. One key difference with study VLA1553-301 is however the definition of baseline serostatus, which is based on CHIKV ELISA antibodies, which is deemed acceptable, since the number of seropositive participants included in the study is very limited. Apart from that, the definition of the PP population is similar to VLA1553-301. The primary analysis was based on a pair-wise comparison of GMTs corresponding to the 3 Lots.

Study participants

A total of 4,128 participants were randomized in study **VLA1553-301** (3,093 in the VLA1553 arm and 1,035 in the placebo arm). Of the randomized participants, 501 were allocated to the Immunogenicity subset (respectively 375 and 126 in the VLA1553 and placebo arm). The Immunogenicity subset includes 110 participants ≥65 years. The PP population consisted in 362 participants (266 and 96 in the VLA1553 and placebo arm, respectively), including 82 participants ≥65 years (59 and 23 in the VLA1553 and placebo arms, respectively). A very high proportion (28%) of the participants were excluded from the Immunogenicity subset to constitute the PP population. The main reason for exclusion was the presence of a major protocol deviation (defined as protocol deviation that could impact characterisation of the immune response). The most frequent were due to participant attending the Visit out of window (exceeding +/- 8 days for Visit 3), participants who missed Visit 3, or vaccination of the participants with investigational product that underwent temperature excursion

during shipment or storage and was deemed not appropriate for further use, as a result from the Product Quality Assessment (i.e., unstable).

In total, 362 participants were included in the PP population of study **VLA1553-302** (122 in Lot 1 arm, 118 in Lot 2 arm, and 122 in Lot 3 arm). The percentage of exclusion from the PP population was much lower than in VLA1553-301 (11%).

In **VLA1553-301**, few participants had a history of vaccination against yellow fever (48/4,115) and Japanese encephalitis (3/4,115), suggesting that the recruited population does not encompass many travelers. Numbers were also very small in **VLA1553-302**.

Several of the studies' sites are located in Florida and Texas where local transmission of Dengue and CHIKV is reported. In VLA1553-301 and VLA1553-302, participants were tested at baseline for antibodies to CHIKV, Mayaro virus (alphavirus circulating in South America), Dengue virus and Zika virus. Of 4,057 participants from **VLA1553-301**, 36 (0.9%), 58 (1.4%), 90 (2.2%) and 282 (7.0%) were respectively baseline seropositive for CHIKV, Mayaro virus, Zika virus and Dengue virus. In study **VLA1553-302**, among those included in the safety analysis set (n=408), 12 (2.9%) participants were baseline seropositive for CHIKV, 12 (2.9%) for Mayaro virus, 5 (1.2%) for Zika virus and 18 (4.4%) for Dengue virus. A high degree of cross-reactivity in antibody responses is expected, especially of CHIKV vs. Mayaro virus.

*Main results of **VLA1553-301**:*

The primary endpoint was met. At 28 days post-vaccination, 98.9% (263/266) of the participants had an antibody titre of at least 150 µPRNT50 (95% CI of 96.7-99.8) in the VLA1553 arm (PP population). The lower bound of the 95% CI around the proportion exceeds by far the non-acceptance criterion of 70%. In total, 3 participants did not reach the threshold of 150 µPRNT50 in the VLA1553 arm. None of the placebo participants reached the threshold.

The percentages remain high up to Day 180 (96.3% [95% CI of 93.1-98.3, n=233/242]). In contrast, only 1.6% (95% CI of 0.4-4.0, n=4/251) of the participants vaccinated with VLA1553 (PP population) had antibody level ≥150 µPRNT50 at Day 8.

In the PP population, Day 8-GMTs was low both for the VLA1553 and placebo arms (<20 µPRNT50 [LLOQ of the assay]). Antibody titres in the placebo arm were low at each timepoint (GMT <20 µPRNT50, maximum µPRNT50 of 22). In the VLA1553 arm, humoral responses peaked at Day 29 with GMT of 3361.6 (95% CI: 2993.8-3774.4). GMT decreased up to Day 180, with a Day 85-GMT of 1083.6 (95% CI: 968.3-1212.6) and a Day 180-GMT of 752.1 (95% CI of 665.9-849.5). GMT at Day 180 were still higher than baseline GMT (<20 µPRNT50).

Proportions of baseline seronegative participants with antibody titres post-vaccination of at least µPRNT50 250 are overall similar to those found when the threshold was set at the µPRNT50 150. CHIKV-specific neutralizing antibody titres post-vaccination increased 3.9-fold (mean value) 7 days after vaccination when compared to baseline and peaked at Day 29 with a 470.8-fold increase versus baseline titres. At Day 180, titres were still 107.2-fold higher than baseline titres.

Results obtained with the IMM, eIMM, sPP and sPP2 were overall similar. Results were also similar in both age categories (18-64 years and ≥65 years). For all the timepoints tested (except Day 8) in both age categories, the lower bound of the 95% CI are above 85%.

*Main results of **VLA1553-302**:*

GMT ratios were 1.08 (Lot 2/Lot 1), 1.06 (Lot 2/ Lot 3) and 1.02 (Lot 3/Lot 1) with 95% CI of 0.81-1.44 (Lot 2/Lot 1), 0.80-1.41 (Lot 2/ Lot 3) and of 0.77-1.36 (Lot 3/Lot 1). The 3 pair-wise (Lot 2/Lot

1, Lot 3/Lot 2, Lot 3/Lot 1) 95% CIs for Day 29-GMT ratios were between 0.67 and 1.5. These margins were pre-defined as acceptance margins for demonstrating equivalence of the 3 Lots.

Day 8-GMTs were very low and similar to baseline (<20 µPRNT50 for the 3 Lots combined). GMTs peaked at Day 29 (2643.2 for the 3 Lot arms combined, 95% CI: 2354.0-2967.9). GMTs decreased at Day 85 when compared to Day 29-GMTs, with a GMT (95% CI) for the 3 Lot arms combined of 846.1 (95% CI: 762.7-938.7). Day 180-GMTs tended to be lower as compared to Day 85 but were still higher than GMTs at baseline (GMT of 708.8, 95% CI: 639.0-786.2 for the 3 Lots).

Proportions of baseline seronegative participants with post-vaccination antibody titres above the defined threshold reasonably likely to predict protection (µPRNT50 ≥150) are in line with VLA1553-301, with 0% (95% CI: 0.0-1.1) and 97.8% (95% CI: 95.6-99.0) of participants reaching this antibody level at Day 8 and Day 29, respectively. Proportions remain high up to Day 180. Results obtained in the FAS, sPP1 and in sPP2 were similar.

Persistence of antibodies:

Antibody results (as measured by µPRNT) observed in studies VLA1553-101 and VLA1553-303 indicate that the single dose regimen induces sustained antibody titres up to 2 years post-vaccination, with a proportion of baseline seronegative participants with antibody titres ≥150 µPRNT50 of 97.1% (95% CI:94.4, 98.7). Participants from VLA1553-303 will be followed for 5 years post-vaccination.

Data on re-vaccination:

Re-vaccination 6 to 12 months post-first dose with the high dose level (3.2x105 TCID50) was assessed in the Phase 1 VLA1553-101 trial. No viraemia was detected after the re-vaccination in any of the 23 participants primed with the medium dose level (a dose comparable to the final dose level) within 14 days after re-vaccination (in contrast with 27/30 after a single final dose of VLA1553), suggesting protection against a challenge with the vaccine virus. Immunogenicity results are hardly interpretable in terms of boostability (anamnestic antibody responses).

Data in baseline CHIKV seropositive participants:

Limited data in participants with pre-existing immunity to CHIKV were obtained in VLA1553-301, VLA1553-302 and VLA1553-321.

The very limited data from VLA1553-301 and VLA1553-302 (n<10 and n<15 respectively, exact number depending on assay/threshold used to define seropositivity) suggest that no booster effect is observed in CHIKV seropositive participants with high baseline CHIKV neutralizing antibody titre. In contrast, VLA1553 might induce an increase in antibody titres in participants with low antibody titres at baseline. These results suggest that that low neutralising antibody titres might not be able to neutralise the attenuated 181/25 CHIKV strain, and hence might not be able to protect from re-infection. Data in CHIKV seropositive participants from study VLA1553-321 in endemic area (n=52) suggest the absence of booster effect, as Day 29 GMT in the VLA1553 arm are similar as Day 1 and Day 8 GMTs. As stratified analyses are not provided, it is not known if a booster effect would be observed in subjects with lower antibody titres.

Concomitant medications in VLA1553-301 and VLA1553-302:

Anti-inflammatory/anti-rheumatic products and analgesics were recorded as concomitant medication in the VLA1553 group in, respectively, 24.8% and 28.2% of the participants of the PP population. Overall, the GMTs and proportion of participants with seroresponse were not influenced by concomitant use of anti-inflammatory and anti-rheumatic products or analgesics in a relevant time period. The immune response in participants who used these concomitant medications was comparable to participants who did not use this concomitant medication. No data are however provided with respect to the impact of

prophylactic administration of antipyretics within 4 hours prior to and during the first 72 hours after vaccination on the immune responses.

Data on cross-neutralisation of wild-type CHIKV strains from studies VLA1553-101 and VLA1553-301:

CHIKV strains of different genotypes are considered to constitute a single serotype and antibodies specific to one genotype are thought to have the ability to also cross-neutralize strains from any other genotype. However, results from the limited literature available suggest that neutralizing antibodies specific to a given genotype (induced either after natural infection or vaccination) might specifically cross-neutralize an heterologous genotype at levels that are not always equivalent in terms of magnitude and kinetic to the one measured against the homologous genotype (Chusri 2014, Chua 2016, Auerswald 2018, Goo 2016).

The primary immunogenicity assay in the pivotal trial VLA1553-301 is a µPRNT assay based on an heterologous strain from the Asian genotype (181/clone 25 strain). The applicant indicated that the aim of using an heterologous strain is to show that vaccine-induced antibodies have the capacity to neutralize an heterologous strain. However, the strain is an attenuated CHIKV strain which might be more easily neutralized in *in vitro* assays compared to virulent wild-type CHIKV isolates/strains.

Sets of sera (from 12 participants randomized to different dose levels of VLA1553-101 [n samples=47], 32 [n samples=72] and 30 participants [n samples=120] of VLA1553-301/-303) were tested for cross-neutralisation of CHIKV 181/clone 25, La Réunion strain, West African strain 37997, an Asian/Caribbean M109 strain or a more recent Brazilian ECSA isolate (7124). Baseline samples and post-vaccination samples (different timepoints) were tested in classical PRNT assays performed in 2 different laboratories (UTMB and OHSU). Results suggest that VLA1553-induced antibodies are able to neutralize wild-type CHIKV strains from the 3 CHIKV genotypes. Although the level of VLA1553-induced antibodies needed to (cross) protect is not known, results suggest that a (similar) protective effect against homotypic and heterotypic CHIKV strains/isolates might be expected. Still the protective effect of VLA1553 remains to be demonstrated.

The applicant plans to test the longitudinal samples from the same 30 participants of studies VLA1553-301/-303 against the West African strain 37997 as well as an Asian/Caribbean strain, which is supported.

Whether these data will be sufficient to demonstrate the capability of the VLA1553-induced antibodies to (cross-)neutralise CHIKV strains from all genotypes should be assessed upon result submission. It is recommended to test for additional more recent isolate(s).

Data on cross-neutralisation of alphaviruses from study VLA1553-301:

Cross-reactivity of the VLA1553-induced antibodies was tested for O´nyong-nyong virus (UgMP30), Mayaro virus (BeAr505411) and Ross River virus (T-48) in classical PRNT. Data were obtained for longitudinal samples from 30 participants seronegative at baseline from studies VLA1553-301/-303. Although the level of VLA1553-induced antibodies needed to (cross-)protect is not known, results suggest that some protective effect against ONNV and MAYV might be expected. Still, this remains to be demonstrated.

Comparison of the responses elicited after VLA1553 vaccination versus natural infection:

VLA1553-induced immune responses were compared to responses elicited after natural infection in sera obtained from different sources (from study of Yoon [n=27], from study of Powers [n=12], analysed at OHSU [n=9], and from UTMB [n=6]). The limited data (obtained with different assays on a limited number of samples) and the difference in time of the sampling between post-vaccination versus post-infection, which is sometimes unknown, hamper the comparison. Overall, antibody titres induced

by VLA1553 are either in the same range or lower than induced after natural infection, which sometimes occurred years before sampling.

This is confirmed in study VLA1553-321 in endemic area. In this study, the Day 29-GMT observed in the baseline CHIKV seronegative participants are comparable to the baseline GMT of baseline CHIKV seropositive participants, i.e. to the titres elicited by the natural infection. The time elapsed between the natural infection and the sampling is unknown (but on average likely much longer than one month), and thus the antibody level reached shortly after natural infection is unknown, but likely much higher than the level induced by the vaccine.

It is considered that only limited data have been generated from samples isolated after natural CHIKV infection. No conclusion can be drawn on the level of neutralizing antibodies induced by VLA1553 compared to the one induced by natural infection.

The applicant intends to test an additional panel of sera collected from 6 baseline CHIKV seropositive participants of VLA1553-321 study in PRNT assays specific to $CHIKV_{LR2006}$, $CHIKV_{Brazil-7124}$, $CHIKV_{181/25}$ and $MAYV_{BeAr505411}$. In addition, negotiations are ongoing to access samples from acute, convalescent (collected 2-3 weeks after acute samples), 3 months, 6 months and 12 months after PCR confirmed CHIKV infection in approx. 20 adolescents (ages 12-14 yrs) of Nicaragua, which will be tested as a minimum against the $CHIKV_{181/25}$ strain and - if feasible– against additional more recent CHIKV strains (e.g., LR2006, Brazil 7124).

VLA1553-induced innate and cellular immune responses were not characterised in the clinical studies and hence not compared to those after natural infection. The applicant will consider collaborations with external partners to study the cellular mediated immune response induced by VLA1553 and the Applicant will also consider studies to better characterize the humoral responses induced by VLA1553, in particular at early time-points after vaccination.

Plan to estimate VLA1553 effectiveness in the post-approval period:

Although immunogenicity results of the VLA1553-301 are compelling, they are based on a neutralizing antibody titre threshold reasonably likely to predict protection (supported by animal and sero-epidemiological data) and not on an established immune correlate of protection. Uncertainties remain around how this threshold actually translates into protection against disease and/or infection. Therefore, the actual protection of VLA1553 remains to be confirmed, and eeffectiveness data are needed as soon as possible to substantiate the clinical benefit of VLA1553.

Two effectiveness studies are planned to be conducted post-approval, an observational test-negative case-control (TNCC) effectiveness study (VLA1553-402) planned to be conducted in Brazil and a multi-country randomized, controlled trial with pragmatic elements to estimate the VE and safety of VLA1553 (study VLA1553-404).

Only concept protocols were submitted and no feasibility evaluations were completed yet.

Study VLA1553-402 will use a test-negative case-control (TNCC) design. In principle, a TNCC design could be appropriate, provided that it is feasible, and that the Applicant can demonstrate that it can lead to reasonably unbiased estimates. The primary objective of VLA1553-402 is to estimate the vaccine effectiveness (VE) of VLA1553 in the prevention of RT-PCR confirmed Chikungunya. The study design does not allow for an estimation of the VE in preventing chronic Chikungunya (arthritis), which greatly contributes to the burden of disease. To be included in the study, participants must have a CHIKV RT-PCR testing result available in routine databases. Participants who meet the eligibility criteria will be classified into cases or controls based on the results of the CHIKV RT-PCR testing available. Vaccinated participants will be defined as those who received VLA1553 ≥14 days before the onset of

symptoms. The target sample size consists in 446 cases and 892 controls. For the data collection (exposure, outcome and covariates), the study will rely on routine databases.

The primary objective of the study VLA1553-404 is to assess vaccine effectiveness in preventing symptomatic virologically-confirmed CHIKV disease among adults, compared to control participants during the same trial period, both overall and by age groups. Secondary objectives include the evaluation of vaccine effectiveness against occurrence of chronic CHIKV disease and CHIKV disease leading to hospitalization in confirmed and suspected cases of CHIKV disease. CHIKV baseline serostatus will be assessed in all participants. Participants will be assigned at random to either the VLA1553 vaccine or a placebo/active control in a 1:1 ratio. The applicant targets active control. After vaccination, participants will be passively followed during all the trial period, with instructions to come back to site in case of chikungunya-like symptoms. A dried blood spot (DBS) will be collected for virological confirmation of CHIKV infection. Participants with confirmed or suspected CHIKV infections will be evaluated for chronic CHIKV disease or CHIKV-related hospitalization. The study duration is currently estimated to be at least 3 years. There will be no immunogenicity assessments in this study. No samples will be taken beside the baseline sample and samples taken in the context of a Visit for chikungunya-like symptoms. A sample size of approximately 20,000 participants is currently targeted. Initial interactions with different countries in different continents are taking place to obtain information historic/current chikungunya epidemiological data and clinical trial infrastructure. Still interactions are not yet much advanced.

Since feasibility of collecting data, epidemiological situation and vaccination uptake are difficult to anticipate and may depend on the country/region, the plan for conducting two studies, with 2 different designs, intended to be conducted at multiple sites in different countries, increases the likelihood to generate VE data.

The designs selected are scientifically appropriate and the combination of both studies is supported.

VLA1553-402 is an observational study, with all the known limitations associated with the non-randomized design (potential bias and confounding, including from unmeasured confounders) and with the reliance on routine care and routine databases. However, it may provide clinically relevant results, which may become available before those of the VLA1553-404 trial. Overall, although the reasons for planning the study in Brazil are acknowledged, and the approach of the Applicant is strongly encouraged, the feasibility of study VLA1553-402 in Brazil remains uncertain. Strong reinforcement and adaptation of the routine care practices and of the surveillance system will be needed before study onset.

VLA1553-404 is expected to generate the highest level of evidence, as it is a randomized trial. The study will address several of the concerns raised for study VLA1553-402, as it will provide estimates of VE against chronic chikungunya and estimates of VE by serostatus at baseline. The study will be challenging on the feasibility point of view. Due inclusion of placebo/comparator (non-chikungunya vaccine) arm, the study poses question in terms of ethical feasibility, particularly in an outbreak setting. The views of local Ethics Committee are not known at this stage. Conducting this study will be challenging due to the sporadic and short-lived nature of chikungunya outbreaks, along with the season-dependent and geographically inconsistent transmission, with very high spatio-temporal heterogeneity of transmission. Although this also applies to study VLA1553-402, this is deemed even more impacting for study VLA1553-404.

Overall, the challenges associated with these studies are acknowledged, but the approach proposed with the combination of two studies is considered the best way forward at the moment and is highly supported.

2.6.7. Conclusions on the clinical efficacy

VLA1553 is a live-attenuated vaccine based on the La Reunion strain (LR2006-OPY1) of East Central South African genotype. It has been evaluated in two Phase 3 studies conducted in adults ≥18 years in the US.

The primary endpoint of the pivotal trial VLA1553-301 was met, with 99% of the baseline CHIKV seronegative participants vaccinated with a single dose of VLA1553 achieving the predefined CHIKV-specific neutralizing antibody titre threshold (μPRNT50 ≥150) at Day 29, and a LB 95% CI at 97%. Only 3/266 VLA1553 vaccinated participants did not reach the threshold. None of the participants reached the threshold in the placebo arm. Proportion of participants achieving the threshold was still high up to 2 years post-vaccination (97.1%, 95% CI of 94.4-98.7).

At Day 8 post-vaccination, no relevant neutralizing antibody response was detected and only 4/251 participants had a μPRNT50≥150.

Neutralizing antibody GMTs peak at Day 29 and decrease up to Day 180 (although remaining above the baseline titres). GMT at 2 years post-vaccination was overall comparable to that observed at Day 180.

No relevant differences in terms of neutralizing antibody responses are observed between age categories (18-64 years and ≥65 years).

Results are consistent across the populations of analyses, and in the sensitivity analyses. Results are also similar in the VLA1553-302 Lot-to-Lot consistency study.

Limited data suggest that VLA1553-induced antibodies are able to cross-neutralize wild-type CHIKV strains from 3 CHIKV genotypes (IOL/ECSA, West African and Asian) and also closely related alphaviruses. More data are still expected with respect to cross-neutralization of wild-type CHIKV strains of different recently circulating genotypes.

There are no efficacy data. The conduct of efficacy trials was considered not feasible pre-authorization due to unpredictable and short-lived outbreaks. There is no established immune correlate of protection (ICP) for Chikungunya.

The primary endpoint of the pivotal trial is based on a CHIKV-specific neutralizing antibody titre threshold considered reasonably likely to predict protection. The threshold is based on both animal challenge studies (using passively transferred sera from human vaccinees) and supported by sero-epidemiological data. Uncertainties remain around how this threshold actually translates into protection against disease (including chronic chikungunya) and/or infection.

Therefore, although immunogenicity results of the pivotal trial VLAA553-301 are compelling and demonstrated that a single dose of VLA1553 induces robust CHIKV-specific neutralizing antibody responses largely achieving the primary endpoint, their clinical relevance remains uncertain. The actual protection conferred by VLA1553 needs to be confirmed.

Two effectiveness studies are planned post-approval, a test-negative case-control effectiveness study (VLA1553-402) planned to be conducted in Brazil and a randomized, controlled trial with pragmatic elements to estimate the VE and safety of VLA1553 (study VLA1553-404) planned to be conducted in different countries/regions. Since feasibility of collecting data, epidemiological situation and vaccination uptake are difficult to anticipate and may depend on the country/region, the plan for conducting two studies, with 2 different designs and intended to be conducted at multiple sites in different countries, increase the likelihood to generate VE data in a timely manner.

The applicant is recommended to conduct and submit the results of study VLA1553-402 (**REC**).

The CHMP considers the following measures necessary to address issues related to efficacy:

Post-authorisation efficacy study (PAES): In order to confirm the efficacy of Ixchiq in individuals 18 years and older, the MAH should conduct, according to an agreed protocol, and submit the results of, a randomized, controlled trial with pragmatic elements to assess the effectiveness of Ixchiq vaccination in the prevention of symptomatic, laboratory confirmed chikungunya after a single vaccination with Ixchiq in adults in endemic areas.

2.6.8. Clinical safety

2.6.8.1. Introduction

The applicant provided a pooled Safety Population consisting of 4,643 participants from the 3 completed clinical VLA1553 studies after 1 vaccination: VLA1553-301 (placebo-controlled Phase 3 US study in adults – lyophilized formulation – targeted dose), VLA1553-302 (lot-to-lot consistency Phase 3 US study in adults – lyophilized formulation – targeted dose), and VLA1553-101 (dose-response Phase 1 US study in adults – liquid formulation – 3 different doses). 3,610 participants were randomized and vaccinated with VLA1553 (3,082, 408, and 120 participants, respectively) and 1,033 participants randomized and vaccinated with placebo (VLA1553-301). All 120 participants in VLA1553-101 were vaccinated with the liquid formulation and 30 participants received the current used dose (arm M), 31 participants received a lower dose (arm L) and 59 participants received a higher dose (arm H). These participants were also included in the pooled safety population.

There is an ongoing placebo-controlled Phase 3 study in adolescents in an endemic country (Brazil) (VLA1553-321) for which supportive safety data in seropositive participants have been provided in this application. The CSR with cut-off date of 26 Jul 2023 has been provided and includes safety data for all participants up to 28 days post-vaccination (Part A). Six month safety data will be provided in Part B post-authorisation.

In addition, there's an ongoing US long-term study VLA1553-303 (antibody persistence and long-term safety Phase 3b study in adults). Only new information on ongoing SAEs and AESI from precursor study VLA1553-301 and new SAEs since the end of study VLA1553-301 were reported in study VLA1553-303.

In the following sections, safety data is presented by pooled safety population and/or per study, as relevant.

3082 participants out of the 3610 vaccinated participants in the pooled safety population are from study VLA1553-301. Therefore, the pooled safety population is representative from this study and the data from this study is not always presented separately. The data are presented for the pooled safety population, and also for the ongoing studies VLA1553-321 and VLA1553-303 independently.

2.6.8.2. Safety data collection

Reactogenicity data were collected through Subject eDiary starting around from vaccination and until 14 days post vaccination in the phase 1 study VLA1553-101 or up to 10 days post vaccination in the phase 3 (studies VLA1553-301 and VLA1553-302). Solicited injection site reactions included injection site pain, tenderness, erythema/redness, induration and swelling. Solicited systemic reaction included fever, fatigue, headache, nausea/vomiting, myalgia/muscle pain, arthralgia/joint pain, and rash. All grade 4 solicited injection and systemic AEs were classified as SAE.

Unsolicited safety information was collected through an eMemory/Subject Diary 2 until the end of study. For each AE the severity, causality, outcome, seriousness, medically attended, action taken to treat AE, action taken on investigational medicinal product and start and stop dates were recorded.

These data were entered into the electronic case report form (eCRF) by the investigator. The United States FDA raised concerns that not each AE eDairy entry was transcribed across to the AE eCRF page and that there could be ambiguities of a Subject eDiary entry was to be considered a solicited event. The re-mapping required by the FDA did not change the safety evaluation of studies VLA1553-301 and VLA1553-302: performed sensitivity analysis (AE re-mapping) showed negligible changes on numbers.

For all subjects, **any AEs and SAEs** were collected during the entire study period (i.e. up to Day 180 for studies VLA1553-301 & VLA1553-302).

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

Sponsor definition in protocols:

In VLA1553-301 and VLA1553-302, a chikungunya-like adverse reaction was defined when a participant reported: fever (≥ 38.0 ° C / 100.4 ° F) and onset of symptoms occurring 2 to 21 days after vaccination for a duration of at least 3 days such as: arthralgia/arthritis and back pain and/or neurological symptoms and/or cardiac symptoms.

In VLA1553-321, a chikungunya-like adverse reaction was defined when a participant reported: fever (≥ 37.8 ° C) and onset of symptoms occurring 2 to 21 days after vaccination for a duration of at least 3 days such as: arthralgia/arthritis, myalgia, neurological symptoms.

In VLA1553-101, a chikungunya-like adverse reaction was defined when a participant reported (onset of symptoms occurring 2 to 28 days after vaccination for a duration of at least 3 days):

- Early systemic symptoms including sudden onset of fever, myalgia, headache, back pain and macular to maculopapular rash, sometimes with cutaneous pruritus (foot arch) and oedema of the face and extremities, polyadenopathies;
- Acute (poly)arthritis most frequently in the extremities (wrists, ankles and phalanges), often symmetric;
- Tenosynovitis;
- Neurological symptoms (e.g. confusion, optic neuritis, meningoencephalitis or polyneuropathy);
- Cardiac symptoms (e.g. myocarditis).

The events have been captured 2 to 21 days post-vaccination (VLA1553-301, VLA1553-302, VLA1553-321) or 2 to 28 days post-vaccination (VLA1553-101). The cluster of symptoms defined above but starting after 28 days until study end is defined as late onset AESI. In study VLA1553-303, any AESI ongoing from study VLA1553-301 was followed-up.

For each study, an independent unblinded Data Safety Monitoring Board (DSMB) met to review accumulating safety data on a regular basis until the last participant had completed the 6-month visit. In addition, the DSMB periodically reviewed accruing safety information throughout the study, as applicable. During the safety reviews, all cases of SAEs, deaths, and solicited and unsolicited AEs, as well as adjudication of all AESI (sponsor definition), were assessed.

Broader definition of AESI

Chikungunya-like adverse reactions have been retrospectively evaluated using a broader definition of AESI (broader than the one initially used in the clinical protocols): occurrence of fever (≥38° C) and at least one other symptom also reported for acute-stage chikungunya illness, including arthralgia or arthritis, myalgia, headache, back pain, rash, lymphadenopathy, and certain neurological, cardiac or ocular symptoms (i.e. optic neuritis, retinitis, and uveitis); within 30 days after vaccination, regardless of time of onset, severity or duration of the individual symptoms.

2.6.8.3. Patient exposure

2.6.8.3.1. Participant population

Completed and Pooled Safety Studies: VLA1553-301, VLA1553-302, and VLA1553-101

Study VLA1553-301 is the phase 3 study with the largest cohort with a safety population of 3,082 participants vaccinated with VLA1553 and 1,033 participants with placebo. In study VLA1553-302 (lot-to-lot consistency study), the safety population consists of: Lot 1 (n= 136), Lot 2 (n= 137) or Lot 3 (n= 135) (total of 408). Each lot received one dose of VLA1553. For study VLA1553-101, only the safety data after the first dose were included in the pooled safety data: 120 participants were vaccinated with either low dose (n=31), medium dose (n=30) or high dose (n=59). The safety data after the re-vaccination is discussed in section 2.6.8.9.6.

In the pooled safety population, the percentage of participants who reached up to 28- and 180-days post vaccination was similar between the VLA1553 and placebo arms. A total of 3,179 (88.1%) participants completed the full study period which is comparable with 921 participants (89.1%) of the placebo arm (Table 32).

The percentage of participants who discontinued from the studies was comparable between the VLA1553 and placebo arms (11.9% vs. 10.9%, respectively), including withdrawals due to death or AE leading to withdrawal, see sections 2.6.8.7.1. and 2.6.8.11., respectively.

Table 32. Pooled Safety Population: Participants Overview

Subject Overview	Pooled VLA1553[a]	Placebo
Subjects Vaccinated, n	3,609[b]	1,034
Pooled Safety Population, n	3,610[c]	1,033[c]
Day 29[d], n (%)	3,442 (95.3)	991 (95.9)
Day 180[d], n (%)	3,196 (88.5)	918 (88.9)
Completed Study, n (%)	3,179 (88.1)	921 (89.1)
Discontinued Study, n (%)	430 (11.9)	113 (10.9)

Abbreviations: eCRF=electronic case report form; GCP=Good Clinical Practice; ID=identification.
a. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
b. One participant in study VLA1553-301 was randomized at two different sites and was vaccinated twice with VLA1553 and thus excluded from the Pooled Safety Population under each participant ID due to violation of GCP.
c. One participant in study VLA1553-301 was randomized to the placebo arm but was vaccinated with VLA1553 and was thus allocated to the actual treatment received (VLA1553) in the Pooled Safety Population.
d. Participants are included at the timepoint if they have data entered in the eCRF at that Visit or an Early Termination visit within the visit window.

Ongoing Studies: VLA1553-303 and VLA1553-321Study VLA1553-321 (Part A data cut-off date: 26 Jul 2023; 28 days follow-up)

A total of 753 adolescents were stratified by their µPRNT baseline serostatus: 614 participants to the seronegative stratum (µPRNT50 ≤40) and 139 participants to the seropositive stratum. Within the

seronegative stratum, 408 participants received VLA1553 (66.4%) and 206 participants received placebo (33.6%). In the seropositive stratum, 94 participants received VLA1553 (67.6%) and 45 participants received placebo (32.4%). Therefore, there the proportion of seropositive participants are very similar in both groups.

In each study arm, there were 2 participants who discontinued the study: 2 lost to follow-up in the VLA1553 arm and 2 withdrawal by participants in placebo arm, all were seronegative. No death or AE leading to discontinuation were reported in this study.

Study VLA1553-303 (cut-off date: 12 Oct 2023; 2 year follow-up)

This is an ongoing open-label trial in adults consisting of seronegative participants who received VLA1553. 393 participants from study VLA 1553-301 were invited to participate into the study. All participants completed scheduled visits at Day 180 (Visit 0) and Year 1 (Visit 1) (Part A analysis), and 317 (87.3%) completed the Year 2 Visit (Part B of the trial). Reasons for trial discontinuation were lost to follow-up (27/47 participants, 57.4%), withdrawal by participant (14/47 participants, 29.8%), moving (3/47 participants, 6.4%), physician decision (1/47 participant, 2.1%), not met inclusion-exclusion criteria (1/47 participant, 2.1%), and death (1/47 participant, 2.1%: severe drug overdose not related to the vaccine). No AE leading to discontinuation were reported in this study.

2.6.8.3.2. Demographic and other characteristics

Completed and pooled Safety Studies: VLA1553-301, VLA1553-302, and VLA1553-101

Table 33. Demographic and Other Baseline Characteristics for Completed Studies: Studies VLA1553-301, VLA1553-302, VLA1553-101 (Pooled and Study Safety Populations)

	Completed Trials				
	Pooled Studies[a]	VLA1553-301		VLA1553-302	VLA1553-101
	VLA1553 (N=3,610)	VLA1553 (N=3,082)	Placebo (N=1,033)	Total (N=408)	Total (N=120)
Gender, n (%)					
Female	1,919 (53.2)	1,682 (54.6)	569 (55.1)	223 (54.7)	14 (11.7)
Male	1,691 (46.8)	1,400 (45.4)	464 (44.9)	185 (45.3)	106 (88.3)
Race, n (%)					
White	2,867 (79.4)	2,456 (79.7)	853 (82.6)	315 (77.2)	96 (80.0)
Black or African American	530 (14.7)	451 (14.6)	122 (11.8)	62 (15.2)	17 (14.2)
Asian	74 (2.0)	51 (1.7)	17 (1.6)	18 (4.4)	5 (4.2)
Other	92 (2.5)	84 (2.7)	31 (3.0)	7 (1.7)	1 (0.8)
American Indian or Alaska Native	33 (0.9)	27 (0.9)	5 (0.5)	5 (1.2)	1 (0.8)
Native Hawaiian or other Pacific Islander	14 (0.4)	13 (0.4)	5 (0.5)	1 (0.2)	0
Age[b] (years)					
Mean (SD)	43.3 (15.14)	45.1 (15.44)	45.0 (15.59)	33.2 (7.40)	32.5 (6.6)
Median	42.0	45.0	45.0	34.0	33.0
Min, Max	18, 88	18, 88	18, 94	18, 45	19, 45
Age Category[b], n (%)					
≥ 18 to 64 years	3,264 (90.4)	2,736 (88.8)	916 (88.7)	408 (100.0)	120 (100.0)
≥ 65 years	346 (9.6)	346 (11.2)	117 (11.3)	0	0
Age Group[b,c], n (%)					
18-45 years	2,084 (57.7)	1,556 (50.5)	525 (50.8)	408 (100.0)	120 (100.0)
46-64 years	1,180 (32.7)	1,182 (32.7)	391 (37.9)	0	0
65-74 years	287 (8.0)	287 (7.9)	97 (9.4)	0	0
75-84 years	54 (1.5)	54 (1.5)	19 (1.8)	0	0
≥85 years	5 (0.1)	5 (0.1)	1 (0.1)	0	0
BMI[d] (kg/m^2)					
Mean (SD)	30.2 (7.39)	30.5 (7.44)	29.97 (7.091)	29.402 (7.4543)	25.8 (2.9)
Median	29.0	29.42	28.86	28.205	25.8
Min, Max	14, 102	14.1, 102.3	16.6, 63.1	13.65, 72.80	19.0, 29.9

Abbreviations: BMI=Body Mass Index; Max=maximum; Min=minimum; SD=standard deviation.
a. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
b. In studies VLA1553-302 and VLA1553-101, the upper age limit was set at 45 years of age, whereas in study VLA1553-301, no upper age limit was established.
c. Hand-calculated age group values for VLA1553 in study VLA1553-301.
d. In study VLA1553-101, a BMI threshold between 18.5 to <30 kg/m^2 was established for eligibility, whereas no such limits were established for studies VLA1553-301 and VLA1553-302.
Source: Module 5.3.5.3, Pooled Analysis, Table P.1.3.1,
Module 5.3.5.1, study VLA1553-301, Table 14.1.2.1,
Module 5.3.1.2, study VLA1553-302, Table 14.1.2.1,
Module 5.3.5.1, study VLA1553-101, Table 3.2.1.1

Gender: In the pooled safety data, there were slightly more female (53.2%) than male (46.8%). For VLA1553-301, similar frequencies are observed in the vaccine (54.6% female vs. 45.4% male) and the placebo arm (55.1% vs. 44.9%). In study VLA1553-302, there were 54.7% of female and 45.3% of male. For VLA1553-101, there were much less female participants enrolled compared to males (11.7% vs 88.3%) because of the exclusion of woman of childbearing potential.

Race: In the pooled safety data, the majority of the participants were white (79.4%). This was consistent amongst all 3 studies and comparable between VLA1553 vaccine arm and placebo in study VLA1553-301. Black or African American was the second most prevalent race (n= 530, 14.7%).

Age: The median age in the pooled safety study is 42.0 year with an age range between 18 and 88 year. The age in VLA1553-302 and VLA 1553-101 was restricted between 18 and 45 year resulting in a slightly lower median of 34.0 year and 33.0 year respectively. In study VLA1553-301, in the VLA1553 arm, there

were: 1,556 (50.5%) participants aged 18-45 year, 1,182 (32.7%) participants aged 46-64 year, 287 (7.9%) participants aged 65-74 year, 54 (1.5%) participants aged 75-84 year, and only 5 (0.1%) participants above 85 years. The age distribution between the vaccinated and placebo arm is considered comparable (50.8%, 37.9%, 9.4%, 1.8%, and 0.1% in each age categories, respectively).

BMI: The median BMI was 29.0 kg/m^2 across the pooled data set. This is comparable between VLA1553-301 and VLA1553-302. Study VLA1553-101 had a BMI threshold between 18.5 to <30kg/m^2 and consequently had a lower median BMI of 25.8 kg/m^2.

Seropositivity (see section 2.6.8.9.2.): In study VLA1553-301, a total of 22 participants tested positive for CHIKV at baseline using baseline threshold µPRNT50 ≥20 (16 in the VLA1553 arm and six in the placebo arm). In study VLA1553-302, a total of 12 participants were seropositive at baseline based on ELISA assay, while 14 participants were tested seropositive at baseline based on µPRNT assay.

Ongoing Studies: VLA1553-303 and VLA1553-321

Table 34. Demographic and Other Baseline Characteristics for Ongoing Studies: Studies VLA1553-303 and VLA1553-321 (Study Safety Populations)

	Ongoing Trials		
	VLA1553-303[a]	VLA1553-321[b]	
	VLA1553 (N=363)	VLA1553 (N=502)	Placebo (N=252)
Gender, n (%)			
Female	207 (57.0)	269 (53.6)	137 (54.4)
Male	156 (43.0)	233 (46.4)	115 (45.6)
Race, n (%)			
White	280 (77.1)	167 (33.3)	78 (31.0)
Black or African American	52 (14.3)	66 (13.1)	31 (12.3)
Asian	6 (1.7)	2 (0.4)	0
Other	20 (5.5)	145 (28.9)	69 (27.4)
American Indian or Alaska Native	2 (0.6)	2 (0.4)	2 (0.8)
Native Hawaiian or other Pacific Islander	3 (0.8)	0	0
Multiracial	0	120 (23.9)	72 (28.6)
Age (years)			
Mean (SD)	47.7 (14.15)	14.5 (1.70)	14.4 (1.66)
Median	49.0	15.0	14.0
Min, Max	18, 78	12, 17	12, 17
Age Category, n (%)			
12 to <18 years	0	502 (100)	252 (100)
≥ 18 to 64 years	310 (85.4)	0	0
≥ 65 years	53 (14.6)	0	0
Body Mass Index (kg/m^2)			
Mean (SD)	31.00 (8.926)	21.63 (4.809)	21.78 (4.754)
Median	29.30	20.36	20.87
Min, Max	17.7, 102.6	13.86, 45.19	14.01, 44.44

Abbreviations: Max=maximum; Min=minimum; SD=standard deviation.
a. Adult participants included in open-label study VLA1553-303 were vaccinated with VLA1553 at Day 1 in study VLA1553-301.
b. Adolescent participant 12 to <18 years of age.
Source: Module 5.3.5.2, study VLA1553-303, Table 3.2.1.1
Module 5.3.5.1, study VLA1553-321, Table 14.1.2.1

Study VLA 1553-303

Participants from study VLA 1553-301 were invited to participate into the study. Therefore, as expected, the characteristics of the population are comparable with study VLA1553-301.

Study VLA1553-321

Study VLA1553-321 is performed in adolescents 12 to 18 year-of-age (yoa). In the VLA1553 arm, the median age is 15 year and there are 53.6% females (46.4% males) (comparable with the placebo arm: median age of 14 year, 54.4% females, 45.6% males).

The study is performed in Brazil and, in the VLA1553 arm, the race is 33.3% white, 28.9% other and 23.9% multiracial (comparable with the placebo arm: 31.0% white, 27.4% other and 28.6% multiracial). The median BMI in the VLA1553 arm is 20.36 kg/m^2 (and 20.87 kg/m^2 in the placebo arm) which is lower compared to the pooled adult data set (29.0 kg/m^2).

614 (81.5%) participants were seronegative for CHIKV serostatus at baseline (µPRNT50 ≤40): 408 in the VLA1553 arm and 206 in the placebo arm. 139 (18.5%) participants were seropositive for CHIKV serostatus at baseline: 94 in the VLA1553 arm and 45 in the placebo arm.

The overall cumulative participant enrolment and participant exposure to VLA1553 within the clinical development program for VLA1553 is provided in the table below. This includes clinical trials VLA1553-101, VLA1553-301, VLA1553-302 and VLA1553-321.

Table 35. VLA1553-321: Participant exposure (cut off 05-Jan-2024)

	Participants enrolled	Participants exposed*	Participants exposed to the proposed dose range	Participants with long term** safety data
Blinded studies (placebo-controlled)	4 869	3 585	3 584	2 724
Blinded studies (active -controlled)	0	0	0	0
Blinded studies (VLA1553-only)	528	528	438	470
Open studies	0	0	0	0
Post marketing	0	0	0	0
Compassionate use	0	0	0	0

* Received at least 1 dose of active treatment

** Refers to 6 months or 12 months continuous exposure data

2.6.8.4. Adverse events

Pooled safety data

The table below provides a summary of the adverse events reported in the safety analysis set up to day 180.

Solicited AEs: Solicited AEs were collected until 14 days post vaccination in the phase 1 study VLA1553-101 or up to 10 days post vaccination in the phase 3 (studies VLA1553-301 and VLA1553-302). In the pooled safety studies, the frequency of solicited AEs was higher in participants vaccinated with VLA1553 compared to the placebo: all solicited AEs (53.7% vs. 32%, respectively), solicited local AEs (15.2% vs. 11.1%) and solicited systemic AEs (51.1% vs. 26.9%). Nearly all solicited AEs were assessed as related to the vaccine.

Unsolicited AEs: Unsolicited AEs and SAEs were collected during the entire study period (i.e. up to Day 180 for studies VLA1553-301 & VLA1553-302). However, the secondary endpoint was the analysis of the frequency and severity of unsolicited AEs within 28 days post-vaccination. Up to Day 180, the frequency of unsolicited AEs was also higher in participants vaccinated with VLA1553 compared to the placebo: all unsolicited (31.6% vs. 23.9%, respectively), related unsolicited (11.6% vs. 4.6%).

Table 36. Summary Table of Adverse Events up to Day 180 (Safety Analysis Set)

Table P.3.1: Summary Table of Adverse Events up to Day 180 (Safety Analysis Set)

	Statistic	VLA1553 (N=3610)	Placebo (N=1033)
Any AE	n (%) Obs [95% CI]	2311 (64.0) 7702 [62.4, 65.6]	462 (44.7) 1070 [41.7, 47.8]
Any related AE	n (%) Obs [95% CI]	1900 (52.6) 5629 [51.0, 54.3]	322 (31.2) 647 [28.4, 34.1]
Any severe AE	n (%) Obs [95% CI]	134 (3.7) 170 [3.1, 4.4]	14 (1.4) 16 [0.8, 2.3]
Any related severe AE	n (%) Obs [95% CI]	86 (2.4) 98 [1.9, 2.9]	1 (0.1) 3 [0.0, 0.5]
Any serious AE	n (%) Obs [95% CI]	52 (1.4) 79 [1.1, 1.9]	8 (0.8) 10 [0.4, 1.5]
Any related serious AE	n (%) Obs [95% CI]	2 (0.1) 2 [0.0, 0.2]	0 (0.0) 0 [0.0, 0.4]
Any medically attended AE	n (%) Obs [95% CI]	445 (12.3) 717 [11.3, 13.4]	117 (11.3) 171 [9.5, 13.4]
Any related medically attended AE	n (%) Obs [95% CI]	70 (1.9) 102 [1.5, 2.4]	7 (0.7) 8 [0.3, 1.4]
Any solicited AE	n (%) Obs [95% CI]	1940 (53.7) 5482 [52.1, 55.4]	331 (32.0) 641 [29.3, 35.0]
Any related solicited AE	n (%) Obs [95% CI]	1806 (50.0) 4988 [48.4, 51.7]	301 (29.1) 579 [26.4, 32.0]
Any solicited local AE	n (%) Obs [95% CI]	549 (15.2) 744 [14.1, 16.4]	115 (11.1) 153 [9.4, 13.2]
Any related solicited local AE	n (%) Obs [95% CI]	534 (14.8) 723 [13.7, 16.0]	114 (11.0) 151 [9.3, 13.1]
Any severe solicited local AE	n (%) Obs [95% CI]	1 (0.0) 1 [0.0, 0.2]	0 (0.0) 0 [0.0, 0.4]
Any related severe solicited local AE	n (%) Obs [95% CI]	1 (0.0) 1 [0.0, 0.2]	0 (0.0) 0 [0.0, 0.4]
Any solicited systemic AE	n (%) Obs [95% CI]	1843 (51.1) 4738 [49.4, 52.7]	278 (26.9) 488 [24.3, 29.7]
Any related solicited systemic AE	n (%) Obs [95% CI]	1697 (47.0) 4265 [45.4, 48.6]	244 (23.6) 428 [21.1, 26.3]
Any severe solicited systemic AE	n (%) Obs [95% CI]	82 (2.3) 91 [1.8, 2.8]	1 (0.1) 3 [0.0, 0.5]
Any related severe solicited systemic AE	n (%) Obs [95% CI]	76 (2.1) 85 [1.7, 2.6]	1 (0.1) 3 [0.0, 0.5]
Any solicited AE with duration >10 days	n (%) Obs [95% CI]	88 (2.4) 124 [2.0, 3.0]	17 (1.6) 21 [1.0, 2.6]
Any unsolicited AE	n (%) Obs [95% CI]	1140 (31.6) 2220 [30.1, 33.1]	247 (23.9) 429 [21.4, 26.6]
Any related unsolicited AE	n (%) Obs [95% CI]	420 (11.6) 641 [10.6, 12.7]	48 (4.6) 68 [3.5, 6.1]
Any severe unsolicited AE	n (%) Obs [95% CI]	56 (1.6) 78 [1.2, 2.0]	13 (1.3) 13 [0.7, 2.1]
Any related severe unsolicited AE	n (%) Obs [95% CI]	11 (0.3) 12 [0.2, 0.5]	0 (0.0) 0 [0.0, 0.4]
Any AESI	n (%) Obs [95% CI]	11 (0.3) 28 [0.2, 0.5]	1 (0.1) 2 [0.0, 0.5]

n...number of subjects with event, percentages are based on N, Obs...number of events (for AESI this is the number of associated symptoms). Part 3/3
Two-sided 95% confidence intervals calculated according to Altman (Wilson score interval).
Adverse events will be considered related if the causality to IMP is reported as "probable" or "possible" or missing causality.
"Severe" adverse events will include events with grade 3 or missing grade.
The Safety Analysis Set (SAS) includes all enrolled subjects in any of the studies who received at least one vaccination.
NC...Not Calculable.

PROGRAM: \PRJ\VALNEVA\VLA1553_POOLED\STAT\SAS\01_Pooled_Analysis\V2_0_006\TLF_AE_Tab\VLA1553_POOLED_TLF_SAF_AE_Tables.sas OWNER: FERLSBACHER
OUTPUT: \PRJ\VALNEVA\VLA1553_POOLED\STAT\SAS\01_Pooled_Analysis\V2_0_006\Output\VLA1553_POOLED_SAF_Tables_30JAN23.RTF RUN: 30JAN23_14:58

2.6.8.5. Solicited AEs

2.6.8.5.1. Pooled Safety Population

Table 37. Solicited Systemic and Injection Site Adverse Events 10 to 14 Days After Single Vaccination (Pooled and Study Safety Populations)

	Adults						
AE Category	**Pooled Studies[a]**	**VLA1553-301[b]**		**VLA1553-302[b,c]**	**VLA1553-101[d]**		
	VLA1553 (N=3,610) n (%)	**VLA1553 (N=3,082) n (%)**	**Placebo (N=1,033) n (%)**	**VLA1553 (N=408) n (%)**	**Arm L (N=31) n (%)**	**Arm M (N=30) n (%)**	**Arm H (N=59) n (%)**
Solicited Systemic Adverse Events							
Headache	1,154 (32.0)	969 (31.4)	151 (14.6)	146 (35.8)	8 (25.8)	8 (26.7)	23 (39.0)
Fatigue	1,063 (29.4)	879 (28.5)	130 (12.6)	155 (38.0)	5 (16.1)	6 (20.0)	18 (30.5)
Myalgia/Muscle Pain	855 (23.7)	735 (23.8)	76 (7.4)	96 (23.5)	1 (3.2)	5 (16.7)	18 (30.5)
Arthralgia/Joint Pain	599 (16.6)	520 (16.9)	50 (4.8)	63 (15.4)	2 (6.5)	4 (13.3)	10 (16.9)
Fever	498 (13.8)	414 (13.4)	8 (0.8)	52 (12.7)	4 (12.9)	6 (20.0)	22 (37.3)
Nausea	411 (11.4)	345 (11.2)	58 (5.6)	53 (13.0)	1 (3.2)	4 (13.3)	8 (13.6)
Rash	85 (2.4)	70 (2.3)	5 (0.5)	13 (3.2)	0 (0.0)	1 (3.3)	1 (1.7)
Vomiting	73 (2.0)	58 (1.9)	10 (1.0)	10 (2.5)	1 (3.2)	2 (6.7)	2 (3.4)
Solicited Injection Site Adverse Events							
Tenderness	390 (10.8)	328 (10.6)	84 (8.1)	58 (14.2)	0 (0.0)	1 (3.3)	3 (5.1)
Pain	219 (6.1)	191 (6.2)	38 (3.7)	26 (6.4)	0 (0.0)	1 (3.3)	1 (1.7)
Erythema/Redness	59 (1.6)	46 (1.5)	15 (1.5)	11 (2.7)	1 (3.2)	1 (3.3)	0 (0.0)
Induration	51 (1.4)	44 (1.4)	8 (0.8)	7 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling	25 (0.7)	21 (0.7)	8 (0.8)	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: AE=adverse event; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose
a. Studies VLA1553-301, VLA1553-302, and VLA1553-101
b. Ten consecutive days post vaccination
c. All lots combined
d. Fourteen consecutive days post vaccination
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.8 and Table P.3.10;
Module 5.3.5.1, study VLA1553-301, Table 14.3.2.5.1 and Table 14.3.2.7.1;
Module 5.3.1.2, study VLA1553-302, Table 14.3.2.3.1 and Table 14.3.2.3.4;
Module 5.3.5.1, study VLA1553-101, Table 3.4.10 and Table 3.4.11.

Table 38. Related Solicited Systemic and Injection Site Adverse Events 10 to 14 Days After Single Vaccination (Pooled and Study Safety Populations) (Pooled and Study Safety Populations)

	Adults						
AE Category	**Pooled Studies[a]**	**VLA1553-301[b]**		**VLA1553-302[b,c]**	**VLA1553-101[d]**		
	VLA1553 (N=3,610) n (%)	**VLA1553 (N=3,082) n (%)**	**Placebo (N=1,033) n (%)**	**VLA1553 (N=408) n (%)**	**Arm L (N=31) n (%)**	**Arm M (N=30) n (%)**	**Arm H (N=59) n (%)**
Related Solicited Systemic Adverse Events							
Headache	1,032 (28.6)	859 (27.9)	128 (12.4)	137 (33.6)	7 (22.6)	7 (23.3)	22 (37.3)
Fatigue	975 (27.0)	799 (25.9)	116 (11.2)	148 (36.3)	5 (16.1)	5 (16.7)	18 (30.5)
Myalgia/Muscle Pain	795 (22.0)	681 (22.1)	70 (6.8)	91 (22.3)	1 (3.2)	4 (13.3)	18 (30.5)
Arthralgia/Joint Pain	540 (15.0)	468 (15.2)	46 (4.5)	58 (14.2)	1 (3.2)	3 (10.0)	10 (16.9)
Fever	447 (12.4)	365 (11.8)	6 (0.6)	51 (12.5)	3 (9.7)	6 (20.0)	22 (37.3)
Nausea	358 (9.9)	297 (9.6)	51 (4.9)	49 (12.0)	1 (3.2)	3 (10.0)	8 (13.6)
Rash	64 (1.8)	52 (1.7)	3 (0.3)	11 (2.7)	0 (0.0)	0 (0.0)	1 (1.7)
Vomiting	54 (1.5)	42 (1.4)	8 (0.8)	8 (2.0)	1 (3.2)	1 (3.3)	2 (3.4)
Related Solicited Injection Site Adverse Events							
Tenderness	381 (10.6)	319 (10.4)	83 (8.0)	58 (14.2)	0 (0.0)	1 (3.3)	3 (5.1)
Erythema/Redness	55 (1.5)	42 (1.4)	15 (1.5)	11 (2.7)	1 (3.2)	1 (3.3)	0 (0.0)
Pain	213 (5.9)	185 (6.0)	37 (3.6)	26 (6.4)	0 (0.0)	1 (3.3)	1 (1.7)
Induration	50 (1.4)	43 (1.4)	8 (0.8)	7 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling	24 (0.7)	20 (0.6)	8 (0.8)	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: AE=adverse event; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose
AEs with causality reported as Possible or Probable were considered as related to investigational medicinal product. AEs with missing causality were classed as related.
a. Studies VLA1553-301, VLA1553-302, and VLA1553-101
b. Ten consecutive days post vaccination
c. All lots combined
d. Fourteen consecutive days post vaccination
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.9 and Table P.3.11;
Module 5.3.5.1, study VLA1553-301, Table 14.3.2.6 and Table 14.3.2.8;
Module 5.3.1.2, study VLA1553-302, Table 14.3.2.3.3 and Table 14.3.2.3.6;
Module 5.3.5.1, study VLA1553-101, Table 3.4.10.1 and Table 3.4.11.1.

Pooled safety population

Solicited local AEs

Pain (6.1% vs. 3.7%), tenderness (10.8% vs. 8.1%) and induration (1.4% vs. 0.8%) were reported slightly more frequently in the VLA1553 arm compared to placebo. Erythema/redness (1.6% vs. 1.5%) and swelling (0.7% vs. 0.8%) were seen equally in the VLA1553 arm compared to placebo.

The range for median onset day for solicited local events was slightly higher in the VLA1553 group (from Day 1 to Day 3) compared to placebo group (from Day 1 to Day 2): tenderness (2.0 vs. 1.5, respectively), pain (3.0 vs. 1.5), erythema/redness (1.0 in both groups), induration (2.0 vs. 1.0), and swelling (1.0 in both groups) (Post hoc analysis).

The frequency of the solicited injection site AEs with a duration longer than 10 days was similar between the VLA1553 and placebo arm (0.4% vs. 0.0%).

Nearly all solicited local adverse events were mild (14.3% vs. 10.6%) or moderate (0.7% vs. 0.5%) in the VLA1553 or placebo arm. Only one severe solicited local AE was reported: pain in the pooled VLA1553 arm.

Most of the solicited local AEs were assessed as related to the vaccine.

Solicited systemic AEs

Headache was the most frequently reported solicited systemic AE with 32.0% in the VLA1553 group vs. 14.6% in placebo, most were mild (26.6%) or moderate (5%). Severe headache was reported in an equal amount in the VLA1553 vs. placebo group, both 0.1%.

This was followed by fatigue (29.4% vs. 12.6%), myalgia (23.7% vs. 7.4%), arthralgia (16.6% vs. 4.8%), fever (13.8% vs 0.8%), nausea (11.4% vs. 5.6%), rash (2.4% vs. 0.5%), and vomiting (2% vs. 1%).

The range for median onset day for solicited systemic events was higher in the VLA1553 group (from Day 4 to Day 6) compared to placebo group (from Day 2.5 to Day 4): fatigue (4.0 vs. 2.0, respectively), headache (4.0 vs. 2.0), myalgia (5.0 vs. 3.0), nausea (5.0 vs. 2.0), arthralgia (5.0 vs. 3.0), fever (5.0 vs. 3.5), rash (6.0 vs. 4.0), and vomiting (5.0 vs. 2.5) (Post hoc analysis).

In VLA1553 arm, the median duration of fever was 2.0 days (vs. 1.5 in the placebo arm), the median duration of myalgia was 2.0 days (vs. 2.0 in the placebo arm), and for arthralgia it was 2.0 days (vs. 3.0 in the placebo arm).

Overall, there was no major difference in the frequency of solicited systemic AEs with a duration longer than 10 days between the pooled VLA1553 arm and the placebo arm (2.2% [1.7%, 2.7%] vs. 1.6% [1.0%, 2.6%]). Only prolonged myalgia showed a difference ≥ 0.5% between both arms: 27/3,610 (0.7%) vs. 2/1,033 (0.2%).

The severity of solicited systemic AEs was mainly mild or moderate. An increase in frequency between the pooled VLA1553 arm and the placebo arm was seen across the increasing grade of severity with mild (37.5% vs. 23.4%), moderate (11.1% vs. 3.1%) and severe (2.3% vs. 0.1%). For the solicited systemic AEs graded as severe in the VLA1553 arm, fever was the most frequently reported (n=60/1.7%) followed by arthralgia (n=10/0.3%), myalgia (n=9/0.2%), fatigue (n=7/0.2%) and headache (n=5/0.1%).

Most of the solicited systemic AEs were assessed as related to the vaccine.

Of the subjects with severe solicited systemic AEs in the VLA1553 group of study VLA1553-301, who were specifically targeted for viraemia testing, viraemia data are available for 62 subjects. For most subjects, viraemia was not detectable at Day 8 or was <LOD/<LOQ; 3 subjects had an inconclusive result at Day 8. Nine out of 62 subjects had quantifiable viraemia results at Day 8. For these 9 participants, there were 11 events from 2 to 7 days after vaccination: 1 participant (14-049) with severe related myalgia, headache and fatigue, and participants with severe arthralgia (1 related), fatigue (1 related), and fever (4 related, 1 not related), the viraemia was highly variable at Day 8, ranging from 3765.6 GCE/mL to 649944.1 GCE/mL; viraemia was undetectable at Day 29 in all subjects. Moreover, very high viraemia was only observed for one participant 6 days after vaccination (baseline serostatus unknown).

This >50 year-old subject received VLA1553 and experienced symptoms of severe arthralgia, moderate fever and back pain, and mild myalgia 2 to 9 days post-vaccination; viraemia was detected on Day 8 (i.e., 7 days post-vaccination) and was not detected at 28 days post-vaccination. Myalgia started 2 days after vaccination, with a duration of 4 days. Related arthralgia and back pain started 5 days after vaccination with a duration of 5 days. The event of fever started 9 days after vaccination with a duration of 1 day. All events resolved and needed no medical attention. The participant underwent viraemia testing following the occurrence of severe arthralgia to gather additional safety data for this individual. There was no recurrence of arthralgia and/or joint issue during the study and the participant completed the study according to schedule.

2.6.8.5.2. Study VLA1553-302

Table 39. Study VLA1553-302: Summary of Solicited Adverse Events (Pooled and Study Safety Populations)

	n (%) [95% CIs][a]					
AE Category	**Pooled Studies[b]**	**VLA1553-302**				
	VLA1553 (N=3,610)	**Total (N=408)**	**Lot 1 (N=136)**	**Lot 2 (N=137)**	**Lot 3 (N=135)**	**p-value[c]**
Any Solicited Systemic AEs	1,843 (51.1) [49.4, 52.7]	233 (57.1) [52.1, 62.0]	73 (53.7) [44.9, 62.3]	78 (56.9) [48.2, 65.4]	82 (60.7) [52.0, 69.0]	0.5045
Any Solicited Systemic AEs by Maximum Severity[d]						
Mild	1,353 (37.5)	177 (43.4)	56 (41.2)	60 (43.8)	61 (45.2)	
Moderate	401 (11.1)	45 (11.0)	12 (8.8)	15 (10.9)	18 (13.3)	
Severe	82 (2.3)	11 (2.7)	5 (3.7)	3 (2.2)	3 (2.2)	
Any Related Solicited Systemic AEs[e]	1,697 (47.0) [45.4, 48.6]	220 (53.9) [48.9, 58.8]	70 (51.5) [42.8, 60.1]	72 (52.6) [43.9, 61.1]	78 (57.8) [49.0, 66.2]	0.5394
Any Related Solicited Systemic AEs by Maximum Severity[d]						
Mild	1,256 (34.8)	165 (40.4)	53 (39.0)	55 (40.1)	57 (42.2)	
Moderate	365 (10.1)	44 (10.8)	12 (8.8)	14 (10.2)	18 (13.3)	
Severe	76 (2.1)	11 (2.7)	5 (3.7)	3 (2.2)	3 (2.2)	
Any Solicited Injection Site AEs	549 (15.2) [14.1, 16.4]	79 (19.4) [15.6, 23.5]	23 (16.9) [11.0, 24.3]	30 (21.9) [15.3, 29.8]	26 (19.3) [13.0, 26.9]	0.5742
Any Solicited Injection Site AEs by Maximum Severity[d]						
Mild	518 (14.3)	76 (18.6)	21 (15.4)	29 (21.2)	26 (19.3)	
Moderate	26 (0.7)	3 (0.7)	2 (1.5)	1 (0.7)	0	
Severe	1 (0.0)	0 (0.0)	0	0	0	
Any Related Solicited Injection Site AEs[e]	534 (14.8) [13.7, 16.0]	79 (19.4) [15.6, 23.5]	23 (16.9) [11.0, 24.3]	30 (21.9) [15.3, 29.8]	26 (19.3) [13.0, 26.9]	0.5742
Any Related Solicited Injection Site AEs by Maximum Severity[d]						
Mild	507 (14.0)	76 (18.6)	21 (15.4)	29 (21.2)	26 (19.3)	
Moderate	26 (0.7)	3 (0.7)	2 (1.5)	1 (0.7)	0	
Severe	1 (0.0)	0	0	0	0	

Abbreviations: AE=adverse event; CI=confidence interval; eCRF=electronic case report form.
a. 95% CIs for pooled studies were calculated according to Altman (Wilson score interval) and for study VLA1553-302 calculated according to Clopper-Pearson exact method.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
c. p-value from Fisher's exact test for difference between Lots.

d. Related AEs are those recorded as 'Probably Related' or 'Possibly Related' on the eCRF. Adverse events from eCRF with missing causality were classified as related.
e. Any AE from eCRF with missing severity was classified as severe.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.1, Table P.3.16, Table P.3.17, Table P.3.18, Table P.3.19,
Module 5.3.1.2, study VLA1553-302, Table 14.3.2.1.1, Table 14.3.2.3.3, Table 14.3.2.3.6, Table 14.3.2.3.7, Table 14.3.2.3.8, Table 14.3.2.3.9, and Table 14.3.2.3.10

Safety was a secondary objective for study VLA1553-302 that was designed to demonstrate lot-to-lot consistency of VLA1553 following 28 days post vaccination. The related solicited systemic and injection site AEs and their severity were equally distributed amongst the 3 different lots.

Of the subjects with severe solicited systemic AEs, specifically targeted for viraemia testing in study VLA1553-302, viraemia data are available for 11 subjects. For 8/11 subjects, viraemia was not detectable at Day 8 or was <LLOQ. Three subjects had quantifiable viraemia results at Day 8. Viraemia was highly variable; viraemia was undetectable at Day 29. The subjects experienced severe solicited systemic events of fever, arthralgia, fatigue, and myalgia (i.e., the same events as in VLA1553-301).

2.6.8.5.3. Study VLA1553-101

Table 40. Study VLA1553-101: Summary of Solicited Adverse Events up to 14 Days Post-vaccination (Pooled and Study Safety Populations)

	n (%) [95% CIs][a]				
AE Category	**Pooled Studies[b]**	**VLA1553-101**			
	VLA1553 (N=3,610)	**Arm L (N=31)**	**Arm M (N=30)**	**Arm H (N=59)**	**p-value[c] Overall**
Any Solicited Systemic AE	1,843 (51.1) [49.4, 52.7]	11 (35.5) [21.1, 53.1]	12 (40.0) [24.6, 57.7]	40 (67.8) [55.1, 78.3]	0.0038
Any Solicited Injection Site AE	549 (15.2) [14.1, 16.4]	1 (3.2) [0.6, 16.2]	2 (6.7) [1.8, 21.3]	6 (10.2) [4.7, 20.5]	0.5509

Abbreviations: AE=adverse event; CI=confidence interval; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose
a. 95% CIs were calculated according to Altman (Wilson score interval).
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
c. p-value Fisher-Freeman-Halton test between Arms L, M, and H.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.8, Table P.3.10,
Module 5.3.5.1, study VLA1553-101, Table 3.4.1

Study VLA1553-101 was a first in human study were 3 escalating dose levels of VLA1553 were evaluated (Arm L 3.2×10E3 TCID50/dose, Arm M 3.2×10E4 TCID50/dose, and Arm H 3.2×10E5 TCID50/dose). Safety and tolerability of VLA1553 was the primary objective. The medium dose (Arm M) was selected for further development.

The frequency of solicited systemic AE increased with increasing dosing: 35.5%, 40% and 67.8% for arm L, arm M and arm H respectively. The same increasing trend was seen for the solicited injection site AE: 3.2%, 6.7% and 10.2% respectively.

The more frequent solicited systemic AE were headache (25.8% arm L, 26.7% arm M, and 39% arm H) and fatigue (16.1% % arm L, 20% arm M, and 30.5% arm H) increasing with increased dosing. The more frequent solicited local AE was tenderness (0% arm L, 3.3% arm M, and 5.1% arm H) increasing with increased dosing.

Of note, the frequencies in arm M are slightly lower than observed in VLA1553-301. However, these results should be interpreted with caution as the number of participants in each arm in study VLA1553-101 is very limited (31 in arm L, 30 in arm M and 59 in arm H).

Of the subjects with severe solicited systemic AEs in study VLA1553-101 (9 subjects with 10 events), 5 subjects (all seronegative at baseline) had a quantifiable viraemia result at Day 3 (after first vaccination or after re-vaccination). For these 5 participants with (probably/possibly related) severe fever around 2/4 days after first vaccination, the viraemia levels were highly variable at Day 3 and ranged from 18442 GCE/mL to 253206 GCE/mL and not depending of the dose. For all subjects, viraemia was not quantifiable/ undetectable at Days 7 and 14.

2.6.8.5.4. Study VLA1553-321 (adolescents)

Table 41. VLA1553-321: All and Related Solicited Systemic and Injection Site Adverse Events 10 Days After Single Vaccination (Study Safety Population)

	Adolescents			
	VLA1553-321			
Adverse Event Category	VLA1553 (N=502) n (%)		Placebo (N=252) n (%)	
	All	Related	All	Related
Solicited Systemic Adverse Events	**319 (63.5)**	**314 (62.5)**	**105 (41.7)**	**102 (40.5)**
Headache	257 (51.2)	252 (50.2)	86 (34.1)	83 (32.9)
Fatigue	112 (22.3)	111 (22.1)	23 (9.1)	22 (8.7)
Myalgia/Muscle Pain	137 (27.3)	132 (26.3)	30 (11.9)	29 (11.5)
Arthralgia/Joint Pain	64 (12.7)	63 (12.5)	14 (5.6)	13 (5.2)
Fever	122 (24.3)	121 (24.1)	9 (3.6)	8 (3.2)
Nausea	80 (15.9)	78 (15.5)	30 (11.9)	29 (11.5)
Rash	19 (3.8)	18 (3.6)	2 (0.8)	2 (0.8)
Vomiting	13 (2.6)	13 (2.6)	8 (3.2)	7 (2.8)
Solicited Injection Site Adverse Events	**161 (32.1)**	**160 (31.9)**	**62 (24.6)**	**62 (24.6)**
Tenderness	100 (19.9)	99 (19.7)	37 (14.7)	37 (14.7)
Pain	97 (19.3)	96 (19.1)	34 (13.5)	34 (13.5)
Erythema/Redness	14 (2.8)	14 (2.8)	3 (1.2)	3 (1.2)
Induration	22 (4.4)	22 (4.4)	11 (4.4)	11 (4.4)
Swelling	8 (1.6)	7 (1.4)	7 (2.8)	7 (2.8)

Adverse events with causality reported as Possible or Probable were considered as related to investigational medicinal product. Adverse events with missing causality were classed as related.
Source: Module 5.3.5.1, study VLA1553-321, Table 14.3.2.6, Table 14.3.2.11, Table 14.3.2.14, and Table 14.3.2.19

In study VLA1553-321 all and related solicited systemic and injection sites AE were collected until 10 days after single vaccination.

Solicited local AEs

Significantly more solicited injection site AEs were reported in the VLA1553 arm compared to placebo (32.1% vs. 24.6%, p<0.0348). Tenderness (19.9% VLA1553 vs. 14.7% in placebo) and pain at injection site (19.3% VLA1553 vs. 13.5% in placebo) were most frequently reported.

The median onset day for solicited local events was Day 1 in both groups for all solicited local AEs (tenderness, pain, erythema/redness, induration, and swelling).

Overall, most solicited injection site AEs were graded as mild (215/754 [28.5%] participants). Five of 754 (0.7%) participants experienced a moderate solicited injection site AE. Only 3/754 (0.4%) participants (2/502 [0.4%] in the VLA1553 arm and 1/252 [0.4%] in the placebo arm) experienced a severe solicited injection site AE. Moderate and severe solicited injection site AEs were reported only in the seronegative stratum.

Most of the solicited local AEs were assessed as related to the vaccine (31.9% vs. 24.6%, in each arm respectively).

Solicited systemic AEs

Significantly more solicited systemic AEs were reported in the VLA1553 arm (63.5%) compared to the placebo arm (41.7%).

Headache was the most observed solicited system AEs: 51.2% in VLA1553 vs. 31.4% in placebo. Most cases were mild or moderate. The median duration was 2.0 days for both study arms. This was followed by myalgia (27.3% vs. 11.9%), fever (24.3% vs. 3.6%) and fatigue (22.3% vs. 9.1%). Median duration

was 2.0 days for myalgia and was 1.0 day for fever (for both study arms). Median duration of fatigue was 2.0 days in the VLA1553 arm and 4.0 days in the placebo arm.

The range for median onset day for solicited systemic events was slightly higher in the VLA1553 group (from Day 3 to Day 5) compared to placebo group (from Day 2 to Day 5): fatigue (3.0 vs. 2.0, respectively), headache (3.0 vs. 2.0), myalgia (3.0 vs. 2.0), nausea (4.0 vs. 2.0), arthralgia (5.0 vs. 3.5), fever (5.0 vs. 3.0), rash (5.0 vs. 1.5), and vomiting (5.0 vs. 5.0) (Post hoc analysis).

Overall, most participants who had solicited systemic AEs experienced events that were graded with a maximum severity of mild (307/754 participants, 40.7%) or moderate (99/754 participants, 13.1%). Eighteen of 754 (2.4%) participants (17/502 [3.4%] in the VLA1553 arm and 1/252 [0.4%] in the placebo arm) experienced at least one AE that was graded severe, predominantly fever (14/502 [2.8%] all in the VLA1553 arm, 13/408 [3.2%] in the seronegative stratum and 1/94 [1.1%] in the seropositive stratum) and headache (4/502 [0.8%] participants in the VLA1553 arm and 1/252 [0.4%] in the placebo arm, all in the seronegative stratum). There was one participant with arthralgia in the VLA1553 arm (seronegative stratum).

Of the subjects with severe solicited systemic AEs in study VLA1553-321 (Part A), viraemia data are available for 2 subjects (both seronegative at baseline). Viraemia was not detectable at Day 29. The subjects experienced severe fever. The fever events started close after vaccination (Day 1 and Day 5) and event duration was 6 days and 1 day, respectively.

Most of the solicited systemic AEs were assessed as related to the vaccine (62.5% vs. 40.5%, in each arm respectively).

2.6.8.6. Unsolicited AEs

2.6.8.6.1. Pooled Safety Population

Unsolicited Adverse Events up to 28 Days Post Vaccination

For the pooled safety population, a difference was noticed in the overall frequency of unsolicited AEs up to 28 days post vaccination between VLA1553 and placebo (23.5% vs. 13.4%), and in particular for chills (2.0% vs. 0.2%), neutropenia (1.7% vs. 0.1%; 6.7% vs. 0.8% if IMM subset denominator is used), diarrhoea (1.4% vs. 0.4%), leukopenia (1.2% vs. 0%, 3.8% vs. 0%, if IMM subset denominator is used) and lymphadenopathy (1.1% vs. 0%).

With regards to neutropenia and leukopenia, it's important to note that in study VLA1553-301. post-vaccination safety laboratory samples only were taken from participants in the IMM subset, as per study protocol. Thus, the total number of participants vaccinated and analysed according to the actual treatment received in the IMM subset was 372 in the VLA1553 arm and 125 in the placebo arm (instead of the safety population: n=4,115; VLA1553, n=3,082; placebo, n=1,033). Leukopenia and neutropenia are discussed in section 3.3.7.14.1.1..

Similar observations were made for related unsolicited AEs up to 28 days post vaccination (11.1% vs. 3.8%). A noticeable difference was noted in chills (1.8% vs. 0.2%), neutropenia (1.6% vs. 0.1%), leukopenia (1.1% vs. 0%), lymphadenopathy (0.8% vs. 0%).

For unsolicited AEs that were reported up to Day 29 (28 days post-vaccination), the median onset day in the VLA1553 group varied depending on the SOC (Post hoc analysis):

- Between Day 4 and Day 6 (i.e., 3 to 5 days post-vaccination): general disorders and administration site conditions, respiratory, thoracic and mediastinal disorders, and ear and labyrinth disorders.
- Between Day 7 and Day 11 (i.e., 6 to 10 days post-vaccination): blood and lymphatic system disorders, gastrointestinal disorders, investigations, skin and subcutaneous tissue disorders, injury, poisoning and procedural complications, metabolism and nutrition disorders, eye disorders, renal and urinary disorders, reproductive system and breast disorders, and endocrine disorders.
- Between Day 11 and Day 29 (i.e., 10 to 28 days post-vaccination): musculoskeletal and connective tissue disorders, infections and infestations, nervous system disorders, psychiatric disorders, vascular disorders, immune system disorders, cardiac disorders, hepatobiliary disorders, congenital, familial and genetic disorders, neoplasms benign, malignant and unspecified.

Unsolicited Adverse Events up to 6 Months Post-vaccination

Table 42. Unsolicited Adverse Events Reported up to 6 Months Occurring at a Frequency of ⩾0.5% in the Pooled VLA1553 Arm Compared Across Studies VLA1553-301, VLA1553-302, and VLA1553-101 (Pooled and Study Safety Populations)

	Adults[a]						
Preferred Term	**Pooled Studies[b,c]**	**VLA1553-301[c]**		**VLA1553-302[c,d]**	**VLA1553-101[e]**		
	VLA1553 (N=3,610) n (%)	**VLA1553 (N=3,082) n (%)**	**Placebo (N=1,033) n (%)**	**VLA1553 (N=408) n (%)**	**Arm L (N=31) n (%)**	**Arm M (N=30) n (%)**	**Arm H (N=59) n (%)**
Chills	74 (2.0)	59 (1.9)	3 (0.3)	7 (1.7)	1 (3.2)	1 (3.3)	6 (10.2)
Arthralgia	71 (2.0)	60 (1.9)	17 (1.6)	10 (2.5)	0 (0.0)	0 (0.0)	1 (1.7)
COVID-19	65 (1.8)	59 (1.9)	22 (2.1)	6 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)
Neutropenia	62 (1.7)	34 (1.1)	1 (0.1)	12 (2.9)	5 (16.1)	1 (3.3)	10 (16.9)
Headache	61 (1.7)	53 (1.7)	19 (1.8)	5 (1.2)	2 (6.5)	0 (0.0)	2 (3.4)
Diarrhoea	56 (1.6)	46 (1.5)	4 (0.4)	4 (1.0)	1 (3.2)	1 (3.3)	3 (5.1)
Back pain	53 (1.5)	44 (1.4)	7 (0.7)	4 (1.0)	2 (6.5)	0 (0.0)	3 (5.1)
Leukopenia	42 (1.2)	18 (0.6)	0 (0.0)	2 (0.5)	6 (19.4)	2 (6.7)	14 (23.7)
Lymphadenopathy	40 (1.1)	29 (0.9)	2 (0.2)	8 (2.0)	0 (0.0)	0 (0.0)	3 (5.1)
Urinary tract infection	35 (1.0)	33 (1.1)	6 (0.6)	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)
Pain in extremity	30 (0.8)	27 (0.9)	8 (0.8)	1 (0.2)	1 (3.2)	1 (3.3)	0 (0.0)
Dizziness	27 (0.7)	22 (0.7)	4 (0.4)	4 (1.0)	1 (3.2)	0 (0.0)	0 (0.0)
Oropharyngeal pain	27 (0.7)	19 (0.6)	5 (0.5)	6 (1.5)	1 (3.2)	0 (0.0)	1 (1.7)
Myalgia	25 (0.7)	21 (0.7)	8 (0.8)	3 (0.7)	1 (3.2)	0 (0.0)	0 (0.0)
Depression	23 (0.6)	22 (0.7)	3 (0.3)	1 (0.2)	0 (0.0)	1 (3.3)	0 (0.0)
Anaemia	21 (0.6)	13 (0.4)	4 (0.4)	7 (1.7)	0 (0.0)	0 (0.0)	1 (1.7)
Pain	21 (0.6)	16 (0.5)	3 (0.3)	2 (0.5)	1 (3.2)	0 (0.0)	2 (3.4)
Nausea	20 (0.6)	17 (0.6)	5 (0.5)	1 (0.2)	0 (0.0)	1 (3.3)	1 (1.7)
Fatigue	19 (0.5)	13 (0.4)	9 (0.9)	4 (1.0)	0 (0.0)	0 (0.0)	2 (3.4)
Neck pain	19 (0.5)	18 (0.6)	2 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Rash	19 (0.5)	15 (0.5)	7 (0.7)	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pyrexia	18 (0.5)	15 (0.5)	5 (0.5)	2 (0.5)	0 (0.0)	1 (3.3)	1 (1.7)
Musculoskeletal stiffness	17 (0.5)	13 (0.4)	5 (0.5)	4 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Upper respiratory tract infection	17 (0.5)	14 (0.5)	7 (0.7)	3 (0.7)	0 (0.0)	0 (0.0)	1 (1.7)

Abbreviations: IMM=immunogenicity; MedDRA=Medical Dictionary for Regulatory Activities; PT=preferred term; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose.
Note: post-vaccination safety laboratory samples were only taken from a subset of participants in study VLA1553-301 (i.e., 375 randomized participants in the VLA1553 arm and 126 randomized participants in the placebo arm), but percentages for laboratory abnormalities reported as adverse events were calculated for the Pooled / Safety Population.
a. Unsolicited adverse events up to 6 months.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
c. Adverse events were coded using MedDRA version 24.1. For each SOC and PT, participants are included only once, even if they experienced multiple events in that SOC or PT.
d. All lots combined.
e. Adverse events were coded using MedDRA version 22.0. For each SOC and PT, participants are included only once, even if they experienced multiple events in that SOC or PT.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.20;
Module 5.3.5.1, study VLA1553-301, Table 14.3.2.25;
Module 5.3.1.2, study VLA1553-302, Table 14.3.2.8.1;
Module 5.3.5.1, study VLA1553-101, Table 7.4.27.1

Table 43. Related Unsolicited Adverse Events Reported up to 6 Months Occurring at a Frequency of ≥ 0.5% in the Pooled VLA1553 Arm Compared Across Studies VLA1553-301, VLA1553-302, and VLA1553-101 (Pooled and Study Safety Populations)

	Adults[a]						
Preferred Term	**Pooled Studies[b,c]**	**VLA1553-301[c]**		**VLA1553-302[c,d]**	**VLA1553-101[e]**		
	VLA1553 (N=3,610) n (%)	**VLA1553 (N=3,082) n (%)**	**Placebo (N=1,033) n (%)**	**VLA1553 (N=408) n (%)**	**Arm L (N=31) n (%)**	**Arm M (N=30) n (%)**	**Arm H (N=59) n (%)**
Chills	64 (1.8)	51 (1.7)	2 (0.2)	6 (1.5)	1 (3.2)	1 (3.3)	5 (8.5)
Neutropenia	59 (1.6)	31 (1.0)	1 (0.1)	12 (2.9)	5 (16.1)	1 (3.3)	10 (16.9)
Leukopenia	40 (1.1)	16 (0.5)	0 (0.0)	2 (0.5)	6 (19.4)	2 (6.7)	14 (23.7)
Lymphadenopathy	28 (0.8)	20 (0.6)	0 (0.0)	6 (1.5)	0 (0.0)	0 (0.0)	2 (3.4)
Diarrhoea	26 (0.7)	22 (0.7)	3 (0.3)	1 (0.2)	0 (0.0)	1 (3.3)	2 (3.4)
Back pain	23 (0.6)	18 (0.6)	1 (0.1)	0 (0.0)	2 (6.5)	0 (0.0)	3 (5.1)
Arthralgia	22 (0.6)	17 (0.6)	5 (0.5)	4 (1.0)	0 (0.0)	0 (0.0)	1 (1.7)
Dizziness	19 (0.5)	14 (0.5)	2 (0.2)	4 (1.0)	1 (3.2)	0 (0.0)	0 (0.0)

Abbreviations: IMM=immunogenicity; MedDRA=Medical Dictionary for Regulatory Activities; PT=preferred term; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose.
Note: post-vaccination safety laboratory samples were only taken from a subset of participants in study VLA1553-301 (i.e., 375 randomized participants in the VLA1553 arm and 126 randomized participants in the placebo arm), but percentages for laboratory abnormalities reported as adverse events were calculated for the Pooled / Safety Population.
a. Unsolicited adverse events up to 6 months.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
c. Adverse events were coded using MedDRA version 24.1. For each SOC and PT, participants are included only once, even if they experienced multiple events in that SOC or PT.
d. All lots combined.
e. Adverse events were coded using MedDRA version 22.0. For each SOC and PT, participants are included only once, even if they experienced multiple events in that SOC or PT.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.22;
Module 5.3.5.1, study VLA1553-301, Table 14.3.2.26;
Module 5.3.1.2, study VLA1553-302, Table 14.3.2.8.2;
Module 5.3.5.1, study VLA1553-101, Table 7.4.28.1

For the pooled safety population, a difference was noticed in the overall frequency of unsolicited AEs up to 6 months post vaccination between VLA1553 and placebo (31.6% vs. 23.9%), and in particular for the SOC 'blood and lymphatic system disorders' (4.2% vs. 1.0%) and 'investigations' (2.9% vs. 1.3%), and for the following unsolicited AEs: chills (2.0% vs. 0.3%), diarrhoea (1.6% vs. 0.4%), neutropenia (1.7% vs. 0.1%), leukopenia (1.2% vs. 0%) and lymphadenopathy (1.1% vs. 0.2%).

The proportion of subjects who experienced musculoskeletal stiffness, joint stiffness, joint swelling, arthritis, or osteoarthritis was comparable between the VLA1553 and placebo group (1.1% and 1.2%). Each event was reported with low and similar frequency in each group (≤0.5% in the VLA1553 group) (Post hoc analysis). Musculoskeletal stiffness (0.5% - 20 events vs. 0.5% - 5 events in each arm respectively), joint stiffness (0.2% - 10 events vs. 0.2% - 2 events), joint swelling (0.1% - 5 events vs. 0.2% - 2 events), arthritis (0.1% - 2 events vs. 0.1% - 1 event) and osteoarthritis (0.3% - 11 events vs. 0.2% - 2 events) were reported with similar frequencies in the pooled VLA 1553 and placebo arm.

No anaphylactic reactions were reported in either arm.

Similar observations were made for related unsolicited AEs up to 6 months post vaccination (11.6% vs. 4.6%). In particular the difference was noted for the blood and lymphatic system disorders (3.3% vs. 0.5%), general disorders and administration site conditions (2.8% vs. 1.2%), investigations (1.2% vs. 0.1%) and respiratory, thoracic and mediastinal disorders (0.8% vs. 0%). Chills (1.8% vs. 0.2%), neutropenia (1.6% vs. 0.1%), leukopenia (1.1% vs. 0%), lymphadenopathy (0.8% vs. 0%) were related unsolicited AEs which were more frequently seen in VLA1553.

All events of arthritis and osteoarthritis were assessed as not related to study vaccination by the investigator. Review of the cases revealed that an alternative aetiology existed (female who are more likely to develop osteoarthritis, overweight or obese, or/and had an osteoarthritis medical history) and/or a relationship to VLA1553 was considered unlikely due to the late onset of the event in relation to VLA1553 vaccination. Viraemia was not assessed.

The proportion of subjects with related events of musculoskeletal stiffness or joint stiffness were comparable between the VLA1553 group and placebo group (0.2% and 0.2%; 0.1% and 0.2%, respectively) (Post hoc analysis). Joint swelling that was assessed as related to study vaccination was

reported in one subject each in the VLA1553 and placebo group. None of the events were serious or medically attended. Viraemia was not assessed. For the related events in the VLA1553 group:

- The events of musculoskeletal stiffness were all of mild severity, with a mean onset 21.6 days post-vaccination (median: 13 days). All events resolved; the duration was between 3 and 77 days.
- The events of joint stiffness were all of mild severity except one event of moderate severity, with a mean onset 25.7 days post-vaccination (median: 18 days). All events resolved; the duration was between 5 and 11 days.
- The event of joint swelling was of mild severity; the onset was 5 days post-vaccination with a duration of 3 days.

Leukopenia and neutropenia are discussed is section 2.6.8.8.1.1..

Overall, in the Pooled Safety Population, the majority of unsolicited AEs up to Day 180 were mild or moderate in severity; the proportion of subjects with mild or moderate unsolicited AEs was similar between the VLA1553 and placebo group (mild AEs: 20.0% vs. 14.4%, moderate AEs: 10.1% vs. 8.2%). The overall frequency of severe unsolicited AEs was low and comparable between the treatment groups (1.6% of subjects in the VLA1553 group and 1.3% of subjects in the placebo group) (Post hoc analysis).

The SOC for which most severe AEs were documented in the VLA1553 group were blood and lymphatic system disorders (VLA1553: 0.2%, placebo: 0%), infections and infestations (VLA1553: 0.2%, placebo: 0.2%), injury, poisoning and procedural complications (VLA1553: 0.2%, placebo: 0.2%), and psychiatric disorders (VLA1553: 0.2%, placebo: 0.2%). The most common severe unsolicited AE in the VLA1553 group was neutropenia (0.1%; 5 subjects with 5 events; none in the placebo group) (Post hoc analysis).

Of the subjects with severe unsolicited AEs in the VLA1553 group of study VLA1553-301, viraemia data are available for 8 subjects; only one subject had a quantifiable viraemia result at Day 8.

In the VLA1553 group, the most common related severe unsolicited AEs were neutropenia (0.1%; 5 subjects with 5 events) and lymphopenia (0.1%, 2 subjects with 2 events; these cases were only reported in the high dose group (Arm H) in study VLA1553-101). Other reported events were: chest discomfort and arthralgia (one subject), chills (one subject), back pain (one subject), and inappropriate antidiuretic hormone secretion (one subject). No related severe unsolicited AEs were reported in the placebo group.

A short summary of the related severe unsolicited AEs is provided in the following section:

- The mean onset of lymphopenia and neutropenia was 6.5 days and 8.8 days post-vaccination. All events were transient with a duration between 4 to 20 days. None of these events were serious or were medically attended. For two subjects with events of neutropenia, viraemia was detected on Day 3) and was either <LOD or undetectable on Days 7 and 14; for one subject viraemia was not detectable at Day 8; and for one subject a viraemia result was not available. For the two subjects with events of lymphopenia, viraemia was detected at Day 3 and was either <LOD or undetectable on Days 7 and 14.
- One subject had chest discomfort and arthralgia that started 3 days post-vaccination and resolved on the same day; the events were non-serious and medically attended. Viraemia was <LOD at Day 8.
- One subject had chills that started 2 days post-vaccination with a duration of 4 days; the event was non-serious and not medically attended. Viraemia was <LOD at Day 8.

- One subject had back pain that started 3 days post-vaccination with a duration of 9 days; the event was non-serious and not medically attended. Viraemia was detectable at Day 3 and was <LOD and not detected at Day 7 and Day 14, respectively.
- One subject was diagnosed with inappropriate antidiuretic hormone secretion (SIADH). The event was serious and is described in section 2.6.8.8.2.

2.6.8.6.2. Study VLA1553-302

Unsolicited Adverse Events *up to 28 Days Post-vaccination*

Table 44. Study VLA1553-302: Summary of Unsolicited Adverse Events up to 28 Days Post-vaccination (Pooled and Study Safety Populations)

	n (%) [95% CIs][a]					
AE Category	**Pooled Studies[b]**	**VLA1553-302**				
	VLA1553 (N=3,610)	**Total (N=408)**	**Lot 1 (N=136)**	**Lot 2 (N=137)**	**Lot 3 (N=135)**	**p-value[c]**
Any Unsolicited AEs	849 (23.5) [22.2, 24.9]	102 (25.0) [20.9, 29.5]	38 (27.9) [20.6, 36.3]	22 (16.1) [10.3, 23.3]	42 (31.1) [23.4, 39.6]	0.0085
Any Related Unsolicited AEs[d]	401 (11.1) [10.1, 12.2]	63 (15.4) [12.1, 19.3]	20 (14.7) [9.2, 21.8]	14 (10.2) [5.7, 16.6]	29 (21.5) [14.9, 29.4]	0.0359

Abbreviations: AE=adverse event; CI=confidence interval; eCRF=electronic case report form.
a. 95% CIs for pooled studies were calculated according to Altman (Wilson score interval) and for study VLA1553-302 calculated according to Clopper-Pearson exact method.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
c. p-value from Fisher's exact test for difference between Lots.
d. Related AEs are those recorded as 'Probably Related' or 'Possibly Related' on the eCRF. Adverse events from eCRF with missing causality were classified as related.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.21, Table P.3.23, Module 5.3.1.2, study VLA1553-302, Table 14.3.2.2.1.

Unsolicited Adverse Events up to 6 Months Post-vaccination

Table 45. Study VLA1553-302: Summary of Unsolicited Adverse Events up to 6 Months (Pooled and Study Safety Populations)

	n (%) [95% CIs][a]					
AE Category	**Pooled Studies[b]**	**VLA1553-302**				
	VLA1553 (N=3,610)	**Total (N=408)**	**Lot 1 (N=136)**	**Lot 2 (N=137)**	**Lot 3 (N=135)**	**p-value[c]**
Any Unsolicited AE	1,140 (31.6) [30.1, 33.1]	139 (34.1) [29.5, 38.9]	51 (37.5) [29.4, 46.2]	37 (27.0) [19.8, 35.3]	51 (37.8) [29.6, 46.5]	0.0979
Any Related Unsolicited AE[d]	420 (11.6) [10.6, 12.7]	67 (16.4) [13.0, 20.4]	22 (16.2) [10.4, 23.5]	15 (10.9) [6.3, 17.4]	30 (22.2) [15.5, 30.2]	0.0413

Abbreviations: AE=adverse event; CI=confidence interval; eCRF=electronic case report form.
a. 95% CIs for pooled studies were calculated according to Altman (Wilson score interval) and for study VLA1553-302 calculated according to Clopper-Pearson exact method.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
c. p-value from Fisher's exact test for difference between Lots.
d. Related AEs are those recorded as 'Probably Related' or 'Possibly Related' on the eCRF. Adverse events from eCRF with missing causality were classified as related.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.20, Table P.3.22, Module 5.3.1.2, study VLA1553-302, Table 14.3.2.8.1 and Table 14.3.2.8.2

For study VLA1553-302, a small difference was noticed in the overall frequency of unsolicited AEs up to 6 months post vaccination between each lot (37.5% in lot 1 vs. 27% in lot 2 vs. 37.8% in lot 3). Similar differences were observed in related unsolicited AEs (16.2% in lot 1 vs. 10.9% in lot 2 vs. 22.2% in lot 3).

Overall, unsolicited AEs were most frequently reported in the following SOCs: Blood and Lymphatic System Disorders (8.6%), Musculoskeletal and Connective Tissue Disorders (6.6%). The most frequent unsolicited AEs were neutropenia (2.9%, range: 2.2% to 3.7% participants across all 3 Lots), lymphadenopathy (2.0%, range: 2.9% and 3.0% participants), anaemia (1.7%, range: 1.5% to 2.2%),

chills (1.7%, range: 0.7% to 3.7%), COVID-19 (1.5%, range: 0.7% to 2.2%), oropharyngeal pain (1.5%, range: 0.7% to 2.2%) and headache (1.2%, range: 1.5% and 2.2%).

Of the subjects with severe unsolicited AEs in study VLA1553-302, viraemia data are available for one subject (seronegative at baseline); viraemia was undetectable at Day 8 and Day 29. The subject experienced severe appendicitis which was assessed as unrelated to VLA1553 vaccination.

2.6.8.6.3. Study VLA1553-101

Table 46. Study VLA1553-101: Summary of Unsolicited Adverse Events (Pooled and Study Safety Populations)

	n (%) [95% CIs][a]				
AE Category	**Pooled Studies[b]**	**VLA1553-101**			
	VLA1553 (N=3,610)	**Arm L (N=31)**	**Arm M (N=30)**	**Arm H (N=59)**	**p-value[c] Overall**
Any Unsolicited AE	1,140 (31.6) [30.1, 33.1]	18 (58.1) [40.8, 73.6]	15 (50.0) [33.2, 66.8]	38 (64.4) [51.7, 75.4]	0.4257
Any Related Unsolicited AE	420 (11.6) [10.6, 12.7]	13 (41.9) [26.4, 59.2]	8 (26.7) [14.2, 44.4]	29 (49.2) [36.8, 61.6]	0.1310

Abbreviation: AE=adverse event; CI=confidence interval; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose
a. 95% CIs were calculated according to Altman (Wilson score interval).
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
c. p-value Fisher-Freeman-Halton test between Arms L, M, and H.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.20, Table P.3.22,
Module 5.3.5.1, study VLA1553-101, Table 3.4.1

Table 47. Study VLA1553-101: Notable Unsolicited Adverse Events (Pooled and Study Safety Populations)

	n (%) [95% CIs][a]			
AE Category	**Pooled Studies[b]**	**VLA1553-101**		
	VLA1553 (N=3,610)	**Arm L (N=31)**	**Arm M (N=30)**	**Arm H (N=59)**
Unsolicited AE				
Leukopenia	42 (1.2) [0.9, 1.6]	6 (19.4) [9.2, 36.3]	2 (6.7) [1.8, 21.3]	14 (23.7) [14.7, 36.0]
Neutropenia	62 (1.7) [1.3, 2.2]	5 (16.1) [7.1, 32.6]	1 (3.3) [0.6, 16.7]	10 (16.9) [9.5, 28.5]
Chills	74 (2.0) [1.6, 2.6]	1 (3.2) [0.6, 16.2]	1 (3.3) [0.6, 16.7]	6 (10.2) [4.7, 20.5]
Diarrhoea	56 (1.6) [1.2, 2.0]	1 (3.2) [0.6, 16.2]	1 (3.3) [0.6, 16.7]	3 (5.1) [1.7, 13.9]
Related Unsolicited AE				
Leukopenia	40 (1.1) [0.8, 1.5]	6 (19.4) [9.2, 36.3]	2 (6.7) [1.8, 21.3]	14 (23.7) [14.7, 36.0]
Neutropenia	59 (1.6) [1.3, 2.1]	5 (16.1) [7.1, 32.6]	1 (3.3) [0.6, 16.7]	10 (16.9) [9.5, 28.5]
Chills	64 (1.8) [1.4, 2.3]	1 (3.2) [0.6, 16.2]	1 (3.3) [0.6, 16.7]	5 (8.5) [3.7, 18.4]

Abbreviations: AE=adverse event; CI=confidence interval; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose
a. 95% CIs were calculated according to Altman (Wilson score interval).
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.20, Table P.3.22,
Module 5.3.5.1, study VLA1553-101, Table 7.4.27.1 and Table 7.4.28.1

For study VLA1553-101, the frequency of unsolicited AEs was similar across the three dose arms: 58.1% in arm L, 50% in arm M and 64.4% in arm H. Similar observations are made for related unsolicited AEs: 41.9% in arm L, 26.7% in arm M, and 49.2% in arm H. However, these results should be interpreted

with caution as the number of participants in each arm in study VLA1553-101 is very limited (31 in arm L, 30 in arm M and 59 in arm H).

Leukopenia (Arm L 19.4%, Arm M 6.7%, Arm H 23.7%) and neutropenia (Arm L 16.1%, Arm M 3.3%, Arm H 16.9%) were the most frequently reported unsolicited AEs (and related unsolicited AEs).

Severe unsolicited AEs were experienced by 3 subjects in Arm L (one AE of back pain, two AEs of neutropenia), 2 subjects in Arm M (one AE of neutropenia and one AE of multiple injuries), and 2 subjects in Arm H (one each in Arms H1 and H2; two AEs of lymphopenia) after a single vaccination. All severe unsolicited AEs except multiple injuries were considered related.

Of the subjects with severe unsolicited AEs in study VLA1553-101 (7 subjects with 7 events), 5 subjects (all seronegative at baseline) had a quantifiable viraemia result at Day 3 (after first vaccination): multiple injuries (1 not-related), back pain (1 probably related 3 days after vaccination), neutropenia (3 probably related, from 7 to 10 after vaccination) and lymphopenia (2 probably related, from 4 to 7 days after vaccination). The viraemia levels were highly variable at Day 3 and ranged from 26508 GCE/mL to 406660 GCE/mL. Viraemia >100000 GCE/mL was only observed in the 2 participants lymphopenia events. For all 7 subjects viraemia was not quantifiable/ undetectable at Days 7 and 14.

Table 48. Summary of Unsolicited Adverse Events Starting at or after Month 12 Re-vaccination by Study Arm (Safety Population)

Overall Starting at or after the Month 12 re-vaccination Subjects with at least one	VLA1553			
	Arm L M12 re-vacc. (N=24) n (%) Obs [95% CI]	Arm M M12 re-vacc. (N=23) n (%) Obs [95% CI]	Arm H1 M12 re-vacc. (N=21) n (%) Obs [95% CI]	p-value (Overall)
Any unsolicited AE	2 (8.3) 3 [2.3, 25.8]	0 (0.0) 0 [0.0, 14.3]	2 (9.5) 2 [2.7, 28.9]	0.4517
Any related unsolicited AE	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A
Any severe unsolicited AE	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A
Any related severe unsolicited AE	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A
Any SAE	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A
Any related SAE	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A
Any related medically attended unsolicited AE	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A
Any unsolicited AESI	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A
Any related unsolicited AESI	0 (0.0) 0 [0.0, 13.8]	0 (0.0) 0 [0.0, 14.3]	0 (0.0) 0 [0.0, 15.5]	N/A

M12 = Month 12; re-vacc. = re-vaccination; n = number of subjects with AE, percentages are based on N; Obs = number of events;
CI = confidence interval; AE = adverse event; AESI = adverse event of special interest; N/A = not applicable
Two-sided 95% CIs calculated according to Altman.
p-value (Overall): Fisher-Freeman-Halton test between Groups L, M, and H1.
Pairwise tests (Fisher exact) performed in case of overall significant p-value.
Source: Section 14, Table 6.4.1.3

Unsolicited AEs starting at or after the Month 12 re-vaccination were reported in 2 (8.3% to 9.5%) subjects each in Arm L and Arm H1. No subjects in Arm M reported any unsolicited AEs at or after the Month 12 re-vaccination. No subjects in the low dose group experienced medically attended solicited or unsolicited AEs starting at or after the re-vaccination at Month 12.

Two AEs in SOC of blood and lymphatic system disorders (leukocytosis and lymphopenia) were reported by 1 (3.2%; Arm L) subject. Sinusitis and pain in extremity each were reported by 1 (4.8%) subject in Arm H1, and asthenia was reported by one (4.2%) subject in Arm L.

None of the reported unsolicited AEs were considered to be related to the vaccination or graded severe. No subjects reported any SAE, related medically attended unsolicited AE or AESI after the Month 12 re-vaccination.

2.6.8.6.4. Study VLA1553-321

Table 49. VLA1553-321: All and Related Unsolicited Adverse Events 28 Days After Single Vaccination Occurring at a Frequency of ⩾0.5% in the VLA1553 Arm Compared With the Placebo Arm (Study Safety Population)

	Adolescents			
Adverse Event Category	**VLA1553-321**			
	VLA1553 (N=502) n (%)		**Placebo (N=252) n (%)**	
Unsolicited Adverse Events	**All**	**Related**	**All**	**Related**
Headache	24 (4.8)	4 (0.8)	17 (6.7)	1 (0.4)
Pyrexia	21 (4.2)	5 (1.0)	7 (2.8)	0
Odynophagia	19 (3.8)	1 (0.2)	9 (3.6)	0
Influenza	16 (3.2)	0	6 (2.4)	0
Eye pain	15 (3.0)	6 (1.2)	0	0
Abdominal pain	13 (2.6)	2 (0.4)	5 (2.0)	0
Cough	13 (2.6)	0	9 (3.6)	0
Neutropenia	12 (2.4)	4 (0.8)	0	0
COVID-19	9 (1.8)	0	2 (0.8)	0
Myalgia	9 (1.8)	1 (0.2)	5 (2.0)	0
Dizziness	8 (1.6)	2 (0.4)	1 (0.4)	0
Dysmenorrhoea	8 (1.6)	0	9 (3.6)	0
Rhinitis	8 (1.6)	1 (0.2)	4 (1.6)	0
Chills	7 (1.4)	3 (0.6)	1 (0.4)	0
Oropharyngeal pain	7 (1.4)	1 (0.2)	3 (1.2)	0
Diarrhoea	6 (1.2)	2 (0.4)	2 (0.8)	0
Leukopenia	5 (1.0)	3 (0.6)	0	0
Nausea	5 (1.0)	2 (0.4)	5 (2.0)	0
Pain in extremity	5 (1.0)	1 (0.2)	0	0
Rhinorrhoea	5 (1.0)	0	7 (2.8)	0
Fatigue	4 (0.8)	1 (0.2)	2 (0.8)	0
Lymphadenopathy	4 (0.8)	0	0	0
Nasal congestion	4 (0.8)	0	3 (1.2)	0
Abdominal pain upper	3 (0.6)	0	0	0
Activated partial thromboplastin time prolonged	3 (0.6)	0	0	0
Anaemia	3 (0.6)	0	1 (0.4)	0
Arthralgia	3 (0.6)	1 (0.2)	3 (1.2)	0
Conjunctival hyperaemia	3 (0.6)	1 (0.2)	0	0
Decreased appetite	3 (0.6)	1 (0.2)	0	0
Dyspnoea	3 (0.6)	1 (0.2)	2 (0.8)	0
Ear pain	3 (0.6)	0	0	0
Eosinophilia	3 (0.6)	0	0	0
Hyperkalaemia	3 (0.6)	1 (0.2)	0	0
Vomiting	3 (0.6)	0	2 (0.8)	0

Note: Adverse events were coded using MedDRA version 24.1. For each system organ class and preferred term, subjects are included only once, even if they experienced multiple events in that system organ class or preferred term.

Source: Module 5.3.5.1, study VLA1553-321, Table 14.3.2.2 and Table 14.3.2.4

In the VLA1553-321 study, Part A analysis, Up to 28 days, there was a difference in the overall incidence in unsolicited and related AEs between VLA 1553 (39.6% and 8.0%, respectively) and the placebo arm (32.5% and 1.2%,respectively).

Overall, headache was the most frequently reported unsolicited AEs in VLA1553 (4.8% vs. 6.7% in placebo), followed by pyrexia (4.2% vs. 2.8%, respectively), odynophagia (3.8% vs. 3.6%), influenza (3.2% vs. 2.4%), eye pain (3.0% vs. 0%), abdominal pain (2.6% vs 2.0%) and cough (2.6% vs 3.6%). Neutropenia was seen in 2.4% (placebo 0%), however this was only performed in the IMM set (n=328 instead of 502) so, when this denominator is used neutropenia, was seen in 3.7% in VLA1553.

There was only one case of joint swelling (verbatim: swollen left ankle) in a subject (seronegative at baseline) in the VLA1553 group. The onset of the event was 12 days post-vaccination with a duration of one day; the event was of mild severity and assessed as not related to VLA1553 vaccination by the investigator. Viraemia was not assessed in this subject.

For unsolicited AEs that were reported up to Day 29, the median onset day in the VLA1553 group varied depending on the SOC (Post hoc analysis):

- Between Day 2 and Day 6 (i.e., 1 to 5 days post-vaccination): eye disorders, reproductive system and breast disorders, skin and subcutaneous tissue disorders, and vascular disorders.
- Between Day 7 and Day 11 (i.e., 6 to 10 days post-vaccination): infections and infestations, gastrointestinal disorders, respiratory, thoracic and mediastinal disorders, blood and lymphatic system disorders, metabolism and nutrition disorders, investigations, ear and labyrinth disorders, and renal and urinary disorders.
- Between Day 11 and Day 29 (i.e., 10 to 28 days post-vaccination): nervous system disorders, general disorders and administration site conditions, musculoskeletal and connective tissue disorders, injury, poisoning and procedural complications, and psychiatric disorders.

Overall, most unsolicited AEs were graded as mild or moderate. Twelve of 754 (1.6%) participants (10/502 [2.0%] in the VLA1553 arm and 2/252 [0.8%] in the placebo arm) experienced at least one unsolicited AE that was graded severe. Overall, the SOC for which most severe unsolicited AEs were documented was blood and lymphatic system disorders (5/754 [0.7%] participants, all in the VLA1553 arm). The most common severe unsolicited AEs were neutropenia (5/754 [0.7%] participants, all in the VLA1553 arm) and headache (2/754 [0.3%] participants, both in the VLA1553 arm).

Of the subjects with severe unsolicited AEs in study VLA1553-321 (Part A), viraemia data are available for 2 subjects (both seropositive at baseline); viraemia was undetectable at Day 8. The subjects experienced severe fever and neutropenia (onset 35 and 42 days post-vaccination) which were assessed as unrelated to VLA1553 vaccination.

Severe unsolicited AEs in the VLA1553 arm:

- The severe unsolicited events of lower limb fracture, activated partial thromboplastin time prolonged, and hyperkalaemia are discussed in the section 2.6.8.7.2. on SAEs.
- The severe events of neutropenia (5 subjects, 5 events) were assessed as not related to VLA1553 vaccination by the investigator. For 3 events that started 14 and 42 days after vaccination, a relationship to VLA1553 vaccination can be considered unlikely due to the timely occurrence of the event relative to the VLA1553 vaccination; these subjects had low neutrophil counts at Visit 0 or pre-vaccination at Visit 1 (FDA toxicity Grade 1 or 2) respectively). Two events of neutropenia were reported on the day of vaccination (both FDA toxicity Grade 3); both subjects had low neutrophil counts at Visit 0 (FDA toxicity Grade 2). Of the neutropenia cases, one subject was baseline seronegative, 4 subjects were seropositive.
- The severe events of headache (2 subjects, 2 events; both subjects were seronegative at baseline) were assessed as related to VLA1553 vaccination by the investigator, although a relationship to VLA1553 vaccination can be considered unlikely due to the timely occurrence of the events relative to the VLA1553 vaccination (onset 13 and 16 days post-vaccination). Both events resolved on the same day of onset and after 2 days post-onset, respectively.

Of the cases in the VLA1553 group with severe unsolicited events, one subject was in the viraemia subset (neutropenia with onset at Day 43); viraemia was not detected at Day 8.

Related unsolicited AEs reported in ≥0.5% for participants were eye pain (1.2% vs. 0%), pyrexia (1.0% vs. 0%), headache (0.8% vs. 0.4%), neutropenia (0.8% vs. 0%), chills (0.6% vs. 0%) and leukopenia (0.6% vs. 0%).

Of note, all these related AEs have been captured as ADR in the SmPC.

2.6.8.7. Serious adverse event/deaths/other significant events

2.6.8.7.1. Deaths

Three deaths were reported in study VLA1553-301 (2 in the VLA1553 arm and 1 in the placebo arm):

- A >50-year-old participant experienced severe coronary artery disease 119 days after study drug administration (VLA1553), which was fatal on the same day. Medical history included hypertension and hypercholesterolemia. The event was assessed by the investigator as not related to vaccination.
- A >50-year-old participant experienced severe COVID-19 165 days after study drug administration (VLA1553), which was fatal on Day 178. The event was assessed by the investigator as not related to vaccination.
- A >60-year-old participant experienced severe mental status changes (anoxic brain injury) 151 days after study drug administration (placebo), which was fatal on Day 161. The event was assessed by the investigator as not related to vaccination.

Viraemia was not assessed at Day 8 and/or Day 29 in these subjects.

In study VLA1553-303, one participant experienced severe drug overdose 552 days after vaccination, which was fatal on the same day (assessed as not related to vaccination).

No death was reported in VLA1553-302, VLA1553-101, and VLA1553-321.

2.6.8.7.2. Other Serious Adverse Events

Across all studies, 1.5% (60/4,112) of participants vaccinated with VLA1553 and 0.7% (9/1,285) of participants vaccinated with placebo reported a total of 87 and 11 SAEs, respectively (table below). Of these SAEs, 3 (0.1%) participants vaccinated with VLA1553 and no participant vaccinated with placebo had SAEs considered to be related to vaccination.

Table 50. Summary of Serious and Other Significant Adverse Events (Safety Population)

	Adults							Adolescents	
AE Category	**VLA1553-301**		**VLA1553-302[a]**	**VLA1553-101[b]**			**VLA1553-303**	**VLA1553-321[c]**	
	VLA1553 (N=3,082) n (%)/E	**Placebo (N=1,033) n (%)/E**	**VLA1553 (N=408) n (%)/E**	**Arm L (N=31) n (%)/E**	**Arm M (N=30) n (%)/E**	**Group H (N=59) n (%)/E**	**VLA1553 (N=363) n (%)/E**	**VLA1553 (N=502) n (%)/E**	**Placebo (N=252) n (%)/E**
Any SAEs	46 (1.5)/73	8 (0.8)/10	5 (1.2)/5	0	1 (3.3)/1	0	4 (1.1)/4	4 (0.8)/4	1 (0.4)/1
Any Related SAEs	2 (0.1)/2	0	0	0	0	0	0	1 (0.2)/1	0
Any AEs Leading to Study Withdrawal	3 (0.1)/3	2 (0.2)/2	0	0	0	1 (1.7)/1	0	0	0
Any Related AEs Leading to Study Withdrawal	0	0	0	0	0	0	0	0	0
Any AESI	10 (0.3)/10	1 (0.1)/1	1 (0.2)/1	0	0	0	0	18 (3.6)/18	3 (1.2)/3
Any Related AESI	9 (0.3)/9	1 (0.1)/1	1 (0.2)/1	0	0	0	0	15 (3.0)/15	1 (0.4)/1
Any Medically attended AEs	386 (12.5)/633	118 (11.4)/172	47 (11.5)/64	2 (6.5)/4	5 (16.7)/5	10 (16.9)/15	0	61 (12.2)/131	25 (9.9)/41
Any Related Medically attended AEs	56 (1.8)/79	7 (0.7)/8	13 (3.2)/21	1 (3.2)/2	0	0	0	41 (8.2)/82	15 (6.0)/22

Abbreviations: AE=adverse event; AESI=adverse event of special interest; E=events; n=number of participants; SAE=serious adverse event; $TCID_{50}$=50% tissue culture infectious dose.
Arm L=3.2×10E3 $TCID_{50}$/dose; Arm M=3.2×10E4 $TCID_{50}$/dose; Arm H=3.2×10E5 $TCID_{50}$/dose
a. All lots combined.
b. SAEs starting before re-vaccination.
c. Includes both early onset AESI (start dates up to 22 days post vaccination) and late onset AESI (i.e., start dates starting after 22 days post vaccination and onwards), data up to 28 days post-vaccination.
Source: Module 5.3.5.1, study VLA1553-301, Table 14.3.2.1.1 and Listing 16.2.7.1;
Module 5.3.1.2, study VLA1553-302, Table 14.3.2.1.1;
Module 5.3.5.1, study VLA1553-101, Table 7.4.1.1 and Listing 4.15;
Module 5.3.1.2, study VLA1553-303, Table 14.3.2.1.1;
Module 5.3.5.1, study VLA1553-321, Table 14.3.2.1

Pooled Safety Population: Studies VLA1553-301, VLA1553-302, and VLA1553-101

Overall, in the pooled safety population, 1.4% (52/3,610) of participants in the pooled VLA1553 arm and 0.8% (8/1,033) of participants in the placebo arm reported a total of 79 and 10 SAEs, respectively. For each SOC, no relevant differences were observed between the 2 groups. For all SOCs, the maximum frequency differences between the 2 groups was 0.1%. The SOC for which most SAEs were documented was Infections and Infestations (0.3% [11/3,610] of participants in the VLA1553 arm and 0.3% [3/1,033] of participants in the placebo arm). The SOCs with the higher differences of SAE frequencies between the 2 groups were:

- Cardiac disorders: 5/3,610 (0.1%: 2 atrial fibrillations, 1 cardiac arrest, 1 cardiomyopathy, and 1 coronary artery disease) VLA1553 vs. 0/1,033 (0%) placebo
- Pregnancy, puerperium and perinatal conditions: 5/3,610 (0.1%: 4 abortions spontaneous, 1 Fetal death) VLA1553 vs. 0/1,033 (0%) placebo

Of these SAEs, 2 (0.1%) participants in the pooled VLA1553 arm (in study VLA1553-301) had SAEs considered to be related to vaccination (versus none in the placebo arm):

- A > 50-year-old participant (seronegative at baseline) experienced mild myalgia 1 day after vaccination, which led to hospitalization from Day 4 to Day 9. Medical history included fibromyalgia. No other trigger for myalgia could be identified and the event was assessed by the investigator as probably related to vaccination. Creatine kinase lab values were within normal range on Day 4. The event was resolved on Day 31. Other solicited systemic events reported were mild headache and mild arthralgia; the onset was 1 to 2 days after vaccination; arthralgia resolved on the same day and headache had a duration of 6 days. Since the myalgia event onset was 1 day after study vaccination and vaccine viraemia was not detectable at Day 8, a prolonged vaccine viraemia can be ruled out as a reason for the prolonged duration of this event (i.e. until 30 days post-vaccination), even though viraemia was not yet tested at Day 29. It can be argued that the prolonged myalgia is attributable to the ongoing chronic condition of

fibromyalgia in this subject which makes myalgia events highly likely. The serious adverse reaction of myalgia does not meet the defined criteria for AESI/ chikungunya like illness. For completeness, the Applicant commits to test samples for viraemia for this subject. (**REC**)

- A >60-year-old participant (serostatus not determined since subject was not in the IMM subset) experienced several solicited AEs 3 days after vaccination, including severe fever (highest temperature was 39.6°C on Day 10 and 38.9°C on Day 11), which resolved on Day 12. At Day 11, he experienced severe atrial fibrillation (AF SAE) and severe hyponatremia, which led to hospitalization from Day 11 to Day 14. The syndrome of inappropriate antidiuretic hormone secretion (SIADH) was diagnosed by a nephrologist. No other cause for SIADH was identified or mentioned by the treating physicians. The applicant considered the event of SIADH more likely to be hypovolemic hyponatremia but agreed to the assessment "related to vaccination" because hypovolemic hyponatremia was most likely developed secondary to the fever observed post-vaccination. The event of SIADH was resolved on Day 24. Retrospectively, SIADH could not be completely ruled out, although a hypovolemic hyponatremia was much more likely. The event of AF was resolved on Day 12 and was assessed as unlikely related to vaccination. The viraemia level was detectable on Day 8 (i.e., 7 days post-vaccination) and was not detected at 28 days post-vaccination. The SAE of hypovolemic hyponatremia does not meet the defined criteria for AESI/ chikungunya like illness.

<u>Cardiac SAEs:</u>

In the pivotal phase 3 study VLA1553-301, cardiac SAEs were seen in 5 (0.2%; 95% CI 0.1, 0.4) subjects (7 events) in the VLA1553 group:

- Participant (also described above for SIADH): The SAE of AF was considered as unlikely related to VLA1553 vaccination by the investigator; as multiple alternative factors existed that might have caused or have contributed to the development of AF in the subject: fluid volume depletion due to diarrhoea and fever (sweating) may have caused an electrolyte imbalance; the observed severe hyponatremia was therefore most probably a secondary event caused by the symptoms of diarrhoea and fever, which caused hypovolemia and maybe AF. There were two known risk factors for AF present in the participant, older age (>65 yoa) and hypertension (reported in the participant's medical history and at the time of the AF event). The participant might have experienced a cardiac side effect to cyclobenzaprine on the days before AF was diagnosed, acknowledging that cyclobenzaprine was only taken for a short period. So, there is a plausible link of the AF SAE 10 days post-vaccination with VLA1553 vaccination and associated vaccine viraemia, but at least one alternative aetiology also existed. The SAE of atrial fibrillation does meet the defined criteria for AESI/chikungunya like illness (broad definition).

Investigators assessed the other cardiac SAEs as unrelated to VLA1553 vaccination; alternative factors for the occurrence of the cardiac events existed, such as older age, ongoing and/or previous comorbidities related to cardiovascular disease (hyperlipidaemia, hypertension, and diabetes), cardiac disorders in the medical history, and overweight/obesity. Furthermore, a relationship to VLA1553 vaccination was considered unlikely due to the timely occurrence of the event relative to the VLA1553 vaccination (most incidents occurred significantly after vaccination).

- Coronary artery disease (CAD) (baseline seronegative [<20 µPRNT50]): the severe event started 119 days after VLA1553 vaccination with duration of one day (not medically attended); the subject was >50 yoa, obese and had a history of hypercholesterolaemia and hypertension; both conditions were ongoing at screening. Viraemia was not assessed in this subject.

- Cardiac arrest (serostatus was not determined since subject was not in IMM subset): the severe event started 32 days after VLA1553 vaccination with duration of one day (medically attended);

the subject was a >70 yoa with normal BMI and had hypertension and hyperlipidaemia in the medical history; both conditions were ongoing at screening; the subject had a medical history including other cardiac conditions or procedures which were resolved at the time of screening (i.e., cardiac murmur, aortic aneurysm, intra-thoracic aortic aneurysm repair, transcatheter aortic valve implantation). Viraemia was not assessed in this subject.

- Cardiomyopathy (serostatus was not determined since subject was not in IMM subset): the severe event started 162 days after VLA1553 vaccination with a duration of 13 days (medically attended); the event of cardiomyopathy was most likely caused by the subject's COVID-19 infection (subject tested positive on Day 163 and had the following symptoms reported: acute respiratory failure, COVID-19 pneumonia, hypoxia and pulmonary embolism). The subject was >30 yoa, obese and medical history included hypertension ongoing at screening. Viraemia was not assessed in this subject.
- Atrial fibrillation (serostatus was not determined since subject was not in IMM subset): the three moderate events of AF started 117, 132, and 164 days after VLA1553 vaccination with a duration of 3, 2, and 6 days (medically attended); the subject was >80 yoa, with overweight and had a medical history of atrial fibrillation which was ongoing at screening; other ongoing medical conditions were hyperlipidaemia and Type 2 diabetes mellitus. Viraemia was not assessed in this subject.

For these four last cases of serious cardiac AEs, viraemia was not assessed in these subjects. The applicant commits to test the Day 8 and Day 29 samples for viraemia for completeness of case assessment. (**REC**)

To ensure identification of all potential cases of cardiac manifestations, additional cardiac-specific safety analyses were conducted on the pooled dataset using the broad SMQs of Cardiac arrythmia and Cardiomyopathy. Overall, the frequency of cardiac events was slightly higher in the pooled VLA1553 arm compared to the placebo arm in study 301: cardiac disorders SOC (0.4% vs. 0.2%, respectively), SMQ Cardiac Arrhythmias (0.5% vs. 0.3%), and SMQ Cardiomyopathy (0.7% vs. 0.5%). For each PT, the maximum frequency differences was 0.1% (Post Hoc analysis).

The SMQs Cardiac Arrythmias and Cardiomyopathy included other PTs in addition to PTs of the SOC Cardiac Disorders; all of these other PTs were reported with low frequency, and most were reported with comparable frequency between the VLA1553 group (pooled) and placebo group. One event within the SMQ Cardiac Arrythmias (PT: syncope) was reported as serious (with moderate severity) in the VLA1553 group; the event onset was 46 days after vaccination and considered unrelated to VLA1553 vaccination with a clear different aetiology. All other non-cardiac events in the SMQs Cardiac Arrythmias and Cardiomyopathy in the VLA1553 group (pooled) were non-serious and non-severe.

The observed small differences might not be representative especially when considering the 3:1 randomization ratio in the pivotal safety study and the fact that the other studies did not have a placebo control group. Overall, these frequencies might be reflective of a general background rate.

Cardiac events have been classified as an important potential risk in the Risk Management Plan. Information on cardiac events will be collected in Post-Authorisation Studies (refer to section 2.7.).

Study VLA1553-321

In study VLA1553-321, a total of 5 participants experienced SAEs (4/502 [0.8%] in the VLA1553 arm and 1/252 [0.4%] in the placebo arm). In the VLA1553 arm, there were 2/405 (0.5%) participants of the seronegative stratum who experienced SAE (one event of pyrexia and one event of hyperkalemia) and 2/97 (2.1%) participants of the seropositive stratum (one event of activated partial thromboplastin

time prolonged and one event of lower limb fracture) (vs. 0% and 2%, respectively, in the placebo arm). The SAE in the placebo arm was prothrombin time prolonged.

SAEs in the VLA1553 arm:

- Subject (>10 yoa, seronegative at baseline) experienced fever of grade 4 according to FDA grading scale (axillary body temperature: 40.2°C) starting 2 days after receiving blinded study vaccine. The investigator considered the event of fever as possibly related to study medication. Four days post-vaccination, the reported body temperature was 37.8°C and 5 days post-vaccination, the first normothermic temperature was reported and the subject was completely asymptomatic. This adverse reaction was part of an AESI in combination with mild arthralgia in arms and hands, mild myalgia, and mild headache. The subject was not in the viraemia subset and viraemia was not determined.

  The symptoms reported by this subject were not exceptional in terms of severity, duration or type of event experienced after VLA1553 vaccination. The events of arthralgia and myalgia were mild and of short duration (3 and 4 days), the event of headache was mild with a duration of 14 days. The event of fever in this subject was reported as serious because the body temperature measurement was grade 4 according to FDA toxicity grading but no other seriousness criteria were met. The subject needed no medical attendance and recovered without sequelae. For completeness, the Applicant commits to test samples for viraemia for this subject). (**REC**)

- A >10 year old participant (seropositive at baseline) experienced severe lower limb fracture 17 days after VLA1553 vaccination. The event was classified as an SAE due to an hospitalization. The investigator assessed the event of lower limb fracture as not related to VLA1553 vaccination; the event can be considered related to an accident while playing soccer.

- A >10 year old participant (seropositive at baseline) experienced activated partial thromboplastin time (APTT) prolonged 7 days after VLA1553 vaccination. The event was classified as an SAE due to due to FDA grading scale definition of life-threatening criterion (Grade 4 event) was met. The investigator assessed the event of APTT prolonged as not related to VLA1553 vaccination; the subject had a prolonged APTT already at Visit 0 (Grade 1) which prolonged further until pre-vaccination at Visit 1 (Grade 3) and post-vaccination Day 8 (Grade 4). At Day 29, APTT returned to the duration measured at Visit 1. The subject was not in the viraemia subset and viraemia was not determined in this subject. This AE was initially reported as serious but was downgraded to an unsolicited AE and later on completely removed from the AE page of the eCRF because it was assessed as not clinically relevant.

- A >10 year old participant (seronegative at baseline) experienced Grade 4 hyperkalaemia 8 days after study drug administration. The event was classified as an SAE due to being medically important. The investigator assessed the event of hyperkalaemia as not related to VLA1553 vaccination; the subject had elevated potassium measurements at Visit 0 and 1 (Grade 1) which further increased up to Day 8 (Grade 4). The levels normalized on Day 29; another increase was measured on study Day 34 (Grade 4) and potassium levels were again normalized on Day 87. The subject was not in the viraemia subset and viraemia was not determined.

Study VLA1553-303

Ten new SAEs were experienced in 9/363 (2.5%) participants during trial VLA1553-303 (7/310 [2.3%] participants of Stratum A [18 to 64 years] and 2/53 [3.8%] participants of Stratum B [≥65 years]). Serious adverse events by SOC and PT are provided in Table below. None of these SAEs were considered related to vaccination.

Table 51. Serious Adverse Events by System Organ Class and Preferred Term (Whole Sample Population)

System Organ Class Preferred Term [n (%) m]	18 to 64 Years (Stratum A) (N=310)	≥65 Years (Stratum B) (N=53)	Total (N=363)
Any Serious Adverse Events	7 (2.3) 8	2 (3.8) 2	9 (2.5) 10
Injury, Poisoning and Procedural Complications	3 (1.0) 3	0	3 (0.8) 3
Gun Shot Wound	1 (0.3) 1	0	1 (0.3) 1
Overdose	1 (0.3) 1	0	1 (0.3) 1
Pelvic Fracture	1 (0.3) 1	0	1 (0.3) 1
Nervous System Disorders	2 (0.6) 2	1 (1.9) 1	3 (0.8) 3
Apallic Syndrome	1 (0.3) 1	0	1 (0.3) 1
Intracranial Aneurysm	0	1 (1.9) 1	1 (0.3) 1
Seizure	1 (0.3) 1	0	1 (0.3) 1
Cardiac Disorders	1 (0.3) 1	1 (1.9) 1	2 (0.6) 2
Coronary Artery Disease	0	1 (1.9) 1	1 (0.3) 1
Myocardial Infarction	1 (0.3) 1	0	1 (0.3) 1
Gastrointestinal Disorders	1 (0.3) 1	0	1 (0.3) 1
Abdominal Pain Upper	1 (0.3) 1	0	1 (0.3) 1
Hepatobiliary Disorders	1 (0.3) 1	0	1 (0.3) 1
Cholecystitis	1 (0.3) 1	0	1 (0.3) 1

n=number of participants; m=number of events; MedDRA=Medical Dictionary for Regulatory Activities dictionary.
Note: Serious adverse events were coded using MedDRA version 24.1. For each System Organ Class and Preferred Term, participants were included only once, even if they experienced multiple events in that System Organ Class or Preferred Term.
At time of database interim lock, the 2 events of apallic syndrome and myocardial infarction reported by participant 1553-1-04-115 were included in the clinical database. However, it was confirmed later that these events occurred after Year 2.
Source: Table 14.3.2.1 and Listing 16.2.6.1

2.6.8.7.3. Adverse events of special interest

AESIs are defined in section 2.6.8.2. and include nonspecific transient muscle pain and joint pain and signs and symptoms suggesting an acute stage CHIKV-associated event.

Sponsor definition

AESIs were reported in 0.3% (11/3,610) of participants in the pooled VLA1553 arm and 0.1% (1/1,033) of participants in placebo arm. No AESI was reported by any participant ≥65 yoa.

The most frequently observed AESI were a combination of pyrexia and arthralgia (and/or with other AEs such as back pain, rash, peripheral oedema, lymphadenopathy). Most symptoms contributing to an AESI were graded as mild or moderate in severity and self-limited after 2 to 4 days. Two participants had prolonged symptoms: 1 participant with fever, arthralgia, and lower back pain, who early discontinued from the study on Day 51; 1 participant with fever, oedema peripheral and arthralgia/arthritis, who was tested positive for genetic predisposition marker for arthralgia/arthritis HLA-B*27. In total, 6 participants in the VLA1553 arm experienced an AESI that was graded severe due to severe pyrexia. All AESIs were considered as related to the vaccination, except one case of pyrexia in combination with arthralgia reported by one participant in the VLA1553 arm with underlying conditions that could explain the event. In study VLA1553-303, there were no ongoing AESI for the 363 participants from precursor study VLA1553-301.

Broader definition

Pooled Safety Population: Studies VLA1553-301, VLA1553-302, and VLA1553-101

Any Chikungunya-like adverse reactions

Table 52. Cases of Chikungunya-like adverse reactions in the VLA1553 pooled group and placebo group (Post Hoc analysis)

	Statistic	301 Placebo (N=1033)	Pooled VLA1553 (N=3610)	Pooled All (N=4643)
Any cases of Chikungunya-like adverse reaction	n (%)	6 (0.6)	436 (12.1)	442 (9.5)
Any cases of Severe Chikungunya-like adverse reaction	n (%)	0 (0.0)	65 (1.8)	65 (1.4)
Any cases of Related Chikungunya-like adverse reaction	n (%)	6 (0.6)	418 (11.6)	424 (9.1)
Any cases of Serious Chikungunya-like adverse reaction	n (%)	0 (0.0)	1 (0.0)	1 (0.0)

The proportion of subjects with Chikungunya-like adverse reactions was 12.1% in the VLA1553 pooled group and 0.6% in the placebo group.

Table 53. AESI Symptoms of Subjects with an AESI (broader definition) (Participants with any AESI) (Post Hoc analysis)

	Statistic	301 Placebo Any AESI symptom (N=6)	Pooled VLA1553 Any AESI symptom (N=436)
Any AESI symptom	n (%) Obs	6 (100) 20	436 (100) 1688
Pyrexia	n (%) Obs	6 (100) 6	436 (100) 437
Headache	n (%) Obs	5 (83.3) 5	340 (78.0) 345
Fatigue	n (%) Obs	5 (83.3) 5	321 (73.6) 322
Myalgia	n (%) Obs	1 (16.7) 1	258 (59.2) 260
Arthralgia	n (%) Obs	2 (33.3) 2	186 (42.7) 192
Chills	n (%) Obs	0 (0.0) 0	37 (8.5) 37
Rash	n (%) Obs	0 (0.0) 0	26 (6.0) 28
Back pain	n (%) Obs	0 (0.0) 0	16 (3.7) 16
Lymphadenopathy	n (%) Obs	0 (0.0) 0	11 (2.5) 12
Dizziness	n (%) Obs	0 (0.0) 0	9 (2.1) 9
Pain	n (%) Obs	0 (0.0) 0	6 (1.4) 6
Paraesthesia	n (%) Obs	0 (0.0) 0	4 (0.9) 4
Hyperhidrosis	n (%) Obs	0 (0.0) 0	3 (0.7) 3
Asthenia	n (%) Obs	0 (0.0) 0	2 (0.5) 2
Oedema peripheral	n (%) Obs	0 (0.0) 0	2 (0.5) 2
Syncope	n (%) Obs	0 (0.0) 0	2 (0.5) 2
Ataxia	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Atrial fibrillation	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Chest pain	n (%) Obs	1 (16.7) 1	0 (0.0) 0
Cold sweat	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Feeling abnormal	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Hypoaesthesia	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Influenza like illness	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Lymph node pain	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Lymphadenitis	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Neuropathy peripheral	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Palpitations	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Rash erythematous	n (%) Obs	0 (0.0) 0	1 (0.2) 1

The following AESI symptoms were commonly identified (in addition to fever) in the VLA1553 pooled group (>1% of the AESIs): headache (78%), fatigue (73.6%), myalgia (59.2%), arthralgia (42.7%), chills (8.5%), rash (6%), back pain (3.7%), lymphadenopathy (2.5%), dizziness (2.1%) and pain (1.4%).

In the VLA1553 pooled group:

- There were 1 case of AESI with atrial fibrillation and 1 case with palpitations. The event of palpitations was considered related to VLA1553 vaccination, was mild in intensity and lasted 1 day. The SAE of severe atrial fibrillation (was considered as unlikely related to VLA1553 vaccination

by the investigator: there is a plausible link of the AF SAE 10 days post-vaccination with VLA1553 vaccination and associated vaccine viraemia, but at least one alternative aetiology also existed.

- Three Chikungunya-like adverse reactions were identified that included the symptoms hypoesthesia, neuropathy peripheral, and ataxia, respectively; the events of hypoesthesia and neuropathy peripheral were assessed unrelated to VLA1553 vaccination by the investigator and were mild in intensity; the duration was 15 and 3 days, respectively; the symptom of ataxia was considered related to VLA1553 vaccination and was of mild intensity with a duration of 8 days; all three events recovered/resolved.

- Two Chikungunya-like adverse reactions that included the symptom of syncope. Both events were mild, assessed unrelated to VLA1553 vaccination and were of short duration (1 day); 1 subject had chills, pyrexia, decreased appetite, headache, nausea before the onset of syncope – due to bed rest, lack of sleep, possible dehydration due to fever, and nausea, the subject might have been susceptible to experience syncope. The other subject had no symptoms occurring at the same time the syncope was experienced; however, the onset of the event was rather late (30 days) after VLA1553 vaccination.

Serious Chikungunya-like adverse reaction

Only 1 Chikungunya-like adverse reaction met the definition of serious: the SAE atrial fibrillation (severe event) of one subject vaccinated with VLA1553 in study VLA1553-301. This subject also had symptoms of fatigue (2 mild and moderate events), headache (2 mild events) and myalgia (1 moderate event). Chikungunya-like adverse reaction duration was 42 days and longest symptom duration was 30 days (mild headache). There were no serious Chikungunya-like adverse reaction reported in the placebo group.

Severe Chikungunya-like adverse reaction

The proportion of subjects with a severe Chikungunya-like adverse reaction was 1.8% in the VLA1553 pooled group; there were no severe Chikungunya-like adverse reaction reported in the placebo group.

Table 54. Severe AESI Symptoms of Subjects with an AESI (broad definition) by Term (Participants with any AESI) (Post Hoc analysis)

	Statistic	301 Placebo Any AESI symptom (N=6)	Pooled VLA1553 Any AESI symptom (N=436)
Any severe AESI symptom	n (%) Obs	0 (0.0) 0	65 (14.9) 72
Pyrexia	n (%) Obs	0 (0.0) 0	54 (12.4) 54
Arthralgia	n (%) Obs	0 (0.0) 0	5 (1.1) 5
Fatigue	n (%) Obs	0 (0.0) 0	4 (0.9) 4
Myalgia	n (%) Obs	0 (0.0) 0	4 (0.9) 4
Headache	n (%) Obs	0 (0.0) 0	3 (0.7) 3
Atrial fibrillation	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Back pain	n (%) Obs	0 (0.0) 0	1) (0.2) 1

In the VLA1553 group, severe pyrexia was reported with a frequency of 12.4%; all other severe symptoms of arthralgia, fatigue, myalgia, headache, atrial fibrillation and back pain were reported with a frequency of below 1.1%.

There were 16/65 subjects with a severe AESI in the VLA1553 group (pooled dataset) who had a positive viraemia result at Day 3 (study VLA1553-101) or Day 8 (study VLA1553-301 and 302; viraemia was not assessed at Day 3 in these studies): 6 subjects were in study VLA1553-101 (3 in the high dose group, 2 in the low dose group and 1 in the medium dose group), 7 subjects were in study VLA1553-301, and 3 subjects were in study VLA1553-302. The severe symptoms present in subjects with viraemia were in the majority of cases fever (11 subjects); other severe symptoms were arthralgia (1 subject), fatigue (1

subject), and back pain (1 subject) or a combination of symptoms (1 subject with fever and atrial fibrillation; 1 subject with arthralgia, fatigue, and myalgia).

Most of these severe AESI symptoms were considered related to VLA1553 vaccination and were self-limited. Most of the severe fever cases resolved after 1 to 3 days except for 2 events with a duration between 6 and 7 days; none of the fever events were serious; similarly, the other severe symptoms of arthralgia, fatigue, and myalgia were of short duration (1 to 5 days) and non-serious; back pain was experienced for 9 days and was non-serious.

Onset and duration of Chikungunya-like adverse reactions

The mean onset Day for Chikungunya-like adverse reactions was Day 3.6 (median: Day 4) in the VLA1553 pooled group and Day 3.3 (Median: Day 2.5) in the placebo group. The mean duration was 9.7 days (median 4 days) in the VLA1553 pooled group and 11.2 days (median 8 days) in the placebo group.

An analysis was conducted to identify Chikungunya-like adverse reactions with symptoms with a duration of at least 30 days which can be considered as prolonged Chikungunya-like adverse reaction (Table 55 below).

Table 55. AESI Symptoms >=30 Days Duration of Subjects with an AESI (broad definition) by Term (Participants with any AESI)

	Statistic	301 Placebo Any AESI symptom (N=6)	Pooled VLA1553 Any AESI symptom (N=436)
Any AESI symptom with duration >= 30 days	n (%) Obs	0 (0.0) 0	16 (3.7) 23
Arthralgia	n (%) Obs	0 (0.0) 0	5 (1.1) 6
Myalgia	n (%) Obs	0 (0.0) 0	5 (1.1) 5
Fatigue	n (%) Obs	0 (0.0) 0	3 (0.7) 3
Headache	n (%) Obs	0 (0.0) 0	3 (0.7) 3
Lymphadenopathy	n (%) Obs	0 (0.0) 0	2 (0.5) 2
Back pain	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Oedema peripheral	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Pyrexia	n (%) Obs	0 (0.0) 0	1 (0.2) 1
Rash erythematous	n (%) Obs	0 (0.0) 0	1 (0.2) 1

Overall, 16 participants experienced a Chikungunya-like adverse reaction with prolonged symptoms; all symptoms were mild or moderate in severity (0.4% of all participants in this group: 16/3610).

Symptoms of six subjects were assessed as not related to VLA1553 vaccination by the investigator. According to the Applicant's assessment, one participant had a medical history potentially explaining the prolonged event.

Day 8 viraemia results are available for four individuals: no viraemia was detected in three individuals; for one individual viraemia was below limit of detection. Thus, for these individuals it is reasonable to assume that the observed prolonged events were not related to VLA1553.

Additionally, for one individual there is a strong indication that events flagged as prolonged during the requested analysis actually resolved within one day.

Causality of Chikungunya-like adverse reactions

Most of the Chikungunya-like adverse reactions were classified as related (i.e., at least one symptom which was assessed as related by the investigator) since the most common symptoms comprising a Chikungunya-like adverse reaction were solicited symptoms (i.e., pyrexia, headache, fatigue, myalgia, and arthralgia). Out of 436 Chikungunya-like adverse reactions in the VLA1553 pooled group, 418 (95.9%) were classified as related.

Study VLA1553-321

Any Chikungunya-like adverse reactions

Table 56. Cases of Chikungunya-like adverse reactions in the VLA1553 pooled group and placebo group (Post Hoc analysis)

	Statistic	VLA1553 (N=502)	Placebo (N=252)	Total (N=754)
Any cases of Chikungunya-like adverse reaction	n (%)	117 (23.3) 117	12 (4.8) 12	117 (23.3) 117
Any cases of Severe Chikungunya-like adverse reaction	n (%)	17 (3.4) 17	0 (0.0) 0	17 (3.4) 17
Any cases of Related Chikungunya-like adverse reaction	n (%)	116 (23.1) 116	10 (4.0) 10	116 (23.1) 116
Any cases of Serious Chikungunya-like adverse reaction	n (%)	1 (0.2) 1	0 (0.0) 0	1 (0.2) 1

The proportion of subjects with Chikungunya-like adverse reactions was 23.3% in the VLA1553 group and 4.8% in the placebo group. The percentages are higher in both treatment groups when compared to the pooled dataset in adults (VLA1553 pooled group: 12.1%; placebo group: 0.6%).

Table 57. AESI Symptoms of Subjects with an AESI (Broad definition) (Participants with any AESI) (Post Hoc analysis)

	Statistic	Placebo Any AESI symptom (N=12)	VLA1553 Any AESI symptom (N=117)
Any AESI symptom	n (%) Obs	12 (100) 36	117 (100) 414
Pyrexia	n (%) Obs	12 (100) 12	117 (100) 124
Headache	n (%) Obs	11 (91.7) 12	102 (87.2) 115
Myalgia	n (%) Obs	3 (25.0) 3	68 (58.1) 71
Fatigue	n (%) Obs	6 (50.0) 6	52 (44.4) 53
Arthralgia	n (%) Obs	2 (16.7) 2	33 (28.2) 33
Rash	n (%) Obs	0 (0.0) 0	12 (10.3) 12
Chills	n (%) Obs	0 (0.0) 0	4 (3.4) 4
Hand dermatitis	n (%) Obs	0 (0.0) 0	1 (0.9) 1
Paraesthesia	n (%) Obs	0 (0.0) 0	1 (0.9) 1
Pruritus	n (%) Obs	1 (8.3) 1	0 (0.0) 0

The reported PTs are consistent with those reported in adults. Similarly to the pooled data set, the following AESI symptoms were commonly identified (in addition to fever) in the VLA1553 group (>1% of the AESIs): headache (87.2%), myalgia (58.1%), fatigue (44.4%), arthralgia (28.2%), rash (10.3%) and chills (3.4%). In the VLA1553 group, the symptom of hand dermatitis was reported in one subject which can be considered as a rash-like symptom.

Serious Chikungunya-like adverse reaction

One Chikungunya-like adverse reaction met the definition of serious. A >10-year-old (baseline seronegative) had mild symptoms of arthralgia, headache, and myalgia. The event of fever was serious, related to VLA1553 and grade 4 according to FDA grading scale (axillary body temperature: 40.2°C) starting two days after receiving blinded study drug. The first normothermic temperature was reported 4 days after fever onset. As per protocol, any grade 4 event required reporting as an SAE, no other seriousness criterion was met. These events were classified by the Applicant as an AESI. The participant recovered without sequelae.

There were no serious Chikungunya-like adverse reaction reported in the placebo group.

Severe Chikungunya-like adverse reaction

The proportion of subjects with a severe Chikungunya-like adverse reaction was 3.4% in the VLA1553 group and none in the placebo group.

Table 58. Severe AESI Symptoms of Subjects with an AESI (broad definition) by Term (Participants with any AESI) (Post Hoc analysis)

	Statistic	Placebo Any AESI symptom (N=12)	VLA1553 Any AESI symptom (N=117)
Any severe AESI symptom	n (%) Obs	0 (0.0) 0	17 (14.5) 21
Pyrexia	n (%) Obs	0 (0.0) 0	14 (12.0) 14
Headache	n (%) Obs	0 (0.0) 0	4 (3.4) 5
Arthralgia	n (%) Obs	0 (0.0) 0	1 (0.9) 1
Myalgia	n (%) Obs	0 (0.0) 0	1 (0.9) 1

In the VLA1553 group, similarly to the pooled data set, severe pyrexia was reported with a frequency of 12%; and arthralgia and myalgia were reported with a frequency of 0.9%. Headache was reported with a frequency of 3.4% (higher than in the pooled data set: 0.7%).

The 14 events of severe fever were all assessed as related to VLA1553 vaccination, were in the majority of cases short in duration, i.e., 1 to 2 days (only 2 events had a duration of 4 and 6 days, respectively). Only one event was serious (see case description for subject above) and only 4 events were medically attended.

The 5 events of severe headache in the VLA1553 group, were assessed as related to VLA1553 vaccination and were all self-limiting with short duration of 1 to 4 days except for 1 subject with an event duration of 11 days; none of the events were serious and only 1 subject needed medical attention (headache duration was 4 days).

There were 3/17 subjects with a severe AESI in the VLA1553 group for whom viraemia was assessed. One subject had non-detectable viraemia at Day 8 and two subjects were viraemic. The severe symptom present in viraemic subjects was fever. The duration was 1 and 6 days; both events were non-serious.

Onset and duration of Chikungunya-like adverse reactions

The mean onset and duration of Chikungunya-like adverse reactions analysed in study VLA1553-321 was comparable to the pooled dataset in adults. The mean onset Day for Chikungunya-like adverse reactions was Day 3.4 (median: Day 3) in the VLA1553 group and Day 2.7 (Median: Day 1.5) in the placebo group. The mean duration was 6.9 days (median 4 days) in the VLA1553 group and 10 days (median 8.5 days) in the placebo group.

There were no prolonged Chikungunya-like adverse reactions (i.e. at least one symptom with a duration of at least 30 days).

Causality of Chikungunya-like adverse reactions

Similar to the pooled dataset, most of the Chikungunya-like adverse reactions were classified as related (i.e., at least one symptom which was assessed as related by the investigator) since the most common symptoms comprising a Chikungunya-like adverse reaction were solicited symptoms (i.e., pyrexia, headache, fatigue, myalgia, and arthralgia). Out of 117 Chikungunya-like adverse reactions in the VLA1553 group, 116 (99.1%) were classified as related.

Study VLA1553-303

Of the overall cases with Chikungunya-like adverse reactions in study VLA155-301, 43 of these subjects (all received VLA1553) enrolled in the follow-up study VLA1553-303. Three of the 43 cases were severe (all assessed as related); the Chikungunya-like adverse reaction onset was 2 to 5 days after vaccination, with a short duration (4 to 6 days). The severe symptoms were arthralgia, myalgia, fatigue, and fever;

the symptom duration was short with 2 to 5 days. All these cases are included in the discussion and summary of Chikungunya-like adverse reactions for the VLA1553 pooled group.

2.6.8.7.4. Medically Attended Adverse Events

Across studies VLA1553-301, VLA1553-302, VLA1553-101, VLA1553-303, and VLA1553-321, MAAEs were reported in 12.4% of participants vaccinated with VLA1553 and 11.2% of participants vaccinated with placebo. Related MAAEs were reported for 2.7% and 1.7%, respectively.

Pooled Safety Population: Studies VLA1553-301, VLA1553-302, and VLA1553-101

Overall, MAAEs up to 6 months occurred in 12.3% participants in the pooled VLA1553 arm and 11.3% in the placebo arm. The SOCs for which most MAAEs were documented were Infections and Infestations (3.9% in the pooled VLA1553 arm and 4.2% in the placebo arm), Musculoskeletal and Connective Tissue Disorders (2.2 vs. 1.6%, respectively), and Injury, Poisoning and Procedural Complications (1.9% vs. 1.9%, respectively). The most common MAAE (≥1% of participants) was headache (1.0% [36/3,610] vs. 0.3% [3/1,033], respectively).

Overall, related MAAEs occurred in 1.9% participants in the VLA1553 arm and 0.7% participants in the placebo arm. The SOCs for which most related MAAEs were documented were Nervous System Disorders (0.7% in the pooled VLA1553 arm and 0.1% in the placebo arm), Musculoskeletal and Connective Tissue Disorders (0.7% vs. 0.3%, respectively), and General Disorders and Administration Site Conditions (0.6% vs. 0.1%, respectively). The most common related MAAEs (≥0.5% of participants) were headache (0.7% [25/3,610] in the VLA1553 arm vs. 0.1% [1/1,033] in the placebo arm) and myalgia (0.6% [21/3,610] in the VLA1553 arm vs. none in the placebo arm).

In study VLA1553-301, most MAAEs were graded as mild or moderate in severity. Overall, 1.2% (38/3,082) in the VLA1553 arm and 1.2% (12/1,033) in the placebo arm experienced at least one MAAE that was graded as severe. The most frequent MAAE graded as severe was pyrexia in 0.1% (4/3,082) in the VLA1553 arm and none in the placebo arm. The majority of the reported MAAEs are solicited AEs already identified as ADRs (please see section 2.6.8.13.).

Of the subjects with related MAAEs in the VLA1553 group of study VLA1553-301, viraemia data are available for 13 subjects including all subjects with related severe MAAEs (5 subjects with 6 events). Since the subjects were not part of the IMM subset, the serostatus at baseline was not determined. Of the subjects with related MAAEs of mild or moderate severity, viraemia was either not detected or was <LOD/<LLOQ at Day 8. For 3 subjects with related severe MAAEs, viraemia was <LOD or <LLOQ at Day 8; the AE reported in these subjects were fever (2 events), arthralgia (1 event), and chest discomfort (1 event). In 2 subjects with related severe MAAEs, viraemia was detectable at Day 8:

- One subject (had a severe event of SIADH (already discussed in section 2.6.8.7.2.). To summarize, the viraemia level of this subject was detectable on Day 8 (i.e., 7 days post-vaccination) and was not detected at 28 days post-vaccination.
- One subject experienced a severe event of myalgia. Viraemia was detectable on Day 8 ; viraemia was not detected on Day 29. The event onset was one day post-vaccination with a duration of 6 days. Since the event onset was closely after the vaccination and event duration was short, a testing of the Day 29 sample to exclude late onset vaccine viraemia is not anticipated.

In study VLA1553-302, the overall frequency of MAAEs graded as mild, moderate, or severe was 3.7% (15/408), 6.4% (26/408), and 1.5% (6/408), respectively (study VLA1553-302, Table 14.3.2.10.2). MAAEs graded as severe were reported for 2.9% (4/136), 0.7% (1/137), and 0.7% (1/135) in Lot A, Lot B, and Lot C, respectively.

Of the subjects with related MAAEs in study VLA1553-302, viraemia data are available for one subject. This subject experienced mild fatigue, headache, and myalgia and severe fever. The events' onset was on Day 5 (i.e., 4 days post-vaccination) and resolved after 2 to 3 days. The vaccine viraemia at Day 8 was <LLOQ and no viraemia was detected on Day 29.

In study VLA1553-101, there were no medically attended unsolicited AE.

Of the subjects with related MAAEs in study VLA1553-101 (2 subjects with 3 events):

- Subject (Arm L; seronegative at baseline) had nausea 2 days after re-vaccination at Month 6. The event was assessed as severe and resolved on the same day. The subject had no detectable viraemia 3, 7, and 14 days after re-vaccination.
- Subject (Arm L; seronegative at baseline) had moderate sinus congestion and oropharyngeal pain 12 days after primary vaccination with a duration of 13 days. Viraemia levels were detectable on Day 3, <LOD on Day 7 and undetectable on Day 14.

Taken together, viraemia was either <LOD or undetectable at the time of the events' onset.

Study VLA1553-321

MAAEs were reported in 61/502 (12.2%) participants in the VLA1553 arm and in 25/252 (9.9%) participants in the placebo arm, of which 41/502 (8.2%) and 15/252 (6.0%) participants, respectively, experienced events that were considered related.Related medically attended solicited AEs were reported in 39/502 (7.8%) and 14/252 (5.6%) participants in the VLA1553 and placebo arms, respectively, while related medically attended unsolicited AEs were reported in 4/502 (0.8%) and 1/252 (0.4%) participants in each arm, respectively.

The SOCs for which most MAAEs were documented were General Disorders and Administration Site Conditions (6.0% in the VLA1553 arm and 3.2% in the placebo arm), Nervous System Disorders (5.0% vs. 3.2%, respectively), and Musculoskeletal and Connective Tissue Disorders (3.0% vs. 2.4%, respectively). The most common MAAEs (≥0.5% of participants) were headache (0.7% [25/3,610] in the VLA1553 arm vs. 0.1% [1/1,033] in the placebo arm) and myalgia (0.6% [21/3,610] in the VLA1553 arm vs. none in the placebo arm).

Of the subjects with related MAAEs in the VLA1553 group of study VLA1553-321, viraemia data are available for 4 subjects of the viraemia subset. In 3/4 subjects, viraemia was not detected at Day 8. The related MAAEs of these subjects were of mild or moderate severity, had an onset between Day 1 and Day 5 and resolved withing 2 days. One subject was viraemic at an acute visit on Day 6 ; viraemia was not detected on Day 8 (Visit 2). The related MAAE was severe solicited fever with onset on Day 1 and with a duration of 6 days. The other (not medically attended) solicited systemic events were all mild (headache, arthralgia, myalgia, nausea, fatigue) with onset between Day 1 and Day 6 and a duration between 1 to 8 days (shortest duration was arthralgia, longest duration was headache).

Study VLA1553-303

MAAEs were not collected for study VLA1553-303.

2.6.8.8. Laboratory findings

2.6.8.8.1. Clinical Laboratory Evaluations

Clinical laboratory data were not part of the pooling exercise. Safety laboratory samples were available from studies VLA1553-301, VLA1553-302, VLA1553-101, and VLA1553-321. In studies VLA1553-301 and VLA1553-321, safety laboratory samples were taken only from the IMM subset:

- In study VLA1553-301, IMM subset: VLA1553, n=372; placebo, n=125.
- In study VLA1553-321 (adolescents in Brazil), IMM subset: VLA1553, n=328; placebo, n=56. The CSR with cut-off date of 26 Jul 2023 has been provided with the Part A analysis: data are available for all participants up to 28 days after vaccination. Six month data will be provided in Part B.

2.6.8.8.1.1. Haematology

2.6.8.8.1.1.1. Study VLA1553-301

The following relevant mean changes from baseline were observed in the VLA1553 arm on Day 8 (not observed in the placebo arm) (SAP study VLA1553-301 Table 14.3.3.1.1, IMM subset):

- Leukocytes: −2.34×10E9/L (vs. 0.03×10E9/L in placebo) and returned to within normal ranges 28 days post vaccination.
- Basophils: −0.03×10E9/L (vs. 0) and returned to within normal ranges 28 days post vaccination.
- Eosinophils: −0.06×10E9/L (vs. 0) and returned to within normal ranges 28 days post vaccination.
- Lymphocytes: −0.54×10E9/L (vs. -0.02×10E9/L) and returned to within normal ranges 28 days post vaccination.
- Neutrophils: −1.68×10E9/L (vs. 0.03×10E9/L) and returned to within normal ranges 28 days post vaccination.
- Platelets: −30.5×10E9/L (vs. 1.4×10E9/L) and returned to within normal ranges 28 days post vaccination.
- Erythrocyte sedimentation rate: 2.04 mm/h (vs. 0.78 mm/h) and returned to within normal ranges 28 days post vaccination.

Table 59. Shift Summary of Haematology Results by Maximum FDA Toxicity Grade for Immunogenicity Subset

Laboratory Parameter Grade	VLA1553 (N=372) n (%)	Placebo (N=125) n (%)
Leukocyte decrease		
Grade 1	99 (27.3)	7 (5.8)
Grade 2	16 (4.4)	0
Grade 3	1 (0.3)	0
Grade 4	0	0
Neutrophil decrease		
Grade 1	100 (27.6)	14 (11.6)

Laboratory Parameter Grade	VLA1553 (N=372) n (%)	Placebo (N=125) n (%)
Grade 2	41 (11.3)	1 (0.8)
Grade 3	11 (3.0)	0
Grade 4	1 (0.3)	0
Lymphopenia		
Grade 1	69 (19.1)	8 (6.6)
Grade 2	15 (4.1)	1 (0.8)
Grade 3	1 (0.3)	0
Grade 4	0	0
Haemoglobin decrease		
Grade 1	49 (13.5)	17 (14.0)
Grade 2	20 (5.5)	6 (5.0)
Grade 3	3 (0.8)	0
Grade 4	0	0

Note: For each laboratory parameter and visit, subjects are included only once, in the maximum Toxicity grade. Total is the number of subjects with at least 1 postbaseline result, and percentages are based on the row total. Only subjects in the immunogenicity subset are included in this tabulation. Grade 0 = Normal; Grade 1 = Mild; Grade 2 = Moderate; Grade 3 = Severe; Grade 4 = Potentially Life Threatening.

The most common FDA Toxicity Grade shifts in haematology parameters were mainly from Grade 0 to Grade 1 or 2. Leukopenia (leukocyte decreased), neutropenia (neutrophile decreased) and lymphopenia (lymphocyte decreased) were more frequently observed in the adults vaccinated with VLA1553 than with placebo.

2.6.8.8.1.1.2. Study VLA1553-302

The following relevant mean changes from baseline were observed in the overall Safety Population 7 days post vaccination: Leukocytes: −2.17×10E9/L and returned to within normal ranges 28 days post vaccination.

- Basophils: −0.03×10E9/L and returned to within normal ranges 28 days post vaccination.
- Eosinophils: −0.05×10E9/L and returned to within normal ranges 28 days post vaccination.
- Lymphocytes: −0.56×10E9/L and returned to within normal ranges 28 days post vaccination.
- Monocytes/Leukocytes: 2.3% and returned to within normal ranges 28 days post vaccination.
- Neutrophils: −1.52×10E9/L and returned to within normal ranges 28 days post vaccination.
- Platelet: −28.7×10E9/L and returned to within normal ranges 28 days post vaccination and increased 6 months post vaccination (mean change from baseline: 37.2×10E9/L).
- Erythrocyte sedimentation rate increased on 7 and 28 days post vaccination (mean change from baseline: 1.2 mm/h and 1.6 mm/h, respectively) and returned to within normal range 84 days post vaccination.

Table 60. Shift Summary of Haematology Results by Maximum FDA Toxicity Grade (Safety Analysis Set)

Laboratory Parameter Grade	VLA1553 lot 1 (N=136) n (%)	VLA1553 lot 2 (N=137) n (%)	VLA1553 lot 3 (N=135) n (%)
Leukocyte decrease			
Grade 1	35 (25.7)	38 (27.9)	44 (33.1)
Grade 2	4 (2.9)	4 (2.9)	2 (1.5)
Grade 3	0	0	0
Grade 4	0	0	0
Neutrophil decrease			
Grade 1	40 (29.4)	43 (31.6)	44 (33.1)
Grade 2	14 (10.3)	15 (11.0)	12 (9.0)
Grade 3	2 (1.5)	2 (1.5)	1 (0.8)
Grade 4	0	0	0
Lymphopenia			
Grade 1	19 (14.0)	29 (21.3)	24 (18.0)
Grade 2	4 (2.9)	6 (4.4)	5 (3.8)
Grade 3	1 (0.7)	1 (0.7)	0
Grade 4	0	0	0
Haemoglobin decrease			
Grade 1	29 (21.3)	16 (11.8)	27 (20.3)
Grade 2	6 (4.4)	7 (5.1)	8 (6.0)
Grade 3	0	2 (1.5)	0
Grade 4	0	0	0

Note: For each laboratory parameter and visit, subjects are included only once, in the maximum Toxicity grade. Total is the number of subjects with at least 1 postbaseline result, and percentages are based on the row total. Only subjects in the immunogenicity subset are included in this tabulation. Grade 0 = Normal; Grade 1 = Mild; Grade 2 = Moderate; Grade 3 = Severe; Grade 4 = Potentially Life Threatening.

The most common FDA Toxicity Grade shifts in haematology parameters were mainly from Grade 0 to Grade 1 or 2. The haematology parameters were similar across the 3 lots.

2.6.8.8.1.1.3. Study VLA1553-101

Parameters outside normal range included haemoglobin, haematocrit, erythrocytes, leukocytes, eosinophils, lymphocytes, monocytes, neutrophils, platelets, and erythrocyte sedimentation rate. There was a temporal relationship between the number of participants presenting with parameters outside normal range and the vaccination. Most values outside the normal range occurred 7 days after the single vaccination.

There was also a dose-related trend. More participants in Arm H (High dose) presented with parameters outside normal range than participants in arm L (Low dose) and in arm M (Medium dose).

Most haematology parameters outside normal range were graded as Grade 1 or Grade 2

The clinical relevance of these data are limited as the number of adults per arm in this phase 1 study was very limited (31 in arm L, 30 in arm M, and 59 in arm H).

2.6.8.8.1.1.4. Study VLA1553-321

The following relevant mean changes from baseline were observed in the VLA1553 arm on Day 8):

- Haemoglobin: −1.07 g/L (vs. -1.89 g/L in placebo) and decreased further to −2.09 g/L by 28 days post vaccination (vs. -1.35).
- Lymphocytes: −0.53×10E9/L (vs. -0.08) and returned to within normal ranges 28 days post vaccination.
- Neutrophils: −1.12×10E9/L (vs. 0.25) and returned to within normal ranges 28 days post vaccination.
- Platelets: −26.33×10E9/L (vs. -2.00) and returned to within normal ranges 28 days post vaccination.

Table 61. Shift Summary of Haematology Results by Maximum FDA Toxicity Grade for Immunogenicity Subset

Laboratory Parameter Grade	VLA1553 (N=328) n (%)	Placebo (N=125) n (%)
Leukocyte decrease		
Grade 1	34 (10.7)	2 (4.1)
Grade 2	3 (0.9)	1 (2.0)
Grade 3	0	0
Grade 4	0	0
Lymphopenia		
Grade 1	16 (5.0)	1 (2.0)
Grade 2	6 (1.9)	1 (2.0)
Grade 3	1 (0.3)	0
Grade 4	0	0
Neutrophil decrease		
Grade 1	57 (17.9)	5 (10.2)
Grade 2	33 (10.4)	5 (10.2)
Grade 3	15 (4.7)	1 (2.0)
Grade 4	0	0
Haemoglobin decrease		
Grade 1	82 (25.8)	19 (38.8)
Grade 2	24 (7.5)	5 (10.2)
Grade 3	0	0
Grade 4	0	0

Note: For each laboratory parameter and visit, subjects are included only once, in the maximum Toxicity grade. Total is the number of subjects with at least 1 postbaseline result, and percentages are based on the row total. Only subjects in the immunogenicity subset are included in this tabulation. Grade 0 = Normal; Grade 1 = Mild; Grade 2 = Moderate; Grade 3 = Severe; Grade 4 = Potentially Life Threatening.

The most common FDA Toxicity Grade shifts in haematology parameters were mainly from Grade 0 to Grade 1 or 2. Leukopenia (leukocyte decreased), neutropenia (neutrophile decreased) and lymphopenia (lymphocyte decreased) were more frequently observed in the adolescents vaccinated with VLA1553 than with placebo.

2.6.8.8.1.1.5. Study VLA1553-303

Clinical laboratory data were not collected in study VLA1553-303.

2.6.8.8.1.2. Clinical Chemistry

2.6.8.8.1.2.1. Study VLA1553-301

A slight increase in c-reactive protein (CRP) was observed in both arms 7 days post vaccination (mean change from baseline: 0.56 mg/L and 0.96 mg/L in the VLA1553 and placebo arms, respectively), which turned back to within normal ranges 28 days post vaccination.

The following relevant mean changes from baseline were observed in the VLA1553 arm on Day 8:

- Alanine aminotransferase (ALT): 4.4 U/L (vs 0.4 U/L in placebo) and returned to within normal range 28 days post vaccination.
- Aspartate aminotransferase (AST): 4.0 U/L (vs. 0.3) and returned to within normal range 28 days post vaccination.
- Bilirubin: −1.43 µmol/L (vs. -0.33) and returned to within normal range 28 days post vaccination.

In the VLA1553 arm versus the placebo arm, the most common FDA Toxicity Grade shifts in chemistry parameters with any worsening from baseline (any grade >0) were the following:

- Alkaline phosphatase (ALP) increase (18.2% [66/372] vs 22.3% [27/125], respectively)
- ALT increase (16.9% [61/372] vs 9.9% [12/125], respectively)
- AST increase (13% [47/372] vs 7.4% [9/125], respectively)
- Hyperkalaemia (11% [40/372] vs 9.9% [12/125], respectively)
- Hypernatremia (8.8% [32/372] vs 13.2% [16/125], respectively)
- Hypokalaemia (7.7% [28/372] vs 13.2% [16/125], respectively)

The most common FDA Toxicity Grade shifts in chemistry parameters were mainly from Grade 0 to Grade 1 or 2. ALT and AST increases were more frequently observed in the adults vaccinated with VLA1553 than with placebo.

2.6.8.8.1.2.2. Study VLA1553-302

The following relevant mean changes from baseline were observed in the overall Safety Population 7 days post vaccination:

- ALT increase: 2.6 U/L and returned to within normal range 28 days post vaccination.
- AST increase: 2.4 U/L and returned to within normal range 28 days post vaccination.
- Bilirubin decrease: −1.8 µmol/L and returned to within normal range 28 days post vaccination.
- CRP increase: 1.0 mg/L and returned to within normal range 28 days post vaccination.

For the 3 lots, the most common FDA Toxicity Grade shifts in chemistry parameters from baseline (any grade >0) were the following:

- ALT increase (14.9% [60/408])
- Hypokalaemia (11.6% [47/408])
- AST increase (10.9% [44/408])
- ALP increase (12.1% [49/408])

The most common FDA Toxicity Grade shifts in chemistry parameters were mainly from Grade 0 to Grade 1 or 2. The chemistry parameters were similar across the 3 lots.

2.6.8.8.1.2.3. Study VLA1553-101

Parameters outside normal range included creatinine, sodium, potassium, calcium, AST, ALT, alkaline phosphatase, bilirubin, and CRP (SAP study VLA1553-101, Table 3.4.47). There was no clear trend when comparing visit period and dose levels with the number of participants presenting with values outside the normal range.

Most clinical chemistry parameters outside normal range were considered Grade 1 to Grade 2.

One participant in Arm L had potassium concentration outside the normal range 28 days post vaccination, which was graded potentially life threatening. However, upon retesting, the potassium value of this participant was within the normal range. The abnormal potassium concentration in the original test was due to a laboratory error.

The clinical relevance of these data are limited as the number of adults per arm in this phase 1 study was very limited (31 in arm L, 30 in arm M, and 59 in arm H).

2.6.8.8.1.2.4. Study VLA1553-321

An increase in CRP was observed in both arms 7 days post vaccination (mean change from baseline: 1.7 mg/L and 5.7 mg/L in the VLA1553 and placebo arms, respectively). By 28 days post vaccination, mean change in CRP from baseline was 0.6 mg/L and 4.3 mg/L in the VLA1553 and placebo arms, respectively.

The following relevant mean changes from baseline were observed in the VLA1553 arm on Day 8:

- AST: 2.4 U/L (vs -1.7 U/L in placebo) and returned to within normal range 28 days post vaccination.
- Bilirubin: −1.70 µmol/L (vs. 0.23) and returned to within normal range 28 days post vaccination.

In the VLA1553 arm versus the placebo arm, the most common FDA Toxicity Grade shifts in chemistry parameters with any worsening from baseline (any grade >0) were the following:

- ALP increase (12% [39/328] vs. 8.9% [5/56], respectively)
- Hypernatremia (9.5% [31/328] vs. 8.9% [5/56], respectively)
- ALT increase (8% [26/328] vs. 5.4% [3/56], respectively)
- Hypokalaemia (5.8% [19/328] vs. 0%, respectively)
- AST increase (5.2% [17/328] vs. 3.6% [2/56], respectively)

The most common FDA Toxicity Grade shifts in chemistry parameters were mainly from Grade 0 to Grade 1 or 2. ALT and AST increases were more frequently observed in the adolescents vaccinated with VLA1553 than with placebo. ALP increases and hypokalaemia were also more frequently observed in the adolescents vaccinated with VLA1553 than with placebo (not observed in study VLA1553-301).

2.6.8.8.1.2.5. Study VLA1553-303

Clinical laboratory data were not collected in study VLA1553-303.

2.6.8.8.1.3. Coagulation and urinalysis

In studies VLA1553-301 and VLA1553-321, no clinically relevant differences were observed between VLA1553 and placebo arms for any coagulation or urinalysis parameters.

2.6.8.8.2. Vital Signs, Physical Findings, and Other Observations Related to Safety

2.6.8.8.2.1. Vitals Signs

In studies VLA1553-301 and VLA1553-321, no clinically relevant differences were observed between VLA1553 and placebo arms for vital signs (including for blood pressure, pulse rate and temperature).

2.6.8.8.2.2. Physical Examination

Clinically significant abnormalities, mainly of abnormal findings in extremities and joints, were observed throughout the studies. No relevant changes in hand stiffness from baseline were observed during the studies. Of note, in the SmPC, arthralgia is recognized as very common ADR of VLA1553.

Study VLA1553-301

Clinically significant abnormalities were observed in 1.4% (56/4,115) of participants throughout the study (1.5% [47/3,082] of participants in the VLA1553 arm vs. 0.9% [9/1,033] of participants in the placebo arm), mainly of abnormal findings in extremities and joints (0.7% [21/3,082] of participants in the VLA1553 arm vs. 0.4% [4/1,033] of participants in the placebo arm).

No relevant changes from baseline up to 7, 28, and 84 days or up to 6 months post vaccination or Early Termination Visit were observed for hand stiffness in both study arms.

Study VLA1553-302

Clinically significant abnormalities were observed in 1.0% (4/408) of participants throughout the study, consisting of abnormal findings in extremities and joints (0.7% [1/136] of participants in Lot 1, 1.4% [2/137] of participants in Lot 2, and 0.7% [1/135] of participants in Lot 3).

No relevant changes from baseline up to 7, 28, and 84 days or up to 6 months post vaccination or Early Termination Visit were observed for hand stiffness in all Lots.

Study VLA1553-101

A number of participants had symptom-driven physical examinations. However, no participant had any complaints within the 1-hour observation time.

Study VLA1553-321

No relevant changes from baseline up to 7 and 28 days post vaccination were observed for hand stiffness in the VLA1553 or placebo arms.

Study VLA1553-303

Physical examination data were not collected in study VLA1553-303.

2.6.8.8.2.3. Maximum Fever Temperature Post-vaccination

Pooled Safety Population: Studies VLA1553-301, VLA1553-302, and VLA1553-101

In the Pooled Safety Population, a total of 498 (13.8%) adult participants in the VLA1553 arm compared with 8 (0.8%) adult participants in the placebo arm had the solicited systemic AE of fever. Most cases of fever in the VLA1553 arm were mild (57.8% [288/498]) to moderate (30.1% [150/498]) in severity. The majority of cases of fever (61.2% [305/498]) in the VLA1553 arm were reported by participants between 18 to 45 yoa. Most cases of fever (89.8% [447/498]) in the VLA1553 arm were related to vaccination.

The median duration of fever in the VLA1553 arm compared with placebo was 2.0 days versus 1.5 days, respectively. Most solicited systemic AEs of fever in the VLA1553 arm resolved within 1 to 2 days. Solicited systemic AEs of fever with duration >10 days after single vaccination were reported for 2 (0.1%) participants in the VLA1553 arm compared with none in the placebo arm. Up to 6 months post vaccination, unsolicited AEs of pyrexia were reported by 18 (0.5%) participants in the VLA1553 arm compared with 5 (0.5%) participants in the placebo arm.

In the Pooled Safety Population, mean maximum fever temperature after single vaccination was 37.2°C in the VLA1553 arm (range: 35.0°C to 40.6°C) versus 36.9°C in the placebo arm (range: 35.0°C to 39.2°C).There were no reports of fever above 40.0°C up to Day 14 in study VLA1553-101. Based on Subject eDiary data, fever above 40°C up to Day 11 after a single vaccination of VLA1553 occurred in four participants in study VLA1553-301 and in one participant in study VLA1553-302. In 4 out of these 5 participants, fever was co-reported with other events (e.g. headache, fatigue, arthralgia, myalgia).

Study VLA1553-321

One related case of fever Grade 4 was reported in combination with arthralgia, myalgia and headache. This event was reported as AESI and is further described in section 2.6.8.7.3.

Study VLA1553-303

Maximum fever temperature data were not collected in study VLA1553-303.

2.6.8.8.2.4. Viraemia Results

Viraemia and shedding were monitored in the Phase 1 study VLA1553-101 at Days 0, 3, 7, and 14 after re-vaccination in serum and urine samples by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).

In the Phase 3 studies VLA1553-301 and VLA1553-302, plasma samples were collected from all participants on vaccination Day 1 and on Days 8 and 29, and if applicable, at the Early Termination Visit for a clinically indicated retrospective investigation of viraemia by RT-qPCR. Viraemia analysis in studies VLA1553-301 and VLA1553-302 was conducted upon request of the DSMB based on clinical indications.

Similarly, in the ongoing VLA1553-321 study, plasma samples have been collected from all subjects for clinically indicated retrospective investigation of viraemia by RT-qPCR. Samples were collected on vaccination Day 1 and on Days 8 and 29, and if applicable, at the Early Termination Visit as performed for VLA1553-301 and VLA1553-302 but also collection of samples is foreseen at Days 85, 180 and 365.

In addition, approximatively 75 subjects of the immunogenicity subset will be included in the viraemia subset (among these approximatively 50 will be randomized to VLA1553 and 25 to placebo). For subjects included in this subset, Day 1 and Day 8 samples will be analysed for viraemia by RT-qPCR and Day 29 sample will be analysed only if Day 8 sample is positive.

Study VLA1553-101

Table 62. Study VLA1553-101: Plasma Viraemia (GCE/mL) Results at Baseline and 3, 7, and 14 Days After Single Vaccination (Safety Population)

Visit		VLA1553		
		Arm L (N=31)	Arm M (N=30)	Arm H (N=59)
Day 0	Subjects at visit	n=31	n=30	n=59
	below LLOQ, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
	below LOD, n (%)	0 (0.0)	0 (0.0)	1 (1.7)
	not detected, n (%)	31 (100)	30 (100)	58 (98.3)
	report result, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
	Mean	n.a.	n.a.	n.a.
	SD	n.a.	n.a.	n.a.
	Median	n.a.	n.a.	n.a.
	Q1 / Q3	n.a. / n.a.	n.a. / n.a.	n.a. / n.a.
	Min / Max	n.a. / n.a.	n.a. / n.a.	n.a. / n.a.
Day 3	Subjects at visit	n=31	n=30	n=59
	below LLOQ, n (%)	2 (6.5)	1 (3.3)	1 (1.7)
	below LOD, n (%)	3 (9.7)	1 (3.3)	2 (3.4)
	not detected, n (%)	1 (3.2)	1 (3.3)	0 (0.0)
	report result, n (%)	25 (80.6)	27 (90.0)	56 (94.9)
	Mean	73,601.2	89,353.7	229,224.1
	SD	152,151.0	107,203.8	332,163.6
	Median	26,508.0	47,810.0	146,548.5
	Q1 / Q3	10,559.0 / 43,882.0	19,009.0 / 119,009.0	62,638.0 / 276,450.5
	Min / Max	3,542.0 / 751,113.0	3,267.0 / 410,728.0	3,739.0 / 1,884,885
Day 7	Subjects at visit	n=30	n=30	n=58
	below LLOQ, n (%)	8 (26.7)	6 (20.0)	5 (8.6)
	below LOD, n (%)	12 (40.0)	6 (20.0)	22 (37.9)
	not detected, n (%)	4 (13.3)	13 (43.3)	27 (46.6)
	report result, n (%)	6 (20.0)	5 (16.7)	4 (6.9)
	Mean	8,814.0	15,725.2	27,028.0
	SD	8,541.9	17,471.2	33,386.1
	Median	4,827.0	6,062.0	13,409.0
	Q1 / Q3	4,305.0 / 9424.0	5,847.0 / 15,470.0	5,470.0 / 48,586.0
	Min / Max	3,756.0 / 25,745.0	5,203.0 / 46,044.0	5,460.0 / 75,834.0
Day 14	Subjects at visit	n=30	n=30	n=56
	below LLOQ, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
	below LOD, n (%)	0 (0.0)	0 (0.0)	2 (3.6)
	not detected, n (%)	30 (100)	30 (100)	54 (96.4)
	report result, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
	Mean	n.a.	n.a.	n.a.
	SD	n.a.	n.a.	n.a.
	Median	n.a.	n.a.	n.a.
	Q1 / Q3	n.a. / n.a.	n.a. / n.a.	n.a. / n.a.
	Min / Max	n.a. / n.a.	n.a. / n.a.	n.a. / n.a.

Abbreviations: GCE=genome copy equivalent; LLOQ=lower limit of qualification; LOD=limit of detection; SD=standard deviation; Q=quartile; Min=minimum; Max=maximum; n.a.=not applicable.
Percentages were based on non-missing observations (total).
LOD: 1,087 GCE/mL. LLOQ: 3,261 GCE/mL.
Source: Module 5.3.5.1, study VLA1553-101, Table 3.4.55.1

In study VLA1553-101, the subjects received VLA1553 at either 3.2 × 103 TCID50/dose (Arm L - low), 3.2 × 104 TCID50/dose (Arm M - medium) or 3.2 × 105 TCID50/dose (Arm H - high) as a first I.M. study vaccination on Day 0 and some participants of Arm H received an I.M. re-vaccination with the high dose at Month 6. All other subjects received an I.M. re-vaccination with the high dose at Month 12.

Viraemia and urinary shedding were assessed as safety endpoints in all participants at baseline and Days 3, 7, and 14 after (re-)vaccination in serum and urine samples. The concentration of detected viral RNA was measured in GCE/mL by a qualified RT-qPCR assay (described in section 2.6.2.). The sensitivity of this assay was 1087 GCE/mL (limit of detection, LOD) and the lower limit of quantification (LLOQ) was 3261 GCE/mL. Any samples with viraemia or shedding results below the LLOQ were not reported.

No participant in any study arm had reportable viraemia results in plasma at baseline.

Three days after first vaccination, highest measured viraemia titres were reached (peak is considered undetermined with the time-points tested). Mean values of plasma viral ribonucleic acid (RNA) were 73,601.2 genome copy equivalent [GCE]/mL in Arm L, 89,353.7 GCE/mL in Arm M, and 229,224.1 GCE/mL in Arm H. At Day 3, at least >80% of subjects had viraemia ≥LLOQ, with 25/31 subjects in Arm L, 27/30 in Arm M, and 56/59 in Arm H having reportable viraemia levels.

Seven days after a single vaccination, the numbers of participants who showed reportable viraemia results were notably decreased in all study arms (respectively 20% (6/30) in Arm L, 17% (5/30) in Arm M, and 7% (4/58) in Arm H), with mean values of plasma viral RNA ranging from 8,814.0 GCE/mL (Arm L) to 27,028.0 GCE/mL (Arm H).

No participant in any dose arm had a reportable viraemia result on Day 14.

After the first study vaccination, only one subject in Arm L had reportable urinary shedding at Day 7 (10,948.0 GCE/mL). No other subject in any study arm showed any reportable urinary shedding (> LOD) at any time-point tested. After the Month 6 or Month 12 re-vaccinations, no subject in any study arm showed any reportable plasma viraemia results nor reportable urinary shedding within 14 days after re-vaccination.

Of the 120 participants tested for viraemia, 12 were non-viraemic and 108 were viraemic at Day 3. The sample size in the non-viraemic group was very small, hence a comparison between the adverse events reported in both groups was limited.

Table 63. Study VLA1553-101: Viraemia Analysis – Summary of Adverse Events by Viraemia Status

	Statistic	Non-viremic (N=12)	Viremic (N=108)
Any solicited AE	n (%) [95% CI] Obs	6 (50.0) [25.4, 74.6] 13	57 (52.8) [43.4, 61.9] 166
Any related solicited AE	n (%) [95% CI] Obs	5 (41.7) [19.3, 68.0] 10	55 (50.9) [41.6, 60.2] 158
Any severe solicited AE	n (%) [95% CI] Obs	2 (16.7) [4.7, 44.8] 3	7 (6.5) [3.2, 12.8] 7
Any unsolicited AE	n (%) [95% CI] Obs	7 (58.3) [32.0, 80.7] 10	64 (59.3) [49.8, 68.1] 170
Any severe unsolicited AE	n (%) [95% CI] Obs	2 (16.7) [4.7, 44.8] 2	5 (4.6) [2.0, 10.4] 5
Any related unsolicited AE	n (%) [95% CI] Obs	2 (16.7) [4.7, 44.8] 3	48 (44.4) [35.4, 53.8] 97
Any related severe unsolicited AE	n (%) [95% CI] Obs	1 (8.3) [1.5, 35.4] 1	5 (4.6) [2.0, 10.4] 5

Study VLA1553-301

Table 64. Study VLA1553-301: Viraemia Analysis – Summary of Adverse Events by Viraemia Status at Day 8 (Safety Population)

AE Category	n (%) [95% CI][a]			
	Non-Viraemic (N=166)	Total Viraemic (N=24)	Low Viraemic (<100×10E3 GCE/mL) (N=22)	High Viraemic (≥100×10E3 GCE/mL) (N=2)
Severe - Any Severe Solicited or Related Severe Unsolicited AEs	52 (31.3) [24.4, 39.0]	9 (37.5) [18.8, 59.4]	7 (31.8) [13.9, 54.9]	2 (100) [15.8, 100.0]
Moderate - Any Moderate Solicited or Related Moderate Unsolicited AEs	58 (34.9) [27.7, 42.7]	6 (25.0) [9.8, 46.7]	6 (27.3) [10.7, 50.2]	0 [0.0, 84.2]
Mild - Any Mild Solicited or Related Mild Unsolicited AEs	56 (33.7) [26.6, 41.5]	9 (37.5) [18.8, 59.4]	9 (40.9) [20.7, 63.6]	0 [0.0, 84.2]
p-value[b]	0.6302		-	

Abbreviations: AE=adverse event; CI=confidence interval; eCRF=electronic case report form; GCE=genome copy equivalents.

a. Two-sided exact Clopper-Pearson 95% CIs.

b. p-value from Fisher's exact test for the association between the AE categories and the total viraemic/Non-viraemic groups.

Note: Only VLA1553 participants were included in the viraemia analysis. For each category, participants were included only once, even if they experienced multiple events in that category. Related AEs are those recorded as 'Probably Related' or 'Possibly Related' on the eCRF. Non-viraemic participants are those with a negative/non-detectable viraemia result at Day 8. Viraemic participants are those with a positive/detectable result at Day 8. The severe AE category was any participant with any severe solicited or related severe unsolicited AE. The moderate and mild categories were selected from the subset of participants with any mild or moderate solicited or related unsolicited AEs, respectively. The moderate and mild categories were demographically matched by age and sex to the severe category.

In study VLA1553-301, viraemia was determined in a cohort of participants with severe solicited or related severe unsolicited AEs (cases, n=61) with age- and gender-matched controls (n=129). Viraemia was seen in a minority (24/190) of tested participants at Day 8, which became undetectable at Day 29. Of the 190 tested participants for this exploratory analysis, 166 were non-viraemic and 24 were viraemic,

including 22 classified as low viraemic (<100×10E3 GCE/mL) and two as high viraemic (≥100×10E3 GCE/mL). Viraemia was resolved in all participants by Day 29.

Nine of 24 viraemic participants at Day 8 reported any severe solicited or related unsolicited AEs, 6 participants reported moderate AEs, and 9 participants reported mild AEs. All 15 participants who reported any moderate or mild AEs (solicited or related unsolicited) had low viraemic loads at Day 8. Two of nine participants with any severe AEs (solicited or related unsolicited) had high viral loads at Day 8:

- One participant reported SIADH (related SAE, completely recovered).
- One participant reported short-term severe arthralgia, moderate pyrexia, moderate back pain, and mild myalgia.

Highest viral loads were detected at Day 3 in the Phase 1 clinical study VLA1553-101, only late viraemia (Day 8) can be compared in this study. Level of viral loads were similar to Phase 1 results, except for 2 participants with high viral loads in the severe category of AEs (i.e., significantly higher viral load was observed compared to Phase 1 results). However, interpretation is limited due to small sample size (24 of 190 participants were viraemic in total).

Study VLA1553-302

Exploratory retrospective viraemia analysis was conducted in 11 participants (five participants in Lot 1, three participants in Lot 2, and three participants in Lot 3). Of these participants, three had positive viraemia at Day 8 (ranging from 4,739.1 GCE/mL to 132,129.2 GCE/mL), which became undetectable at Day 29.

Of these, most of the reported AEs were either mild or moderate; there were 5 severe events reported: fever (2 events), arthralgia (1 event), fatigue (1 event), and myalgia (1 event). The same severe solicited AEs in similar number (9 viraemic subjects with 10 severe solicited systemic events) were reported in study VLA1553-301: fever (5 events), arthralgia (2 events), fatigue (2 events), and myalgia (1 event).

Of the subjects in VLA1553-302, who had no detectable vaccine viraemia at Day 8, severe solicited systemic events were also reported in these subjects (7 events of fever, and 1 event of headache).

In general, viraemic load at Day 8 was not related to AE severity.

The clinical relevance of these data are limited due to the small sample size.

Study VLA1553-321

Table 65. Viraemia Results by Time Point and µPRNT Baseline Stratification Serostatus (Safety Population – Restricted to the Viraemia Subset)

	Stratum: Seronegative by µPRNT		Stratum: Seropositive by µPRNT		Stratum: Total	
Time point Statistic	VLA1553 (N=43)	Placebo (N=20)	VLA1553 (N=9)	Placebo (N=4)	VLA1553 (N=52)	Placebo (N=24)
Visit 1 - Day 1						
n	42	20	9	4	51	24
Mean (std)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
Median	0.00	0.00	0.00	0.00	0.00	0.00
Q1, Q3	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.00
Min, Max	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0
Visit 2 - Day 8						
n	31	20	9	3	40	23
Mean (std)	8037.04 (34266.442)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	6228.71 (30245.243)	0.00 (0.000)
Median	0.00	0.00	0.00	0.00	0.00	0.00
Q1, Q3	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.00	0.00, 0.00
Min, Max	0.0, 190038.3	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 190038.3	0.0, 0.0
Visit 3 - Day 29						
n	15	0	0	2	15	2
Mean (std)	0.00 (0.000)			0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
Median	0.00			0.00	0.00	0.00
Q1, Q3	0.00, 0.00			0.00, 0.00	0.00, 0.00	0.00, 0.00
Min, Max	0.0, 0.0			0.0, 0.0	0.0, 0.0	0.0, 0.0

std = standard deviation; µPRNT = Micro Plaque Reduction Neutralization Test
Note: This table is restricted to the viremia subset. Viremia is measured in genome copy equivalents (GCE). A result of 'Not Detected' is set as 0 GCE for this summary. Other non-numeric results are classed as missing.

Source: Listing 16.2.13, Dataset: ADMB, Program: t-admb-veremia.sas, Output: T-14-03-06-01-viremia-by-timepoint.rtf, Generated on: 2023-10-19T05:28
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In the viraemia subset of the Safety population (that included 51 subjects administered VLA1553 and 24 placebo subjects of the immunogenicity subset), viraemia was not detected in any of the participant on Day 1.

In addition, viraemia was not detected in any of the subject of the placebo arm on Day 8 and Day 29 (irrespective of baseline serostatus) and it was not detected in any of the 9 baseline seropositive subjects of the VLA1553 arm on Day 8.

Concerning baseline seronegative subjects administered VLA1553, on Day 8, 5/31 tested participants had a quantifiable positive viraemia result. Of these 5 subjects with vaccine viraemia, all of the reported AEs were mild except of 1 event of severe fever.

Overall, the proportion of subjects with reportable viraemia at Day 8 is comparable to the proportion reported for the VLA1553-101 study (this comparison is purely indicative as sample size for viraemia analyses were limited in both trials and formal comparison across trials is complex, in particular when trials are performed in subjects of different age ranges and in different geographical non-endemic and endemic regions). Indeed, 5/40 subjects had quantifiable viraemia, which would correspond to 12.5% of viraemia positive subjects. In study VLA1553-101, 17% (5/30) in Arm M had a reportable viraemia seven days after a single vaccination with a dose comparable to the one administered in study VLA1553-321.

In terms of viral loads measured on Day 8, it is however noted that this ranged from 4,882.1 to 190,038.3 GCE/mL as compared to a min/max range between 5,203.0 and 46,044.0 GCE/mL in VLA1553-101.

The maximum vaccine viraemia level of 190,038.3 GCE/mL was detected in the viraemia cohort for one subject that was seronegative at baseline), the Applicant considers that this elevated vaccine viraemia levels on Day 8 was not associated with increase in any solicited and/or unsolicited AE in the AE profile of this subject.

In VLA1553-321, for subjects developing fever after vaccination, a so-called "acute visit" within 7 days of illness onset and a "convalescent" visit 3 weeks after the "acute" visit were foreseen, with sampling for retrospective viraemia analysis in paired acute/convalescent samples. It is noted that some participants of the viraemia subset also underwent acute/convalescent visits but the Applicant did not

submit separately details nor corresponding viraemia results for subjects not included in the viraemia subset and that underwent acute/convalescent visits. The applicant is required to submit the corresponding viraemia results. This should include sequencing data if CHIKV symptoms occurred at early time-points after vaccination to enable discrimination between vaccine viraemia and *wt* CHIKV infection (**REC**).

In VLA1553-321, also clinically indicated retrospective analysis of viraemia was foreseen for all participants as performed in studies VLA1553-301 and VLA1553-302. No data were found in the submitted CSR of VLA1553-321, the Applicant should submit and discuss corresponding data. Day 29 viraemia data should also be submitted on all subjects with AESI symptoms with onset after Day 8 (including subjects with CHIK-like adverse reaction using the broader definition) even when viraemia at Day 8 results were negative in order to exclude late onset vaccine viraemia (**REC**).

Study VLA1553-303

Viraemia data were not collected in study VLA1553-303.

AESI (Sponsor definition)

Pooled Dataset

There were no AESI according to Sponsor definition reported in Study VLA1553-101.

For all AESI cases identified according to the Sponsor's AESI definition in Study VLA1553-301 and -302 viraemia was assessed at Day 8 (and Day 29 if Day 8 sample was positive in VLA1553-301; Day 8 and Day 29 was assessed for the AESI case in study VLA1553-302).

Overall, subjects with an AESI (Sponsor's definition) had no detectable viraemia at Day 8 or viraemia was <LOD at Day 8; one subject had an inconclusive result (IR).

The onset of an AESI was defined as the start date of the first symptom (or more than one symptom if onset of these were on the same day) within the cluster of AESI symptoms. According to this definition, all AESIs had an onset between Day 2 to Day 5 (i.e., 1 to 4 days post-vaccination). Symptoms which started after the onset of the first symptom(s) had an onset between Day 2 and Day 5 except for one AESI with the first symptom (oedema peripheral) starting 10 days post-vaccination. Per definition (and not yet implemented for studies VLA1553-301 and VLA1553-302) for all subsequently planned and/or ongoing studies (e.g., VLA1553-321), late onset AESI was defined as an AESI with onset after Day 22 (i.e. after 21 days post-vaccination). According to this definition, none of the AESI of VLA1553-301 and VLA1553-302 met the criterion of a late onset AESI as all AESIs started early within the 10-day post-vaccination solicited adverse event period.

For the subject in VLA1553-301 who had an inconclusive viraemia test result at Day 8 (AESI), the Applicant commits to retest the Day 8 sample. **(REC)**

In study VLA1553-321, there were 6 subjects who had AESI symptoms with onset after Day 8. The applicant commits to test the Day 29 sample to determine the presence of late vaccine viraemia and to submit the data with the VLA1553-321 CSR Part C latest by 30-Nov-2024 as part of the agreed EMA PIP. **(REC)**

Chikungunya-like adverse reactions (broad definition)

There were overall 436/3610 (12.1%) of subjects with Chikungunya-like adverse reactions in the VLA1553 pooled group (Pooled Safety Population). Of these, 123 had available viraemia test results for Day 3 (only study VLA1553-101), Day 7/Day 8, Day 14 (only study VLA1553-101), or Day28/Day 29 (as applicable). From the 123 subjects for whom viraemia was determined, the following number of

subjects had a quantifiable result available: Day 3 n=26, Day 7/8 n=12, Day 14: n=0, and Day 28/29: n=0. The viraemia levels were highly variable at Day 3 and Day 7/8 and ranged from 18442 GCE/mL to 1559538 GCE/mL (Day 3) and from 5480 GCE/mL to 2492499.4 GCE/mL (Day 7/8).

The following highest viraemia at Day 3 were observed (>300000 GCE/mL) (viraemia not detected or < LoD at day 8):

- in a >20 -year-old participant who had fever, fatigue and myalgia (grade 2) 2 days after vaccination (total duration of 2 days) et who discontinued from arm H of study VLA1553-101 16 days after vaccination (withdraw of consent, reason not provided by Applicant).
- in a >30-year-old participant who had fever and myalgia (grade 2) 3 days after vaccination (total duration of 2 days) et who completed arm H of study VLA1553-101.
- in a >20-year-old participant who had back pain, fatigue and myalgia (grade 2) 4 days after vaccination (total duration of 2 days) et who completed arm H of study VLA1553-101.

The following highest viraemia at Day 8 were observed (>300000 GCE/mL) (viraemia not detected at day 829):

- in a >50-year-old participant who had arthralgia, back pain, myalgia (grade 3) 2 days after vaccination (total duration of 8 days) and who completed study VLA1553-301 (case discussed previously).
- in a >60 year-old participant who had atrial fibrillation, fatigue, headache, myalgia (grade 3) 3 days after vaccination (total duration of 41 days) and who completed study VLA1553-301 (case discussed previously with hypovolemic hyponatremia).

In study VLA1553-321, there were overall 117/502 (23.3%) subjects with Chikungunya-like adverse reactions in the VLA1553 group (Safety Population). Of these, 8 had available viraemia test results for Day 8. From the 8 subjects for whom viraemia was determined, 2 subjects had a quantifiable result available. Viraemia. Viraemia was not detectable at Day 29.

2.6.8.9. Safety in special populations

Safety data in different subgroups (age groups, serostatus at baseline, pregnancy, immunosuppressed participants) is presented next. With a small number of participants in some subgroups, datas need to be interpreted with caution. Only the stratification groups with an important number of participants will be discussed here.

2.6.8.9.1. Age

Safety analyses by age were performed on the Pooled Safety Population (studies VLA1553-301, VLA1553-302, and VLA1553-101). Safety data in adolescents up to 28 days post-vaccination from study VLA 1553-321 are provided separately for reference.

When comparing the safety pooled analysis by broad age groups (18-64 yoa and more than 55 yoa) solicited local and systemic AEs were less frequent in participants older than 65 yoa compared to participants 18-64 yoa. The frequency of unsolicited (all, related and severe) AEs was comparable in both categories. SAEs and MAAEs were more frequent in participants ≥65 yoa (3.5% and 17.6%, respectively) compared to participants 18-64 yoa (1.2% and 11.8%, respectively).

2.6.8.9.1.1. Solicited Adverse Events

Due to the small number of participants ⩾85 yoa (n=5), solicited AEs for this age group could not be interpreted or compared with the other five age groups: 12 to <18 (n=502), 18 to 45 (n=2,084), 46 to 64 (n=1,180), 65 to 74 (n=287), and 75 to 84 yoa (n=54).

Table 66. Solicited Systemic and Solicited Injection Site Adverse Events by Preferred Term and Age Group (Pooled safety population)

	VLA1553				
AE Category	**Adolescents[a]**	**Adults - Pooled Studies[b]**			
	12-<18 years (N=502) n (%)	**18-45 years (N=2,084) n (%)**	**46-64 years (N=1,180) n (%)**	**65-74 years (N=287) n (%)**	**75-84 years (N=54) n (%)**
Solicited Systemic	**319 (63.5)**	**1,131 (54.3)**	**561 (47.5)**	**129 (44.9)**	**19 (35.2)**
Fever	122 (24.3)	305 (14.6)	153 (13.0)	35 (12.2)	4 (7.4)
Nausea	80 (15.9)	271 (13.0)	109 (9.2)	28 (9.8)	3 (5.6)
Vomiting	13 (2.6)	54 (2.6)	12 (1.0)	4 (1.4)	3 (5.6)
Headache	257 (51.2)	745 (35.7)	327 (27.7)	68 (23.7)	12 (22.2)
Fatigue	112 (22.3)	669 (32.1)	303 (25.7)	77 (26.8)	14 (25.9)
Myalgia	137 (27.3)	492 (23.6)	275 (23.3)	74 (25.8)	13 (24.1)
Arthralgia	64 (12.7)	346 (16.6)	203 (17.2)	43 (15.0)	6 (11.1)
Rash	19 (3.8)	66 (3.2)	16 (1.4)	3 (1.0)	0 (0.0)
Solicited Injection Site	**161 (32.1)**	**364 (17.5)**	**148 (12.5)**	**34 (11.8)**	**3 (5.6)**
Pain	97 (19.3)	139 (6.7)	68 (5.8)	12 (4.2)	0 (0.0)
Tenderness	100 (19.9)	272 (13.1)	95 (8.1)	22 (7.7)	1 (1.9)
Erythema/Redness	14 (2.8)	36 (1.7)	20 (1.7)	3 (1.0)	0 (0.0)
Induration	22 (4.4)	32 (1.5)	12 (1.0)	5 (1.7)	2 (3.7)
Swelling	8 (1.6)	17 (0.8)	5 (0.4)	3 (1.0)	0 (0.0)

Abbreviation: AE=adverse event.
a. Study VLA1553-321 in adolescents.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101 in adults.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.8.1 and Table P.3.10.1;
Module 5.3.5.1, study VLA1553-321, Table 14.3.2.6 and Table 14.3.2.14

Solicited systemic AEs

A decrease of frequency of solicited systemic AEs was observed with increasing age: 12 to <18 (63.5%), 18 to 45 (54.3%), 46 to 64 (47.5%), 65 to 74 (44.9%), and 75 to 84 yoa (35.2%). In particular, the frequency of headache and fever appeared to decrease with increasing age.

In all adult categories, the most frequent solicited systemic AEs were headache, fatigue and myalgia (and then arthralgia and fever). Vomiting and rash were infrequently reported, with frequencies ranging from 1.0% to 5.6% and 0% to 3.8%, respectively, across all age groups.

The overall frequency of solicited systemic AEs with duration longer than 10 days was similar across age groups (none reported in the age group of 75 to 84 yoa). The events of myalgia, headache, arthralgia, and fatigue with duration longer than 10 days accounted for 81.5% of the 65 reported solicited systemic AEs in the 18-to-45-yoa group and 81.1% of the 37 solicited systemic AEs in the 46-to-64-yoa group, while myalgia and fatigue accounted for 75.0% of the eight solicited systemic AEs in the 65-to-74-yoa group.

Regardless of age group, most participants had solicited systemic AEs graded as mild or moderate in maximum severity. A decrease of frequency of severe solicited systemic AEs was observed with increasing age: 18 to 45 (2.8%), 46 to 64 (1.7%), 65 to 74 (1%), 75 to 84 yoa (0%), and ⩾ 85 yoa (0%). No participant experienced nausea, vomiting, or rash graded as severe. Fever was the most frequent severe solicited systemic AE in the age groups of 12 to <18, 18 to 45, and 46 to 64 yoa. Severe fever was reported for one participant in the 65-to-74-yoa group.

Solicited injection site AEs

Similarly, a decrease of frequency of solicited injection site AEs was observed with increasing age: 12 to <18 (32.1%), 18 to 45 (17.5%), 46 to 64 (12.5%), 65 to 74 (11.8%), and 75 to 84 yoa (5.6%). In

particular, the frequency of pain and tenderness at the injection site appeared to decrease with increasing age.

In the 3 adult categories between 18 to 74 yoa, the most frequent solicited injection site AEs were pain and tenderness. In the 75 to 84 yoa group, they were only 1 tenderness AE and 2 induration AEs but the number of participants in this group is too low to be representative.

Erythema/redness, induration and swelling were infrequently reported, with frequencies ranging from 0% to 2.8%, 1% to 4.4% and 0% to 1.6%, respectively, across all age groups.

The overall frequency of solicited injection site AEs with duration longer than 10 days was similar across age groups (none reported in the age group of 75 to 84 yoa). The event of tenderness with duration longer than 10 days accounted for 75.0% (6/8) of the reported solicited injection site AEs in the 18-to-45-year age group and 80.0% (4/5) of the solicited injection site AEs in the 46-to-64-year age group. In the 65-to-74-year age group, one participant had a solicited injection site AE of tenderness, which accounted for 100% of the solicited injection site AEs in this age group.

Regardless of age group, most participants had solicited injection site AEs graded as mild or moderate in maximum severity. Solicited injection site AEs graded as severe were induration (1 participant) and erythema/redness (1 participant) reported in the 12 to <18 yoa group and pain (1 participant) reported in the 46 to 64 yoa group. There were no solicited injection site AEs graded as severe in the 18 to 45, 65 to 74, 75 to 84 yoa or ≥ 85 yoa groups).

Most of the solicited systemic and solicited injection site AEs were assessed as related by the investigator.

Table 67. Related Solicited Systemic and Solicited Injection Site Adverse Events by Preferred Term and Age Group

	VLA1553				
AE Category	**Adolescents[a]**	**Adults - Pooled Studies[b]**			
	12-<18 years (N=502) n (%)	**18-45 years (N=2,084) n (%)**	**46-64 years (N=1,180) n (%)**	**65-74 years (N=287) n (%)**	**75-84 years (N=54) n (%)**
Solicited Systemic	**314 (62.5)**	**1,041 (50.0)**	**516 (43.7)**	**120 (41.8)**	**17 (31.5)**
Fever	121 (24.1)	271 (13.0)	140 (11.9)	31 (10.8)	4 (7.4)
Nausea	78 (15.5)	238 (11.4)	98 (8.3)	20 (7.0)	2 (3.7)
Vomiting	13 (2.6)	40 (1.9)	11 (0.9)	2 (0.7)	1 (1.9)
Headache	252 (50.2)	664 (31.9)	296 (25.1)	60 (20.9)	10 (18.5)
Fatigue	111 (22.1)	619 (29.7)	280 (23.7)	65 (22.6)	11 (20.4)
Myalgia	132 (26.3)	454 (21.8)	259 (21.9)	70 (24.4)	11 (20.4)
Arthralgia	63 (12.5)	311 (14.9)	185 (15.7)	37 (12.9)	6 (11.1)
Rash	18 (3.6)	50 (2.4)	11 (0.9)	3 (1.0)	0 (0.0)
Solicited Injection Site	**160 (31.9)**	**356 (17.1)**	**142 (12.0)**	**33 (11.5)**	**3 (5.6)**
Pain	96 (19.1)	135 (6.5)	66 (5.6)	12 (4.2)	0 (0.0)
Tenderness	99 (19.7)	266 (12.8)	93 (7.9)	21 (7.3)	1 (1.9)
Erythema/Redness	14 (2.8)	35 (1.7)	17 (1.4)	3 (1.0)	0 (0.0)
Induration	22 (4.4)	32 (1.5)	11 (0.9)	5 (1.7)	2 (3.7)
Swelling	7 (1.4)	16 (0.8)	5 (0.4)	3 (1.0)	0 (0.0)

Abbreviation: AE=adverse event.
a. Study VLA1553-321 in adolescents.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101 in adults.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.9.1 and Table P.3.11.1;
Module 5.3.5.1, study VLA1553-321, Table 14.3.2.11 and Table 14.3.2.19

2.6.8.9.1.2. Unsolicited Adverse Events

Table 68. Unsolicited Adverse Events Reported up to 6 Months Occurring in ≥1% of Participants by Age Group

	VLA1553			
Preferred Term	**Adolescents[a]**	**Adults - Pooled Studies[b]**		
	12-<18 years (N=502) n (%)	**18-45 years (N=2,084) n (%)**	**46-64 years (N=1,180) n (%)**	**65-74 years (N=287) n (%)**
COVID-19	<<>>	40 (1.9)	24 (2.0)	1 (0.3)
Urinary tract infection	<<>>	16 (0.8)	14 (1.2)	5 (1.7)
Arthralgia	<<>>	32 (1.5)	30 (2.5)	9 (3.1)
Back pain	<<>>	32 (1.5)	18 (1.5)	2 (0.7)
Pain in extremity	<<>>	15 (0.7)	10 (0.8)	5 (1.7)
Chills	<<>>	42 (2.0)	27 (2.3)	5 (1.7)
Fatigue	<<>>	9 (0.4)	6 (0.5)	4 (1.4)
Neutropenia	<<>>	38 (1.8)	16 (1.4)	7 (2.4)
Leukopenia	<<>>	27 (1.3)	9 (0.8)	5 (1.7)
Lymphadenopathy	<<>>	32 (1.5)	7 (0.6)	1 (0.3)
Diarrhoea	<<>>	24 (1.2)	22 (1.9)	7 (2.4)
Headache	<<>>	36 (1.7)	19 (1.6)	6 (2.1)
C-reactive protein increased	<<>>	4 (0.2)	1 (0.1)	3 (1.0)
Oropharyngeal pain	<<>>	21 (1.0)	3 (0.3)	3 (1.0)
Rash	<<>>	9 (0.4)	4 (0.3)	4 (1.4)
Type 2 diabetes mellitus	<<>>	1 (0.0)	2 (0.2)	3 (1.0)
Hypertension	<<>>	5 (0.2)	7 (0.6)	3 (1.0)

Abbreviation: COVID-19=Coronavirus Disease 2019.
a. Study VLA1553-321 in adolescents.
b. Studies VLA1553-301, VLA1553-302, and VLA1553-101 in adults.
Note: Adverse events were coded using Medical Dictionary for Regulatory Activities version 24.1. For each preferred term, participants were included only once, even if they experienced multiple events in that preferred term.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.20.1; Module 5.3.5.1, study VLA1553-321-Part A, Table <<>> (will be submitted at Day 91 of the EMA marketing authorization procedure)

In the safety pooled analysis, unsolicited AEs up to 6 months were experienced by 31.0% (645/2,084), 31.4% (371/1,180), 35.5% (102/287), and 35.2% (19/54) of participants in the age groups of 18 to 45, 45 to 64, 65 to 74, and 75 to 84 respectively There were experienced by 60% (3/5) of participants ≥ 85 yoa.

Due to the low number of reported unsolicited AEs (19 events) in the 75 to 84 yoa group, the table focuses on the 18 to 45, 45 to 64, and 65 to 74 yoa groups.

The most frequently reported unsolicited AEs (≥2% of participants) were chills (2.0%) in the 18 to 45 yoa group; arthralgia (2.5%), chills (2.3%), and COVID-19 (2.0%) in the 46 to 64 yoa group; arthralgia (3.1%), diarrhoea (2.4%), neutropenia (2.4%), and headache (2.1%) in the 65 to 74 yoa group; diarrhoea (5.6%), osteoarthritis (3.7%), dizziness (3.7%), nasopharyngitis (3.7%) in the 75 to 84 yoa group.

At the end of the study, 6.8%, 9.7%, 13.2% and 11.1% of the participants in the 18 to 45 yoa, 46 to 64 yoa, 65 to 74, and 75 to 84 yoa groups had a total of 197, 155, 47 and 12 ongoing unsolicited AEs, respectively.

No unsolicited adverse events have been reported in the adolescent population up to 28 days post-vaccination up to the DLP of the CSR of part A.

Table 69. Related Unsolicited Adverse Events Reported Up to 6 Months Occurring in ≥1% of Participants by Age Group

Preferred Term	n (%) [95% CIs][a]				
	Adolescents[b]	Adults - Pooled Studies[c] - VLA1553			
	12-<18 years (N=502)	18-45 years (N=2,084)	46-64 years (N=1,180)	65-74 years (N=287)	75-84 years (N=54)
Neutropenia	<<>>	36 (1.7) [1.3, 2.4]	16 (1.4) [0.8, 2.2]	6 (2.1) [1.0, 4.5]	1 (1.9) [0.3, 9.8]
Leukopenia	<<>>	26 (1.2) [0.9, 1.8]	9 (0.8) [0.4, 1.4]	4 (1.4) [0.5, 3.5]	1 (1.9) [0.3, 9.8]
Chills	<<>>	36 (1.7) [1.3, 2.4]	25 (2.1) [1.4, 3.1]	3 (1.0) [0.4, 3.0]	0 (0.0) [0.0, 6.6]
Diarrhoea	<<>>	8 (0.4) [0.2, 0.8]	13 (1.1) [0.6, 1.9]	4 (1.4) [0.5, 3.5]	1 (1.9) [0.3, 9.8]

Abbreviation: CI=confidence interval.
a. 95% CIs calculated according to Altman (Wilson score interval)].
b. Study VLA1553-321 in adolescents.
c. Studies VLA1553-301, VLA1553-302, and VLA1553-101 in adults.
Note: Adverse events were coded using Medical Dictionary for Regulatory Activities version 24.1. For each preferred term, participants were included only once, even if they experienced multiple events in that preferred term.
Source: Module 5.3.5.3, Pooled Analysis, Table P.3.28.1; Module 5.3.5.1, study VLA1553-321-Part A, Table <<>> (will be submitted at Day 91 of the EMA marketing authorization procedure)

Related unsolicited AEs up to 6 months were experienced by 12.6% (263/2,084), 10.5% (124/1,180), 9.1% (26/287), 13% (7/54) and 0% (0/5) of participants in the age groups of 18 to 45, 45 to 64, 65 to 74, 75 to 84 and ≥ 85 yoa respectively. Across age groups, related unsolicited AEs of neutropenia and chills were reported by ≥1% of participants in the 18-to-45, 46-to-64, and 65-to-74 yoa groups.

Most of the unsolicited and related unsolicited AEs up to Day 180 were of mild or moderate severity for both age subgroups (18-64 and ≥65 years) in the VLA1153 pooled group and placebo group. The proportion of subjects with severe unsolicited AEs in the VLA1553 pooled group was higher in subjects ≥65 yoa (2.3%) when compared to the younger subjects (aged 18-64 years: 1.5%). Similar rates were observed in the placebo group (3.4% and 1.0%, respectively). The proportion of subjects with related severe unsolicited AEs in the VLA1553 pooled group was comparable between the younger and older subjects (0.3% and 0.6%). No related severe unsolicited AEs were reported in the placebo group. The increase frequency of severe events with older age (observed in both groups) is considered reflective of the general health status of older versus younger study population.

2.6.8.9.1.3. AEs of special interest, serious adverse events and deaths, other significant events

In the pooled analysis, SAEs up to 6 months were experienced by 1.1% (22/2,084), 1.5% (18/1,180), 2.1% (6/287), 9.3% (5/54) and 20% (1/5) of participants in the age groups of 18 to 45, 45 to 64, 65 to 74, 75 to 84 and ≥ 85 yoa respectively.

In the adolescent population, 5 SAEs have been reported in the ongoing study VLA1553-321 up to 28 days post-vaccination (4/502 [0.8%] in the VLA1553 arm and 1/252 [0.4%] in the placebo arm) (see section 2.6.8.7.).

AESIs up to 6 months were experienced by 0.3% (6/2,084), 0.4% (5/1,180), 0% (0/287), 0% (0/54) and 0% (0/5) of participants in the age groups of 18 to 45, 45 to 64, 65 to 74, 75 to 84 and ≥ 85 yoa respectively (sponsor definition). Most of the AESI symptoms were mild or moderate; 6/11 subjects had a severe AESI symptom of fever; the onset was either at the day of vaccination or started within 4 days post-vaccination. The duration was mostly short with 1-3 days except for one event which had a duration of 7 days. None of the events were serious and only 2 subjects needed medically attention.

In study VLA1553-321, up to 28 days, AESIs were reported by 3.8% (19/502) of the adolescents vaccinated by VLA1553 and by 0.8% (2/252) in the placebo arm.

MAAEs up to 6 months were experienced by 11.2% (233/2,084), 12.8% (151/1,180), 17.8% (51/287), 14.8% (8/54) and 40% (2/5) of participants in the age groups of 18 to 45, 45 to 64, 65 to 74, 75 to 84 and ≥ 85 yoa respectively.

Similar observations were done for MAAEs and SAEs up to Day 180; most of the events were mild or moderate in severity, regardless of age and treatment group. The proportion of subjects with severe MAAEs or severe SAEs was comparable between the younger and older subjects in the VLA1553 pooled group (MAAEs: 1.1% and 1.4%; SAEs: 0.9% and 1.2%) and was comparable to the proportion of younger and older subjects with severe MAAEs or severe SAEs in the placebo group (MAAEs: 0.9% and 3.4%; SAEs: 0.5% and 1.7%).

In study VLA1553-321, up to 28 days. MAAEs were reported in 61/502 (12.2%) participants in the VLA1553 arm and in 25/252 (9.9%) participants in the placebo arm (see section 2.6.8.7.3.).

The table below shows an overview of AEs by age range. Because of the limited number of participants older than 75-year-old, the interpretation of the data should be cautious. The safety profile is, overall, similar between people older than 65-year-old and those between 65 and 74 year-old.

Table 70. AEs by age range (Pooled Safety Analysis Set)

	VLA1553 Age <65 (N=3264)	VLA1553 Age 65-74 (N=287)	VLA1553 Age 75-84 (N=54)	VLA1553 Age 85+ (N=5)	Placebo Age <65 (N=916)	Placebo Age 64-74 (N=97)	Placebo Age 75-84 (N=19)	Placebo Age 85+ (N=1)
Total AEs	2093 (64.1)	182 (63.4)	31 (57.4)	5 (100)	406 (44.3)	46 (47.4)	9 (47.4)	1 (100)
Serious AEs	40 (1.2)	6 (2.1)	5 (9.3)	1 (20.0)	6 (0.7)	2 (2.1)	0 (0.0)	0 (0.0)
Fatal	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Hospitalization/prolong existing hospitalization	32 (1.0)	6 (2.1)	5 (9.3)	1 (20.0)	6 (0.7)	2 (2.1)	0 (0.0)	0 (0.0)
Life-threatening	2 (0.1)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)
Disability/incapacity	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Other, medically significant	6 (0.2)	2 (0.7)	1 (1.9)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
AE leading to drop-out	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)

Psychiatric disorders	51 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	13 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)
Nervous system disorders	1122 (34.4)	76 (26.5)	14 (25.9)	2 (40.0)	153 (16.7)	14 (14.4)	2 (10.5)	0 (0.0)
Accidents and injuries	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cardiac disorders	8 (0.2)	3 (1.0)	1 (1.9)	1 (20.0)	2 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)
Vascular disorders	18 (0.6)	6 (2.1)	1 (1.9)	0 (0.0)	7 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
Cerebrovascular disorders	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Infections and infestations	223 (6.8)	20 (7.0)	5 (9.3)	0 (0.0)	63 (6.9)	4 (4.1)	4 (21.1)	0 (0.0)
Anticholinergic syndrome	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Quality of life decreased	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures	50 (1.5)	9 (3.1)	3 (5.6)	0 (0.0)	11 (1.2)	2 (2.1)	1 (5.3)	0 (0.0)
AEs appearing more frequently in older patients*	1594 (48.8)	130 (45.3)	18 (33.3)	4 (80.0)	249 (27.2)	22 (22.7)	4 (21.1)	1 (100)

n...number of subjects with event, percentages are based on N, Obs...number of events.
Two-sided 95% confidence intervals calculated according to Altman.
*Sum of AEs appearing with a frequency of >=1% in subjects >= 65 years of age in the VLA1553 group of the pooled dataset: Arthralgia, Pain in extremity, Chills, Fatigue, Neutropenia, Leukopenia, C-reactive protein increased, Headache, Rash, and Type 2 diabetes mellitus (PTs in this category and which were already included in one of the other categories of this table where not again included).

2.6.8.9.2. Serostatus at Baseline

Safety data by baseline serostatus is available for studies VLA1553-301, VLA1553-302 and VLA1553-321.

2.6.8.9.2.1. Study VLA1553-301

In study VLA1553-301, a total of 22 participants tested positive for CHIKV at baseline using baseline threshold µPRNT50 ≥20 (16 in the VLA1553 arm and six in the placebo arm). Overall, the frequency of solicited AEs (local and systemic) were lower in the seropositive participants (6.3% and 37.5%, respectively) compared to the seronegative participants (12.4% and 50.1%). The frequency of unsolicited AEs (all and related) were slightly higher in the seropositive participants (50% and 25%, respectively) compared to the seronegative participants (42.9% and 19.1%). No SAEs nor AESI were reported in seropositive participants. However, these safety data should be interpreted with caution, due to the very low number of participants in this analysis.

2.6.8.9.2.2. Study VLA1553-302

In study VLA1553-302, a total of 12 participants were seropositive at baseline based on ELISA assay, while 14 participants were tested seropositive at baseline based on µPRNT assay. Safety data were analysed in these two groups of participants. Within each group, no between-Lot difference was observed. In both groups, there were no severe AE, no SAE, no AESI and no AE leading to withdrawal. There was only 1 MAAE in the seropositive group at baseline based on ELISA assay (lot 3). Except for unsolicited AEs, the rates of AEs were higher in baseline seropositive participants based on µPRNT assay than in baseline seropositive participants based on ELISA assay. No comparison was provided to seronegative participants. However, this safety data should be interpreted with caution, due to the very low number of seropositive participants in this analysis.

2.6.8.9.2.3. Study VLA1553-321

For the ongoing study VLA1553-321 (adolescents in Brazil), the CSR with cut-off date of 26 Jul 2023 including safety data for all participants up to 28 days after vaccination. Six month data will be provided in Part B.

A total of 614 (81.5%) participants were seronegative for CHIKV serostatus at baseline (µPRNT): 408 in the VLA1553 arm and 206 in the placebo arm. 139 (18.5%) participants were seropositive for CHIKV serostatus at baseline (µPRNT): 94 in the VLA1553 arm and 45 in the placebo arm.

AEs were reported at a numerically higher frequency in the VLA1553 arm of the seronegative stratum than the VLA1553 arm of the seropositive stratum (80.1% and 70.2% participants, respectively). For both the seronegative and seropositive strata, a significantly higher proportion of participants in the VLA1553 arm experienced an AE than in the placebo arm (seronegative: 80.1% vs. 62.6%, respectively; seropositive: 70.2% vs. 55.6% participants for placebo).

Related AEs were seen numerically more frequently in the VLA1553 arm of the seronegative stratum (74.3%) than in the VLA1553 arm of the seropositive stratum (51.1%). Within the seronegative stratum, there was a significant difference in the number of participants who experienced a related AE between the VLA1553 arm and the placebo arm (74.3% vs. 50.0%, respectively). No significant difference was seen between the two arms in the seropositive stratum (51.1% vs. 37.8%).

Severe AEs were seen at a similar frequency in the VLA1553 arm of each stratum (5.1% in seronegative vs. 7.4% in seropositive). In the seronegative stratum, the proportions of participants that experienced severe AEs and related severe AEs were numerically higher in the VLA1553 arm than in the placebo arm (severe AE: 5.1% vs. 1.0%, respectively; related severe AE: 4.7% vs. 1.0%). Only 1 participant experienced related severe AEs in the VL1553 arm of the seropositive stratum (none in the placebo arm).

The proportion of participants that experienced solicited AEs was higher in the VLA1553 arm of the seronegative stratum (74.3%) than the VLA1553 arm of the seropositive stratum (53.2%). Similarly, related solicited AEs were seen more frequently in the VLA1553 arm of the seronegative stratum (73.5%) than the VLA1553 arm of the seropositive stratum (51.1%).

The proportion of participants who experienced unsolicited AEs was similar in the VLA1553 arms of each stratum (seronegative VLA1553 arm: 39.5%; seropositive: 40.4%). Related unsolicited AEs were reported more frequently in participants in the VLA1553 arm of the seronegative stratum compared to the VLA1553 arm of the seropositive stratum (9.6% and 1.1%, respectively). Within the seronegative stratum a significant difference was observed between treatment arms (VLA1553: 9.6%; placebo: 0.5%). No significant difference between treatment arms in the seropositive group was observed (VLA1553 1.1% and placebo 4.4%).

In the VLA1553 arm, severe unsolicited AEs were mostly reported in the seropositive stratum: 4/408 [1%] in seronegative participants vs. 6/94 [6.4%] in seropositive.

In the VLA1553 arm, there were 2/408 (0.5%) participants of the seronegative stratum who experienced SAE and 2/94 (2.1%) participants of the seropositive stratum (vs. 0% and 2.2%, respectively, in the placebo arm).

In the VLA1553 arm, the proportion of participants with AESI was higher for the seronegative vs. the seropositive strata (4.4% - 18 events and 0%, respectively, as assessed by DSMB, sponsor definition).

For the analysis of chikungunya-like adverse reactions (broad definition) by serostatus, please see section 2.6.8.9.3.4.

MAAEs were reported in 13.5% participants in the seronegative stratum and in 6.4% participants in the seropositive stratum, of which 9.6% and 2.1%, respectively, experienced events that were considered related.

Table 71. Overall Summary of the safety profile by Baseline µPRNT Serostatus (Safety Population)

	Stratum: Seronegative by µPRNT			Stratum: Seropositive by µPRNT		
Category [n (%) m]	VLA1553 (N=408)	Placebo (N=206)	Total (N=614)	VLA1553 (N=94)	Placebo (N=45)	Total (N=139)
Any Adverse Events	327 (80.1) 1230	129 (62.6) 382	456 (74.3) 1612	66 (70.2) 187	25 (55.6) 76	91 (65.5) 263
95% CI	75.9, 83.9	55.6, 69.2	70.6, 77.7	59.9, 79.2	40.0, 70.4	56.9, 73.3
P-value[a]			*<0.0001*			0.1266
Any Related Adverse Events	303 (74.3) 969	103 (50.0) 246	406 (66.1) 1215	48 (51.1) 114	17 (37.8) 39	65 (46.8) 153
95% CI	69.7, 78.4	43.0, 57.0	62.2, 69.9	40.5, 61.5	23.8, 53.5	38.3, 55.4
P-value[a]			*<0.0001*			0.1514
Any Severe Adverse Events	21 (5.1) 25	2 (1.0) 2	23 (3.7) 27	7 (7.4) 7	2 (4.4) 2	9 (6.5) 9
95% CI	3.2, 7.8	0.1, 3.5	2.4, 5.6	3.0, 14.7	0.5, 15.1	3.0, 11.9
P-value[a]			*0.0114*			0.7180
Any Related Severe Adverse Events	19 (4.7) 23	2 (1.0) 2	21 (3.4) 25	1 (1.1) 1	0	1 (0.7) 1
95% CI	2.8, 7.2	0.1, 3.5	2.1, 5.2	0.0, 5.8	0.0, 7.9	0.0, 3.9
P-value[a]			*0.0175*			>0.9999
Any Serious Adverse Events	2 (0.5) 2	0	2 (0.3) 2	2 (2.1) 2	1 (2.2) 1	3 (2.2) 3
95% CI	0.1, 1.8	0.0, 1.8	0.0, 1.2	0.3, 7.5	0.1, 11.8	0.4, 6.2
P-value[a]			0.5534			>0.9999
Any Related Serious Adverse Events	1 (0.2) 1	0	1 (0.2) 1	0	0	0
95% CI	0.0, 1.4	0.0, 1.8	0.0, 0.9	0.0, 3.8	0.0, 7.9	0.0, 2.6
P-value[a]			>0.9999			NC
Any Adverse Events of Special Interest as assessed by the DSMB	18 (4.4) 18	1 (0.5) 1	19 (3.1) 19	0	2 (4.4) 2	2 (1.4) 2
95% CI	2.6, 6.9	0.0, 2.7	1.9, 4.8	0.0, 3.8	0.5, 15.1	0.2, 5.1
P-value[a]			*0.0058*			0.1032
Any Solicited Adverse Events	303 (74.3) 922	104 (50.5) 253	407 (66.3) 1175	50 (53.2) 123	17 (37.8) 36	67 (48.2) 159
95% CI	69.7, 78.4	43.5, 57.5	62.4, 70.0	42.6, 63.6	23.8, 53.5	39.7, 56.8
P-value[a]			*<0.0001*			0.1042
Any Related Solicited Adverse Events	300 (73.5) 913	102 (49.5) 245	402 (65.5) 1158	48 (51.1) 113	16 (35.6) 35	64 (46.0) 148
95% CI	69.0, 77.7	42.5, 56.5	61.6, 69.2	40.5, 61.5	21.9, 51.2	37.6, 54.7
P-value[a]			*<0.0001*			0.1030

Any Severe Solicited Adverse Events	18 (4.4) 21	2 (1.0) 2	20 (3.3) 23	1 (1.1) 1	0	1 (0.7) 1
95% CI	2.6, 6.9	0.1, 3.5	2.0, 5.0	0.0, 5.8	0.0, 7.9	0.0, 3.9
P-value[a]			*0.0279*			>0.9999
Any Related Severe Solicited Adverse Events	18 (4.4) 21	2 (1.0) 2	20 (3.3) 23	1 (1.1) 1	0	1 (0.7) 1
95% CI	2.6, 6.9	0.1, 3.5	2.0, 5.0	0.0, 5.8	0.0, 7.9	0.0, 3.9
P-value[a]			*0.0279*			>0.9999
Any Solicited Injection Site Adverse Event	133 (32.6) 203	54 (26.2) 81	187 (30.5) 284	28 (29.8) 38	7 (15.6) 9	35 (25.2) 47
95% CI	28.1, 37.4	20.3, 32.8	26.8, 34.3	20.8, 40.1	6.5, 29.5	18.2, 33.2
P-value[a]			0.1147			0.0945
Any Severe Solicited Injection Site Adverse Event	2 (0.5) 2	1 (0.5) 1	3 (0.5) 3	0	0	0
95% CI	0.1, 1.8	0.0, 2.7	0.1, 1.4	0.0, 3.8	0.0, 7.9	0.0, 2.6
P-value[a]			>0.9999			NC
Any Solicited Systemic Adverse Event	277 (67.9) 719	89 (43.2) 172	366 (59.6) 891	42 (44.7) 85	15 (33.3) 27	57 (41.0) 112
95% CI	63.1, 72.4	36.3, 50.3	55.6, 63.5	34.4, 55.3	20.0, 49.0	32.7, 49.7
P-value[a]			*<0.0001*			0.2689
Any Severe Solicited Systemic Adverse Event	16 (3.9) 19	1 (0.5) 1	17 (2.8) 20	1 (1.1) 1	0	1 (0.7) 1
95% CI	2.3, 6.3	0.0, 2.7	1.6, 4.4	0.0, 5.8	0.0, 7.9	0.0, 3.9
P-value[a]			*0.0162*			>0.9999
Any Unsolicited Adverse Events	161 (39.5) 308	65 (31.6) 129	226 (36.8) 437	38 (40.4) 64	17 (37.8) 40	55 (39.6) 104
95% CI	34.7, 44.4	25.3, 38.4	33.0, 40.8	30.4, 51.0	23.8, 53.5	31.4, 48.2
P-value[a]			0.0627			0.8536
Any Related Unsolicited Adverse Events	39 (9.6) 56	1 (0.5) 1	40 (6.5) 57	1 (1.1) 1	2 (4.4) 4	3 (2.2) 5
95% CI	6.9, 12.8	0.0, 2.7	4.7, 8.8	0.0, 5.8	0.5, 15.1	0.4, 6.2
P-value[a]			*<0.0001*			0.2449
Any Severe Unsolicited Adverse Events	4 (1.0) 4	0	4 (0.7) 4	6 (6.4) 6	2 (4.4) 2	8 (5.8) 8
95% CI	0.3, 2.5	0.0, 1.8	0.2, 1.7	2.4, 13.4	0.5, 15.1	2.5, 11.0
P-value[a]			0.3063			>0.9999
Any Medically Attended Adverse Events	55 (13.5) 124	23 (11.2) 38	78 (12.7) 162	6 (6.4) 7	2 (4.4) 3	8 (5.8) 10
95% CI	10.3, 17.2	7.2, 16.3	10.2, 15.6	2.4, 13.4	0.5, 15.1	2.5, 11.0
P-value[a]			0.4441			>0.9999
Any Related Medically Attended Adverse Events	39 (9.6) 79	15 (7.3) 22	54 (8.8) 101	2 (2.1) 3	0	2 (1.4) 3
95% CI	6.9, 12.8	4.1, 11.7	6.7, 11.3	0.3, 7.5	0.0, 7.9	0.2, 5.1
P-value[a]			0.3702			>0.9999
Any Related Medically Attended Solicited Adverse Events	37 (9.1) 72	14 (6.8) 21	51 (8.3) 93	2 (2.1) 3	0	2 (1.4) 3
95% CI	6.5, 12.3	3.8, 11.1	6.2, 10.8	0.3, 7.5	0.0, 7.9	0.2, 5.1
P-value[a]			0.3581			>0.9999
Any Adverse Event Leading to Study Withdrawal	0	0	0	0	0	0
95% CI	0.0, 0.9	0.0, 1.8	0.0, 0.6	0.0, 3.8	0.0, 7.9	0.0, 2.6
P-value[a]			NC			NC

2.6.8.9.3. Subgroup analyses by sex, race, ethnicity, dose, BMI, and medical history

Analysis by sex, race, ethnicity, dose, BMI and medical history were performed in the pooled safety population. Overall, no clinically meaningful differences could be identified in the subgroup analyses by sex, race, ethnicity, dose, BMI or medical history in VLA1553 vaccinated subjects of the Pooled Safety Population.

A summary of the results is provided in the below sections.

2.6.8.9.3.1. Solicited Adverse Events

A summary of solicited local adverse event reported in different subgroups is presented in Table 72

Table 72. Subgroup analyses of solicited local reactions in Pooled Safety Population (Safety Population) (Post Hoc analysis)

Subgroup	VLA1553 (N=3610)	Placebo (N=1033)
Any solicited local AE	**n/N (%)**	**n/N (%)**
Overall	549/3610 (15.2)	115/1033 (11.1)
By Sex		
Male	215/1691 (12.7)	49/464 (10.6)
Female	334/1919 (17.4)	66/569 (11.6)
By Race		
White	448/2867 (15.6)	98/853 (11.5)
Black or African American	60/530 (11.3)	8/122 (6.6)
Asian	19/74 (25.7)	1/17 (5.9)
American Indian or Alaska Native	3/33 (9.1)	2/5 (40.0)
Native Hawaiian or other Pacific Islander	2/14 (14.3)	0/5 (0.0)
Other	17/92 (18.5)	6/31 (19.4)
By Ethnicity		
Hispanic or Latino	69/608 (11.3)	18/177 (10.2)
Non-Hispanic or Latino	478/2961 (16.1)	96/840 (11.4)
By Dose		
3.2x10E3 TCID50	1/31 (3.2)	n/a
3.2x10E4 TCID50	544/3519 (15.5)	n/a
3.2x10E5 TCID50	4/59 (6.8)	n/a
By BMI		

Subgroup	VLA1553 (N=3610)	Placebo (N=1033)
BMI <25 kg/m2	134/884 (15.2)	25/270 (9.3)
BMI ≥ 25 kg/m2 and BMI<30 kg/m2	164/1126 (14.6)	35/318 (11.0)
BMI ≥ 30 kg/m2 and BMI<35 kg/m2	123/833 (14.8)	25/231 (10.8)
BMI ≥35 kg/m2	126/763 (16.5)	30/210 (14.3)
By Medical History		
Immune systems Disorders	223/1181 (18.9)	56/382 (14.7)
Metabolism and nutrition disorders	137/948 (14.5)	32/298 (10.7)
Vascular disorders	75/671 (11.2)	21/215 (9.8)
Gastrointestinal Disorders	148/717 (20.6)	31/219 (14.2)
Musculoskeletal and connective tissue disorders	130/701 (18.5)	28/217 (12.9)
Nervous system disorders	119/653 (18.2)	25/205 (12.2)
n: number of subjects with event, percentages are based on N.		

Overall, in the VLA1553-Pooled group:

- Female participants (17.4%: 334/1919) had slightly higher solicited local reaction rate compared to males (12.7%: 215/1691).
- Most solicited local reactions were reported in white participants (15.6%: 448/2867), who constituted the stratum that included the majority of participants.
- They were reported with slightly lower rate in Hispanic or Latino (11.3%: 69/608) compared to non-Hispanic or Latino (16.1%: 478/2961).
- They were reported at a similar rate when stratified by BMI <25 kg/m^2, ≥ 25 kg/m^2 to <30 kg/m^2, ≥ 30 kg/m^2 to <35 kg/m^2, and ≥35 kg/m^2
- They were reported with similar rate by participants with medical history of immune systems disorders, gastrointestinal disorders, musculoskeletal and connective tissue disorders, and nervous system disorders (between 18.2% and 20.6%), which was slightly higher than the rate of those with medical history of metabolism and nutrition disorders (14.5%) or vascular disorders (11.2%).

A summary of solicited general adverse event in different subgroups is presented in Table 73.

Table 73. Subgroup analyses of solicited systemic adverse reactions in Pooled Safety Population (Safety Population) (Post Hoc analysis)

Subgroup	VLA1553 (N=3610)	Placebo (N=1033)
Any solicited systemic AE	**n/N (%)**	**n/N (%)**
Overall	1843/3610 (51.1)	278/1033 (26.9)
By Sex		
Male	865/1691 (51.2)	106/464 (22.8)
Female	978/1919 (51.0)	172/569 (30.2)
By Race		
White	1540/2867 (53.7)	228/853 (26.7)
Black or African American	199/530 (37.5)	32/122 (26.2)
Asian	38/74 (51.4)	5/17 (29.4)
American Indian or Alaska Native	14/33 (42.4)	3/5 (60.0)
Native Hawaiian or other Pacific Islander	6/14 (42.9)	1/5 (20.0)
Other	46/92 (50.0)	9/31 (29.0)
By Ethnicity		
Hispanic or Latino	268/608 (44.1)	32/177 (18.1)
Non-Hispanic or Latino	1556/2961 (52.5)	242/840 (28.8)
By Dose		
3.2x10E3 TCID50	11/31 (35.5)	n/a
3.2x10E4 TCID50	1791/3519 (50.9)	n/a
3.2x10E5 TCID50	40/59 (67.8)	n/a
By BMI		
BMI <25 kg/m2	473/884 (53.5)	60/270 (22.2)
BMI ≥ 25 kg/m2 and BMI<30 kg/m2	574/1126 (51.0)	67/318 (21.1)
BMI ≥ 30 kg/m2 and BMI<35 kg/m2	404/833 (48.5)	71/231 (30.7)
BMI ≥35 kg/m2	388/763 (50.9)	80/210 (38.1)
By Medical History		
Immune systems Disorders	691/1181 (58.5)	127 (33.2)

Subgroup	VLA1553 (N=3610)	Placebo (N=1033)
Metabolism and nutrition disorders	483/948 (50.9)	92 (30.9)
Vascular disorders	317/671 (47.2)	57 (26.5)
Gastrointestinal Disorders	411/717 (57.3)	61 (27.9)
Musculoskeletal and connective tissue disorders	388/701 (55.3)	67 (30.9)
Nervous system disorders	370/653 (56.7)	68 (33.2)
n: number of subjects with event, percentages are based on N.		

Overall, in the VLA1553-Pooled group:

- Female participants (51%) had similar solicited systemic reaction rate compared to males (51.2%).
- Most solicited systemic reactions were reported in white participants (53.7%), who constituted the stratum that included the majority of participants.
- They were reported with slightly lower rate in Hispanic or Latino (44.1%) compared to non-Hispanic or Latino (52.5%).
- They were reported at a similar rate when stratified by BMI <25 kg/m^2, ≥ 25 kg/m^2 to <30 kg/m^2, ≥ 30 kg/m^2 to <35 kg/m^2, and ≥35 kg/m^2.
- They were reported with similar rate by participants with medical history of immune systems disorders, gastrointestinal disorders, musculoskeletal and connective tissue disorders, and nervous system disorders (between 55.3% and 58.5%), which was slightly higher than the rate of those with medical history of metabolism and nutrition disorders (50.9%) or vascular disorders (47.2%).

2.6.8.9.3.2. Unsolicited Adverse Events

A summary of unsolicited adverse events reported 28 days post-vaccination in different subgroups is presented in Table 74.

Table 74. Subgroup analyses of unsolicited adverse events up to Day 29 (28 days postvaccination) in Pooled Safety Population (Safety Population)

Subgroup	VLA1553 (N=3610)	Placebo (N=1033)
Any unsolicited AE up to Day 29	**n/N (%)**	**n/N (%)**
Overall	849/3610 (23.5)	138/1033 (13.4)
By Sex		
Male	385/1691 (22.8)	42/464 (9.1)
Female	464/1919 (24.2)	96/569 (16.9)

Subgroup	VLA1553 (N=3610)	Placebo (N=1033)
By Race		
White	704/2867 (24.6)	117/853 (13.7)
Black or African American	95/530 (17.9)	13/122 (10.7)
Asian	18/74 (24.3)	2/17 (11.8)
American Indian or Alaska Native	7/33 (21.2)	0/5 (0.0)
Native Hawaiian or other Pacific Islander	3/14 (21.4)	1/5 (20.0)
Other	22/92 (23.9)	5/31 (16.1)
By Ethnicity		
Hispanic or Latino	127/608 (20.9)	19/177 (10.7)
Non-Hispanic or Latino	715/2961 (24.1)	116/840 (13.8)
By Dose		
3.2x10E3 TCID50	16/31 (51.6)	n/a
3.2x10E4 TCID50	800/3519 (22.7)	n/a
3.2x10E5 TCID50	33/59 (55.9)	n/a
By BMI		
BMI <25 kg/m2	228/884 (25.8)	39/270 (14.4)
BMI ≥ 25 kg/m2 and BMI<30 kg/m2	254/1126 (22.6)	39/318 (12.3)
BMI ≥ 30 kg/m2 and BMI<35 kg/m2	178/833 (21.4)	29/231 (12.6)
BMI ≥35 kg/m2	188/763 (24.6)	31/210 (14.8)
By Medical History		
Immune systems Disorders	338/1181 (28.6)	62/382 (16.2)
Metabolism and nutrition disorders	240/948 (25.3)	44/298 (14.8)
Vascular disorders	165/671 (24.6)	27/215 (12.6)
Gastrointestinal Disorders	202/717 (28.2)	35/219 (16.0)
Musculoskeletal and connective tissue disorders	212/701 (30.2)	42/217 (19.4)
Nervous system disorders	174/653 (26.6)	39/205 (19.0)
n: number of subjects with event, percentages are based on N.		

- Overall, in the VLA1553-Pooled group: Female participants (24.2%) reported unsolicited events at a similar rate compared to males (22.8%).
- Most of them were reported in white participants (24.6%), who constituted the stratum that included the majority of participants.
- They were reported with slightly lower rate in Hispanic or Latino (20.9%) compared to non-Hispanic or Latino (24.1%).
- They were reported at a similar rate when stratified by BMI <25 kg/m^2, ≥ 25 kg/m^2 to <30 kg/m^2, ≥ 30 kg/m^2 to <35 kg/m^2, and ≥35 kg/m^2.
- They were reported with similar rate by participants with medical history of immune systems disorders, gastrointestinal disorders, musculoskeletal and connective tissue disorders, nervous system disorders, metabolism and nutrition disorders, or vascular disorders.

2.6.8.9.3.3. AEs of special interest and serious adverse events

The analysis of AESIs (sponsor definition) reported in different subgroups shows that:

- Female participants (0.3%) had similar rate of AESIs compared to males (0.3%).
- Most of them were reported in white participants (0.4%), who constituted the stratum that included the majority of participants.
- They were reported with slightly higher rate in Hispanic or Latino (1%) compared to non-Hispanic or Latino (0.1%).
- They were reported at a similar rate when stratified by BMI <25 kg/m^2, ≥ 25 kg/m^2 to <30 kg/m^2, ≥ 30 kg/m^2 to <35 kg/m^2, and ≥35 kg/m^2.
- They were reported with similar rate by participants with medical history of immune systems disorders, gastrointestinal disorders, musculoskeletal and connective tissue disorders, nervous system disorders, metabolism and nutrition disorders, or vascular disorders.

With regards to SAEs:

- Female participants (1.7%) reported SAEs at a similar rate compared to males (1.2%).
- Most of them were reported in white participants (1.6%), who constituted the stratum that included the majority of participants.
- They were reported with slightly lower rate in Hispanic or Latino (0.7%) compared to non-Hispanic or Latino (1.6%).
- They were reported at a similar rate when stratified by BMI <25 kg/m^2, ≥ 25 kg/m^2 to <30 kg/m^2, ≥ 30 kg/m^2 to <35 kg/m^2, and ≥35 kg/m^2.
- They were reported with similar rate by participants with medical history of immune systems disorders, gastrointestinal disorders, musculoskeletal and connective tissue disorders, nervous system disorders, metabolism and nutrition disorders, or vascular disorders.

2.6.8.9.3.4. Chikungunya-like adverse reactions (broad definition) by sex, race, ethnicity, serostatus, and BMI

Analysis by sex, race, ethnicity, dose, BMI and medical history were performed for chikungunya like adverse reactions (broader definition) in studies VLA1553-301, VLA1553-321 and in the pooled safety population.

Study VLA1553-301 and pooled safety population

A summary of chikungunya like adverse reactions n in different subgroups is presented in Table 75.

Table 75. Subgroup analyses of Chikungunya-like adverse reactions (VLA1553-301 and Pooled Safety Population) (Post Hoc analysis)

	Placebo-VLA1553-301 N=1033	VLA1553-Pooled all three studies N=3610
	n/N* (%)	n/N* (%)
By Age		
18-64 years	6/916 (0.7)	399/3264 (12.2)
>=65 years	0/117 (0.0)	37/346 (10.7)
By Sex		
Male	1/464 (0.2)	254/1691 (15.0)
Female	5/569 (0.9)	182/1919 (9.5)
By Race		
White	4/853 (0.5)	362/2867 (12.6)
Black or African American	2/122 (1.6)	48/530 (9.1)
Asian	0/17 (0.0)	11/74 (14.9)
American Indian or Alaska Native	0/5 (0.0)	3/33 (9.1)
Native Hawaiian or Other Pacific Islander	0/5 (0.0)	1/14 (7.1)
Other	0/31 (0.0)	11/92 (12.0)
By Ethnicity		
Hispanic or Latino	0/177 (0.0)	67/608 (11.0)
Non-Hispanic or Latino	6/840 (0.7)	365/2961 (12.3)
By Dose		
3.2x10E3 TCID50	n/a	4/31 (12.9)
3.2x10E4 TCID50	n/a	411/3519 (11.7)
3.2x10E5 TCID50	n/a	20/59 (33.9)
By BMI		
BMI <25 kg/m2	1/270 (0.4)	114/884 (12.9)
BMI ≥ 25 kg/m2 and BMI<30 kg/m2	2/318 (0.6)	136/1126 (12.1)
BMI ≥ 30 kg/m2 and BMI<35 kg/m2	1/231 (0.4)	90/833 (10.8)
BMI ≥35 kg/m2	2/210 (1.0)	96/763 (12.6)
By Medical History		
Immune systems Disorders	2/382 (0.5)	155/1181 (13.1)
Metabolism and nutrition disorders	3/298 (1.0)	114/948 (12.0)
Vascular disorders	3/215 (1.4)	76/671 (11.3)
Gastrointestinal Disorders	1/219 (0.5)	78/717 (10.9)
Musculoskeletal and connective tissue disorders	2/217 (0.9)	72/701 (10.3)
Nervous system disorders	4/205 (2.0)	63/653 (9.6)
n=number of participants with event; N=total number of participants *Percentages are based on subgroup N.		

Overall, in the VLA1553-Pooled group:

- The rate of Chikungunya-like adverse reactions was comparable between the younger (18-64 yoa) (12.2%: 399/3264 participants) and subjects 65 yoa or older (10.7%: 37/346).
- Female participants (9.5%: 182/1919) had slightly lower Chikungunya-like adverse reactions rates compared to males (15.0%: 254/1691).
- Most Chikungunya-like adverse reactions were reported in white participants (12.6%: 362/2867), who constituted the stratum that included the majority of participants.

- They were reported with similar rate in Hispanic or Latino (11%: 67/608) or non-Hispanic or Latino (12.3%: 365/2961).
- Chikungunya-like adverse reactions were reported at a similar rate when stratified by BMI <25 kg/m^2, ≥ 25 kg/m^2 to <30 kg/m^2, ≥ 30 kg/m^2 to <35 kg/m^2, and ≥35 kg/m^2 and by medical history of immune systems disorders, metabolism and nutrition disorders, vascular disorders, gastrointestinal disorders, musculoskeletal and connective tissue disorders, and nervous system disorders.

In conclusion, no clinically meaningful differences could be identified in the subgroup analyses by age, sex, race, ethnicity, BMI, and medical history in VLA1553 vaccinated participants with Chikungunya-like adverse reactions.

Study VLA1553-321

A summary of chikungunya like adverse reactions in different subgroups is presented in Table 76.

Table 76. Subgroup analyses of Chikungunya-like adverse reactions (Safety Population) (Post Hoc analysis)

	Placebo N=252	VLA1553 N=502
	n/N* (%)	n/N* (%)
By Sex		
Male	8/115 (7.0)	61/233 (26.2)
Female	4/137 (2.9)	56/269 (20.8)
By Race		
White	5/78 (6.4)	44/167 (26.3)
Black or African American	2/31 (6.5)	18/66 (27.3)
Asian	0/0 (0.0)	0/2 (0.0)
American Indian or Alaska Native	0/2 (0.0)	2/2 (100)
Multiracial	1/72 (1.4)	21/120 (17.5)
Other	4/69 (5.8)	32/145 (22.1)
By Ethnicity		
Hispanic or Latino	8/172 (4.7)	92/358 (25.7)
Non-Hispanic or Latino	4/79 (5.1)	24/140 (17.1)
By Baseline Serostatus		
Seropositive	4/45 (8.9)	6/94 (6.4)
Seronegative	8/206 (3.9)	111/408 (27.2)
By BMI		
BMI <25 kg/m2	8/198 (4.0)	89/406 (21.9)
BMI ≥ 25 kg/m2 and BMI<30 kg/m2	1/40 (2.5)	20/62 (32.3)
BMI ≥ 30 kg/m2 and BMI<35 kg/m2	3/11 (27.3)	6/24 (25.0)
BMI ≥35 kg/m2	0/3 (0.0)	2/10 (20.0)

*Percentages are based on subgroup N.

n=number of participants with event; N=total number of participants

In the VLA1553 group:

- Similarly to the pooled dataset in adults, female participants (20.8%: 56/269) had slightly lower Chikungunya-like adverse reaction rates compared to males (26.2%: 61/233).

- Chikungunya-like adverse reactions were mostly reported in black or African American (27.3%: 18/66), white participants (26.3%: 44/167), other (22.1%: 32/145) and multiracial (17.5%: 21/120).

- They were reported with similar slightly higher rate in Hispanic or Latino (25.7%: 92/358) compared to non-Hispanic or Latino (17.1%: 24/140). (In the pooled data set in adults, this rate was comparable between each ethnicity).

- The rate of Chikungunya-like adverse reactions was higher in the seronegative participants at baseline (27.2%: 11/408 participants) compared to the seropositive at baseline (6.4%: 6/94). This finding is in agreement with the safety conclusion of VLA1553-321 Part A analysis by

serostatus at baseline: in the VLA1553 arm, the frequency of solicited systemic AEs was higher in the seronegative stratum compared to the seropositive stratum.

2.6.8.9.4. Fertility, Pregnancy and Lactation

Pregnancy

Animal studies with VLA1553 do not indicate direct or indirect harmful effects with respect to pregnancy, embryo/fetal development, parturition or postnatal development (see section 2.5.4.2.).

Adults:

Pregnant and lactating women have been excluded from clinical trials so far. There were no reports of pregnancy in studies VLA1553-101 and VLA1553-321. Nevertheless, 18 pregnancies were recorded for participants: 15 pregnancies in study VLA1553-301 (13 in the VLA1553 arm and two in the placebo arm) and 3 in study VLA1553-302. Pregnancy outcomes for the 16 participants who were vaccinated with VLA1553 during pregnancy were as follow: 10 were live birth without congenital anomalies, 5 spontaneous abortions (thereof one foetal death i.e. turner syndrome, 45 X generic disorder), 1 lost to follow up.

No spontaneous abortion were assessed as related to the vaccine. The DSMB conducted a detailed review of all available data on the reported miscarriages and did not identify any safety concerns.

VLA1553-321 Study (beyond Part A, data cut-off 14-Feb-2024)

Overall, 3 pregnancies were reported during the study: whereof 2 pregnancies have well progressed up to birth (2 born healthy babies) and one pregnancy is currently ongoing.

An independent DSMB regularly reviewed pregnancy safety information for study VLA1553-321 until the last participant had completed Day 180/ Visit 5. None of the data reviews raised any safety issue.

Breastfeeding

It is unknown whether VLA1553 is excreted in human milk.

Fertility

Animal studies do not indicate direct or indirect harmful effects with respect to reproductive toxicity.

'Safety in pregnant or breastfeeding women' is considered an important potential risk in the RMP and will be further characterised in the planned post-authorisation safety studies (see section 2.7.).

2.6.8.9.5. Immunosuppression

There is no safety data of VLA5553 on participants with immunosuppression.

As for other live attenuated vaccines,administration to individuals who are immunodeficient or immunosuppressed due to disease or medical therapy is contraindicated. Safety in patients with autoimmune or inflammatory disorders has been identified as a missing information in the RMP will be further characterised in the planned post-authorisation safety studies (see section 2.7.).

2.6.8.9.6. Re-vaccination - study VLA1553-101

In study VLA1553-101, participants received a re-vaccination by intramuscular (I.M.) injection into the musculus deltoideus.

- Arm L (Low dose): VLA1553 3.2 × 103 50% tissue culture infectious dose (TCID50) on Day 0 (0.1 mL of 3.2 × 104 TCID50/mL) and 3.2 × 105 TCID50/dose at Month 12 (1 mL of 3.2 × 105 TCID50/mL)

- Arm M (Medium dose): VLA1553 3.2 × 104 TCID50 (1 mL of 3.2 × 104 TCID50/mL) on Day 0 and 3.2 × 105 TCID50/dose at Month 12
- Arm H (High dose): VLA1553 3.2 × 105 TCID50 on Day 0 and 3.2 × 105 TCID50/dose at Month 12 (Arm H1) or at Month 6 (Arm H2)

Solicited AEs

Two (7.7%) subjects in Arm H2 reported 3 solicited AEs within 14 days after the Month 6 re-vaccination . All 3 were solicited systemic AEs were considered related to the vaccination: one case of fatigue (4%) graded as mild and 2 cases (8%) of nausea both graded as severe.

Solicited AEs after the Month 12 re-vaccination were reported in 1 (4.2%) and 3 (14.3%) subjects in Arms L and H1, respectively. No subject in Arm M reported solicited AEs after the re-vaccination. All reported solicited AEs were considered related to the vaccination. One (4.2%) subject in Arm L (pain) and 2 (9.5%) subjects in Arm H1 reported 3 solicited local AEs (swelling, tenderness, joint pain/arthralgia), all of which were graded mild. Only 1 (4.8%) subject in Arm H1 reported a solicited systemic AE, which was grade moderate (headache).

Unsolicited AEs

Unsolicited AEs starting at or after the Month 6 re-vaccination were reported in 5 (19.2%) subjects in Arm H2. The most frequent unsolicited SOC was gastrointestinal disorder, reported by 2 (7.7%) subjects. The most frequently reported unsolicited AE was diarrhoea, reported by 2 (7.7%) subjects. Three AEs in SOC of blood and lymphatic system disorders (leukocytosis, monocytosis, and neutrophilia) were reported by 1 (3.8%) subject. One (3.8%) subject reported two AEs of urinary tract infection. None of the AEs were considered related to the vaccination or graded severe. No medically attended unsolicited AE or unsolicited AESI was reported after the Month 6 re-vaccination. One (3.8%) subject in Arm H2 reported one SAE of supraventricular extrasystoles 62 days after re-vaccination, which was considered to be not related to the vaccination since the patient had alternative factors that could explain the event (concurrent medical history of sinus bradycardia ongoing at Visit 1) and there was no temporal association with the time of vaccination.

Up to 12 months after the single vaccination, unsolicited AEs were reported in 17 (54.8%), 15 (50.0%), and 36 (61.0%) subjects in Arms L, M, and H, respectively 5. The most frequent unsolicited SOC was blood and lymphatic system disorders, reported by four (13.3%; Arm M) to 18 (30.5%; Arm H) subjects. The most frequently reported unsolicited AE was leukopenia, reported by two (6.7%; Arm M) to 14 (23.7%; Arm H) subjects, followed by neutropenia, reported by one (3.3%; Arm M) to ten (16.9%; Arm H) subjects. No dose-related trends were observed for any of the unsolicited AEs. Of all subjects who reported any unsolicited AEs, 13 (41.9%), 8 (26.7%), and 29 (49.2%) subjects in Arms L, M, and H without H2, respectively, experienced at least one unsolicited AE that was considered related to the vaccination.

Across the study arms, 2 (3.4% in Arm H and 6.7% in Arm M) to 3 (9.7%; Arm L) subjects reported 7 unsolicited AEs graded as severe, including neutropenia (in one subject in Arm M and two subjects in Arm L), lymphopenia (in two subjects in Arm H), back pain (reported by one subject in Arm L), and multiple injuries (related to car accident; reported by one subject in Arm M). All severe AEs were considered related to the vaccination except one AE (multiple injuries) reported by one subject in Arm M. One (3.3%) subject in Arm M reported one SAE (multiple trauma related to car accident) and 2 (6.5%) subject in Arm L reported 4 medically attended unsolicited AE. The two subjects experienced four events of medically attended unsolicited AEs after single vaccination up to Month 12 (starting before the re-vaccination at Month 12). All events were assessed as not related to VLA1553 vaccination by the investigator. One subject had a urinary tract infection starting 182 days after the first VLA1553 vaccination (viraemia was shown on Day 3 and Day 7 post vaccination, no viraemia was shown at later

time points or post re-vaccination). The other subject had an event of acute sinusitis, oropharyngeal pain and sinus congestion starting 12 days after vaccination; no viraemia was detectable at Day 7 and at later visits (Post Hoc analysis). Given that both are events of infectious aetiology with symptoms not aligning with chikungunya, a causal link to VLA1553 is excluded.

No unsolicited AESI was reported.

Unsolicited AEs starting at or after the Month 12 re-vaccination were reported in 2 (8.3% to 9.5%) subjects each in Arm L and Arm H1. No subjects in Arm M reported any unsolicited AEs at or after the Month 12 re-vaccination. No subjects in the low dose group experienced medically attended solicited or unsolicited AEs starting at or after the re-vaccination at Month 12.

Two AEs in SOC of blood and lymphatic system disorders (leukocytosis and lymphopenia) were reported by 1 (3.2%; Arm L) subject. Sinusitis and pain in extremity each were reported by 1 (4.8%) subject in Arm H1, and asthenia was reported by one (4.2%) subject in Arm L.

None of the reported unsolicited AEs were considered to be related to the vaccination or graded severe. No subjects reported any SAE, related medically attended unsolicited AE or AESI after the Month 12 re-vaccination.

2.6.8.10. Safety related to drug-drug interactions and other interactions

2.6.8.10.1. Concomitant Use With Other Vaccines

No clinical study has been carried out to evaluate the effect on the immune response of other vaccines given before, after, or at the same time. "Co-administration with other vaccines" is considered missing information in the RMP and will be further addressed in PASS. The proposed routine risk minimisation measures are acceptable.

2.6.8.10.2. Concomitant Use With Other Drugs

In the pooled safety dataset, in the VLA1553 group (3610 participants), 870 participants (24.1%) were taking non-steroidal anti-inflammatory drugs (NSAIDs), 859 antipyretics (23.8%), and 72 immunosuppressants (2%). In the placebo group (1033 participants), 159 participants (15.4%) were taking NSAIDs, 173 antipyretics (16.7%), and 18 immunosuppressants (1.7%).

Table 77. Summary Table of Adverse Events up to Day 180 by Concomitant Medications for VLA1553 Subjects of Study VLA1553-Pooled (Safety Population) (Post Hoc analysis)

	Statistic	Non-steroidal anti-infl. drugs taken (N=870)	Non-steroidal anti-infl. drugs not taken (N=2740)	Antipyretics taken (N=859)	Antipyretics not taken (N=2751)	Immunosuppressants taken (N=72)	Immunosuppressants not taken (N=3538)
Any AE	n (%) Obs [95% CI]	752 (86.4) 3085 [84.0, 88.6]	1559 (56.9) 4617 [55.0, 58.7]	742 (86.4) 2940 [83.9, 88.5]	1569 (57.0) 4762 [55.2, 58.9]	67 (93.1) 304 [84.8, 97.0]	2244 (63.4) 7398 [61.8, 65.0]
Any related AE	n (%) Obs [95% CI]	631 (72.5) 2175 [69.5, 75.4]	1269 (46.3) 3454 [44.5, 48.2]	609 (70.9) 2029 [67.8, 73.8]	1291 (46.9) 3600 [45.1, 48.8]	50 (69.4) 156 [58.0, 78.9]	1850 (52.3) 5473 [50.6, 53.9]
Any severe AE	n (%) Obs [95% CI]	65 (7.5) 83 [5.9, 9.4]	69 (2.5) 87 [2.0, 3.2]	59 (6.9) 74 [5.4, 8.8]	75 (2.7) 96 [2.2, 3.4]	9 (12.5) 12 [6.7, 22.1]	125 (3.5) 158 [3.0, 4.2]
Any related severe AE	n (%) Obs [95% CI]	46 (5.3) 53 [4.0, 7.0]	40 (1.5) 45 [1.1, 2.0]	33 (3.8) 37 [2.7, 5.3]	53 (1.9) 61 [1.5, 2.5]	4 (5.6) 4 [2.2, 13.4]	82 (2.3) 94 [1.9, 2.9]
Any serious AE	n (%) Obs [95% CI]	20 (2.3) 30 [1.5, 3.5]	32 (1.2) 49 [0.8, 1.6]	28 (3.3) 45 [2.3, 4.7]	24 (0.9) 34 [0.6, 1.3]	5 (6.9) 5 [3.0, 15.2]	47 (1.3) 74 [1.0, 1.8]
Any related serious AE	n (%) Obs [95% CI]	2 (0.2) 2 [0.1, 0.8]	0 (0.0) 0 [0.0, 0.1]	2 (0.2) 2 [0.1, 0.8]	0 (0.0) 0 [0.0, 0.1]	0 (0.0) 0 [0.0, 5.1]	2 (0.1) 2 [0.0, 0.2]
Any medically attended AE	n (%) Obs [95% CI]	201 (23.1) 343 [20.4, 26.0]	244 (8.9) 374 [7.9, 10.0]	206 (24.0) 351 [21.2, 26.9]	239 (8.7) 366 [7.7, 9.8]	36 (50.0) 73 [38.7, 61.3]	409 (11.6) 644 [10.5, 12.7]
Any related medically attended AE	n (%) Obs [95% CI]	36 (4.1) 54 [3.0, 5.7]	34 (1.2) 48 [0.9, 1.7]	35 (4.1) 51 [2.9, 5.6]	35 (1.3) 51 [0.9, 1.8]	3 (4.2) 4 [1.4, 11.5]	67 (1.9) 98 [1.5, 2.4]
Any solicited AE	n (%) Obs [95% CI]	660 (75.9) 2119 [72.9, 78.6]	1280 (46.7) 3363 [44.9, 48.6]	641 (74.6) 2010 [71.6, 77.4]	1299 (47.2) 3472 [45.4, 49.1]	52 (72.2) 150 [61.0, 81.2]	1888 (53.4) 5332 [51.7, 55.0]
Any related solicited AE	n (%) Obs [95% CI]	616 (70.8) 1937 [67.7, 73.7]	1190 (43.4) 3051 [41.6, 45.3]	596 (69.4) 1811 [66.2, 72.4]	1210 (44.0) 3177 [42.1, 45.8]	48 (66.7) 131 [55.2, 76.5]	1758 (49.7) 4857 [48.0, 51.3]
Any solicited local AE	n (%) Obs [95% CI]	202 (23.2) 276 [20.5, 26.1]	347 (12.7) 468 [11.5, 14.0]	188 (21.9) 258 [19.2, 24.8]	361 (13.1) 486 [11.9, 14.4]	18 (25.0) 22 [16.4, 36.1]	531 (15.0) 722 [13.9, 16.2]
Any related solicited local AE	n (%) Obs [95% CI]	197 (22.6) 269 [20.0, 25.5]	337 (12.3) 454 [11.1, 13.6]	185 (21.5) 250 [18.9, 24.4]	349 (12.7) 473 [11.5, 14.0]	18 (25.0) 22 [16.4, 36.1]	516 (14.6) 701 [13.5, 15.8]
Any severe solicited local AE	n (%) Obs [95% CI]	0 (0.0) 0 [0.0, 0.4]	1 (0.0) 1 [0.0, 0.2]	0 (0.0) 0 [0.0, 0.4]	1 (0.0) 1 [0.0, 0.2]	0 (0.0) 0 [0.0, 5.1]	1 (0.0) 1 [0.0, 0.2]
Any related severe solicited local AE	n (%) Obs [95% CI]	0 (0.0) 0 [0.0, 0.4]	1 (0.0) 1 [0.0, 0.2]	0 (0.0) 0 [0.0, 0.4]	1 (0.0) 1 [0.0, 0.2]	0 (0.0) 0 [0.0, 5.1]	1 (0.0) 1 [0.0, 0.2]
Any solicited systemic AE	n (%) Obs [95% CI]	643 (73.9) 1843 [70.9, 76.7]	1200 (43.8) 2895 [41.9, 45.7]	617 (71.8) 1752 [68.7, 74.7]	1226 (44.6) 2986 [42.7, 46.4]	48 (66.7) 128 [55.2, 76.5]	1795 (50.7) 4610 [49.1, 52.4]
Any related solicited systemic AE	n (%) Obs [95% CI]	589 (67.7) 1668 [64.5, 70.7]	1108 (40.4) 2597 [38.6, 42.3]	567 (66.0) 1561 [62.8, 69.1]	1130 (41.1) 2704 [39.3, 42.9]	43 (59.7) 109 [48.2, 70.3]	1654 (46.7) 4156 [45.1, 48.4]
Any severe solicited systemic AE	n (%) Obs [95% CI]	48 (5.5) 54 [4.2, 7.2]	34 (1.2) 37 [0.9, 1.7]	37 (4.3) 40 [3.1, 5.9]	45 (1.6) 51 [1.2, 2.2]	4 (5.6) 4 [2.2, 13.4]	78 (2.2) 87 [1.8, 2.7]
Any related severe solicited systemic AE	n (%) Obs [95% CI]	44 (5.1) 50 [3.8, 6.7]	32 (1.2) 35 [0.8, 1.6]	32 (3.7) 35 [2.7, 5.2]	44 (1.6) 50 [1.2, 2.1]	4 (5.6) 4 [2.2, 13.4]	72 (2.0) 81 [1.6, 2.6]
Any solicited AE with duration >10 days	n (%) Obs [95% CI]	41 (4.7) 54 [3.5, 6.3]	47 (1.7) 70 [1.3, 2.3]	42 (4.9) 53 [3.6, 6.5]	46 (1.7) 71 [1.3, 2.2]	7 (9.7) 9 [4.8, 18.7]	81 (2.3) 115 [1.8, 2.8]
Any unsolicited AE	n (%) Obs [95% CI]	449 (51.6) 966 [48.3, 54.9]	691 (25.2) 1254 [23.6, 26.9]	416 (48.4) 930 [45.1, 51.8]	724 (26.3) 1290 [24.7, 28.0]	56 (77.8) 154 [66.9, 85.8]	1084 (30.6) 2066 [29.1, 32.2]
Any related unsolicited AE	n (%) Obs [95% CI]	159 (18.3) 238 [15.8, 21.0]	261 (9.5) 403 [8.5, 10.7]	137 (15.9) 218 [13.7, 18.5]	283 (10.3) 423 [9.2, 11.5]	16 (22.2) 25 [14.2, 33.1]	404 (11.4) 616 [10.4, 12.5]
Any severe unsolicited AE	n (%) Obs [95% CI]	21 (2.4) 29 [1.6, 3.7]	35 (1.3) 49 [0.9, 1.8]	25 (2.9) 34 [2.0, 4.3]	31 (1.1) 44 [0.8, 1.6]	6 (8.3) 8 [3.9, 17.0]	50 (1.4) 70 [1.1, 1.9]
Any related severe unsolicited AE	n (%) Obs [95% CI]	3 (0.3) 3 [0.1, 1.0]	8 (0.3) 9 [0.1, 0.6]	2 (0.2) 2 [0.1, 0.8]	9 (0.3) 10 [0.2, 0.6]	0 (0.0) 0 [0.0, 5.1]	11 (0.3) 12 [0.2, 0.6]
Any AESI	n (%) Obs [95% CI]	6 (0.7) 16 [0.3, 1.5]	5 (0.2) 12 [0.1, 0.4]	6 (0.7) 14 [0.3, 1.5]	5 (0.2) 14 [0.1, 0.4]	2 (2.8) 6 [0.8, 9.6]	9 (0.3) 22 [0.1, 0.5]

n...number of subjects with event, percentages are based on N, Obs...number of events (for AESI this is the number of associated symptoms). Part 3/3
Two-sided 95% confidence intervals calculated according to Altman (Wilson score interval).
Adverse events will be considered related if the causality to IMP is reported as "probable" or "possible" or missing causality.
"Severe" adverse events will include events with grade 3 or missing grade.
The Safety Population includes all enrolled subjects in any of the studies who received at least one vaccination.
NC...not calculable. Participants may appear in several subgroups. Medication intake started before or at Day 180. Subjects may appear in several subgroups.

Non-steroidal anti-inflammatory drugs (NSAIDs)

In the pooled safety dataset, in the VLA1553 group, 24.1% of participants used NSAIDs at any time during study participation. Compared with those who had not done it, they were more likely to have reported solicited systemic AEs (73.9% vs. 43.8%, respectively), solicited injection site AEs (23.2% vs. 12.7%), and unsolicited AEs (51.6% vs. 25.2%).

In the placebo group, similar pattern was observed. Compared with those who had not done it, participants who used NSAIDs were more likely to have reported solicited systemic AEs (40.3% vs. 24.5%, respectively), solicited injection site AEs (14.5% vs. 10.5%), and unsolicited AEs (47.2% vs. 19.7%).

Of the subjects who took NSAIDs as concomitant medication in study VLA1553-101, 31 subjects had a quantifiable viraemia result at Day 3; the viraemia levels were highly variable at Day 3 and ranged from 4225 GCE/mL to 1559538 GCE/mL. Of the subjects who took NSAIDs as concomitant medication in study VLA1553-301, 12 subjects (with serostatus not determined or seronegative) had a quantifiable viraemia

result at Day 8; the viraemia levels were highly variable at Day 8 and ranged from 3765.6 GCE/mL to 2492499.4 GCE/mL. Of the subjects who took NSAIDs as concomitant medication in study VLA1553-302, 3 subjects (with serostatus seronegative) had a quantifiable viraemia result at Day 8; the viraemia levels were highly variable at Day 8 and ranged from 4739.1 GCE/mL to 132129.2 GCE/mL. There were no subjects in study VLA1553-321 with a quantifiable viraemia result and who used NSAIDs as concomitant medication.

Antipyretics

In the pooled safety dataset, in the VLA1553 group, 23.8% of participants used antipyretics at any time during study participation. Compared with those who had not done it, they were more likely to have reported solicited systemic AEs (71.8% vs. 44.6%, respectively), solicited injection site AEs (21.9% vs. 13.1%), and unsolicited AEs (48.4% vs. 26.3%).

In the placebo group, similar pattern was observed. Compared with those who had not done it, participants who used antipyretics were more likely to have reported solicited systemic AEs (43.4% vs. 23.6%, respectively), solicited injection site AEs (13.9% vs. 10.6%), and unsolicited AEs (46.8% vs. 19.3%).

Of the subjects who took antipyretics as concomitant medication in study VLA1553-101, 16 subjects (with serostatus seronegative) had a quantifiable viraemia result at Day 3; the viraemia levels were highly variable at Day 3 and ranged from 4225 GCE/mL to 306760 GCE/mL. Of the subjects who took antipyretics as concomitant medication in study VLA1553-301, 7 subjects (with serostatus not determined or seronegative) had a quantifiable viraemia result at Day 8; the viraemia levels were highly variable at Day 8 and ranged from 3280.4 GCE/mL to 649944.1 GCE/mL. There were no subjects in studies VLA1553-302, and VLA1553-321 with a quantifiable viraemia result and who used antipyretics as concomitant medication.

Immunosuppressants

In the pooled safety dataset, in the VLA1553 group, 2% of participants used immunosuppressants at any time during study participation. Compared with those who had not done it, they were more likely to have reported solicited systemic AEs (66.7% vs. 50.7%, respectively), solicited injection site AEs (25% vs. 15%), and unsolicited AEs (77.8% vs. 30.6%).

In the placebo group, similar pattern was observed. Compared with those who had not done it, participants who used immunosuppressants were more likely to have reported solicited systemic AEs (27.8% vs. 26.9%, respectively), solicited injection site AEs (16.7% vs. 11%), and unsolicited AEs (83.3% vs. 22.9%).

In the VLA1553 group, the frequency of unsolicited AEs for participants who used immunosuppressants (77.8%) is particularly high compared to the participant who took NSAIDs (51.6%) or antipyretics (48.4%). However, in the placebo group, the frequency of unsolicited AEs for participants who used immunosuppressants (83.3%) is also particularly high compared to the participant who took NSAIDs (47.2%) or antipyretics (46.8%).

For AESI (sponsor definition), 2 AESI occurred in persons who were classified to have taken immunosuppressants, while 9 occurred in individuals who did not receive immunosuppressants. Note that this refers to use of immunosuppressants at any point in time during the trial, not at baseline when receiving the vaccine, since immunosuppression was an exclusion criterion (only two individuals were excluded from the PP due to intake of prohibited medication). Of the subjects with Chikungunya-like adverse reactions (VLA1553 pooled group), 12 subjects used immunosuppressants as concomitant medication, while 424 Chikungunya-like adverse reactions occurred in individuals who did not receive

immunosuppressants. Immunosuppressants (mainly corticosteroids for systemic use) were mostly used to treat adverse events.

There were no subjects in studies VLA1553-101, VLA1553-301, VLA1553-302, and VLA1553-321 with a quantifiable viraemia result and who used systemic immunosuppressants as concomitant medication.

Overall, for the pooled safety dataset, in the VLA1553 group, the frequencies of any AEs and of any solicited AEs were slightly higher for participants who used NSAIDs or antipyretics 1-7 or 1-29 days post-vaccination compared to those who used them 30-180 days post-vaccination. Similar pattern was observed in the placebo group. Because of the low number of participants who used immunosuppressants per categories, it is difficult to make strong conclusions for them.

It is probable that these concomitant medications were used in reaction to adverse events, not as an underlying medication modifying the adverse event profile (and not for prophylaxis).

2.6.8.11. Discontinuation due to adverse events

In study VLA1553-301, excluding death cases (described in section 2.6.8.8.1.), only 2 participants discontinued due to AE:

- One subject (VLA1553 group) had influenza with onset 4 days after vaccination; the event was assessed as mild and not related to vaccination, with a duration of 14 days; the outcome was recovered/resolved.
- One subject (placebo group) experienced cerebellar haemorrhage with onset 19 days after vaccination; the event was assessed as severe and not related to vaccination, with a duration of 16 days; the outcome was recovered/resolved.

In study VLA1553-101, 2 participants in Arm H experienced AEs leading to withdrawal/discontinuation: one case of autoimmune thyroiditis (verbatim: Hashimoto's Disease) and one case of syncope (verbatim: vasovagal episode). One subject (seronegative at baseline, assigned to Arm H) experienced autoimmune thyroiditis 320 days after first vaccination. The event was assessed as mild and not related to vaccination; the subject started a medical procedure to treat this condition and withdrew from study.

None of the AEs leading to withdrawal were considered as related to vaccination.

No participants experienced an AE leading to withdrawal/discontinuation from the studies VLA1553-302, VLA1553-321, or VLA1553-303.

2.6.8.12. Post marketing experience

Ixchiq was approved by the US FDA on 9 November 2023. No post-authorisation exposure data is available.

2.6.8.13. Adverse drug reactions in SmPC

The applicant has proposed ADRs based on the frequencies of related solicited and unsolicited AEs from the pooled safety population. Following assessment, the ADRs included in the SmPC are based on the frequencies of all local and systemic solicited AEs and all remaining unsolicited AEs (related and unrelated).

2.6.9. Discussion on clinical safety

Participants Pooled Safety Population

The Pooled Safety Population consisted of 4,643 adult participants from the 3 completed clinical VLA1553 studies after 1 vaccination: VLA1553-301, VLA1553-302, and VLA1553-101. 3,610 participants were randomized and vaccinated with VLA1553 (3,082, 408, and 120 participants, respectively) and 1,033 participants randomized and vaccinated with placebo (VLA1553-301). In VLA1553-101, the formulation and doses used are different from the targeted formulation / dose but only a small number of participants has been vaccinated in this study and the formulations have been shown to be comparable (for the medium dose based on quality data and limited non-clinical data).

Overall, baseline demographic characteristics (gender, race, age and BMI) are comparable between participants vaccinated with VLA1553 and placebo in the pooled studies (with slightly more females than males, majority of white people, median age of 42 and 45 respectively, and median BMI of 29).

Of note, the BMI range in the VLA1553-301 goes up to 102.3 (only healthy people were included and there was no exclusion criteria based on BMI). The immunogenicity and reactogenicity can be altered in case a vaccine is not administered intramuscular as indicated, e.g., subcutaneous due to increased skin-to-deltoid-muscle distance in obese participants.

Ongoing studies and follow-up

There are 2 relevant studies ongoing: a supportive placebo-controlled Phase 3 study VLA1553-321 in adolescents in an endemic country (Brazil) and a US long-term study VLA1553-303 in adults (for 363 participants of study VLA1553-301). Only new information on ongoing SAEs and AESI from precursor study VLA1553-301 and new SAEs since the end of study VLA1553-301 were reported in study VLA1553-303 up to 2 years post-vaccination. The long-term safety follow-up has been recognised as a missing information in the RMP.

For study VLA1553-321, the CSR with cut-off date of 26 Jul 2023 has been provided with the Part A analysis, which includes data for all participants up to 28 days after vaccination. The applicant commits to submit the Part B CSR (6-month follow-up) as soon as it is finalized (expected by 30-Jun-2024), and the Part C CSR (1 year follow-up final CSR) latest by 30-Nov-2024 as part of the agreed EMA PIP. This report will include the post-hoc analysis for chikungunya-like adverse reactions (broad definition). (**REC**)

Completed studies

Solicited AEs

Reactogenicity data were collected from vaccination and until 14 days post vaccination in the phase 1 study VLA1553-101 or up to 10 days post vaccination in the phase 3 (studies VLA1553-301 and VLA1553-302). In the pooled safety studies, the frequency of solicited systemic and local AEs was higher in participants vaccinated with VLA1553 compared to the placebo. Most solicited AEs were assessed as related to the vaccine.

Solicited injection site reactions: Tenderness (10.8% versus 8.1%), pain (6.1% versus 3.7%), and induration (1.4% vs. 0.8%) were reported slightly more frequently in the VLA1553 arm compared to placebo. Erythema/redness (1.6% versus 1.5%) and swelling (0.7% versus 0.8%) were seen equally in the VLA1553 arm compared to placebo. The frequency of the solicited injection site AEs with a duration longer than 10 days was similar between the VLA1553 and placebo arm (0.4% versus 0.0%). The majority of the solicited local adverse events were mild in both VLA1553 and placebo arms. Only one

severe solicited local AE was reported: pain in the pooled VLA1553 arm. These events are reflected in section 4.8 of the SmPC.

Solicited systemic reactions: In the VLA1553 arm, the most reported were headache (32%), fatigue (29.4%) and myalgia (23.7%), followed by arthralgia (16.6%), fever (13.8%), nausea (11.4%), rash (2.4%) and vomiting (2%) (all reported at a higher frequency than in the placebo arm). In VLA1553 arm, the median duration of fever was 2.0 days (vs. 1.5 in the placebo arm), for myalgia it was 2.0 days (vs. 2.0 in the placebo arm), and for arthralgia it was 2.0 days (vs. 3.0 in the placebo arm). These events are reflected in section 4.8 of the SmPC.

Overall, there was no major difference in the frequency of solicited systemic AEs with a duration longer than 10 days between both arms (2.2% versus 1.6%). Only prolonged myalgia showed a difference ≥ 0.5% between both arms: 27/3,610 (0.7%) vs. 2/1,033 (0.2%). The severity of solicited systemic AEs was mainly mild or moderate. There were 2.3% of severe solicited systemic AEs in VLA1553 arm (vs. 0.1% in the placebo arm): fever (1.7%) was the most frequently reported followed by arthralgia (0.3%), myalgia (0.2%), fatigue (0.2%) and headache (0.1%). The difference between VLA1553 and placebo arms regarding (severe) solicited systemic AEs is considered expected upon administration of a live-attenuated vaccine such as VLA1553.

Overall, occurrence of severe solicited systemic events was not associated to a certain viraemia level at Day 8, since the viraemia levels varied considerably between subjects. Moreover, same types of severe solicited events (fever, arthralgia, fatigue, myalgia, and headache) were also documented for subjects without detectable viraemia at Day 8 or with viraemia levels <LOD/LOQ at Day 8. Therefore, severe solicited systemic events were reported in subjects irrespective if viraemia at Day 8 was present or not. It should be however considered that after natural CHIKV infection, associations between symptoms and viral loads have been reported (e.g. Raghavendhar 2019; Tun 2022) and that viral loads peak in the first ~4 days after symptoms onset (e.g. Panning et al., 2008). To conclude on a potential association between adverse reactions and vaccine viraemia, studies adequately designed and powered with vaccine viraemia screenings performed prospectively at early time-points after vaccination should be performed. The difficulties in conducting such studies is acknowledged.

Of note, in study VLA1553-101, the frequencies of solicited local and systemic AEs increased with increasing dosing.

Unsolicited AEs

Unsolicited safety information (any AEs and SAEs) was collected during the entire study period until the end of study (i.e. up to Day 180 for studies VLA1553-301 & VLA1553-302). Up to Day 180, in the pooled safety studies, the frequency of unsolicited AEs was higher in participants vaccinated with VLA1553 compared to the placebo (31.6% vs. 23.9%, respectively), and in particular for: chills (2.0% versus 0.3%), diarrhoea (1.6% versus 0.4%), neutropenia (1.7% versus 0.1%), leukopenia (1.2% versus 0%) and lymphadenopathy (1.1% versus 0.2%). These events have been included in section 4.8 of the SmPC.

No relevant difference was observed between both arm for musculoskeletal stiffness (0.5% - 20 events vs. 0.5% - 5 events in each arm respectively), joint stiffness (0.2% - 10 events vs. 0.2% - 2 events), joint swelling (0.1% - 5 events vs. 0.2% - 2 events), arthritis (0.1% - 2 events vs. 0.1% - 1 event) and osteoarthritis (0.3% - 11 events vs. 0.2% - 2 events). As chikungunya infection can cause arthritis and VLA1553 is a live attenuated vaccine, arthritis is an important potential risk in the RMP.

Up to Day 180, the frequency of related unsolicited AEs was also higher in participants vaccinated with VLA1553 compared to the placebo (11.6% vs. 4.6%, respectively). In particular the difference was noted for the blood and lymphatic system disorders (3.3% vs. 0.5%), general disorders and administration site conditions (2.8% vs. 1.2%), investigations (1.2% vs. 0.1%) and respiratory, thoracic and mediastinal disorders (0.8% vs. 0%). Chills (1.8% vs. 0.2%), neutropenia (1.6% vs. 0.1%), leukopenia (1.1% vs.

0%), lymphadenopathy (0.8% vs. 0%) were related unsolicited AEs which were more frequently seen in VLA1553 (please refer to the clinical laboratory evaluation sub-section below).

Most unsolicited AEs in the pooled dataset up to Day 180 were mild or moderate in severity; the proportion of subjects with mild, moderate, or severe unsolicited AEs was similar between the VLA1553 and placebo group. In the VLA1553 group, the most common related severe unsolicited AEs in the VLA1553 group were neutropenia (0.1%; 5 subjects with 5 events) and lymphopenia (0.1%, 2 subjects with 2 events; these cases were only reported in the high dose group (Arm H) in study VLA1553-101). Other reported events were: chest discomfort and arthralgia (one subject), chills (one subject), back pain (one subject), and inappropriate antidiuretic hormone secretion (one subject). No related severe unsolicited AEs were reported in the placebo group. In the VLA1553 group, the 11 related severe unsolicited AE cases were transient. Viraemia was detected on Day 3 for subjects with related severe lymphopenia and neutropenia, and for the subject with related severe back pain; and viraemia was <LOD or undetectable at Day 7 or 8 and/or 14 for all related severe unsolicited AEs. All events were non-serious except the case of SIADH. A detailed review of this case suggests a diagnosis of hypovolemic hyponatremia as more likely.

Of note, in study VLA1553-101, no dose dependent relationship was observed on the frequencies of unsolicited AEs.

Deaths, SAEs and AESIs

Three deaths were reported in study VLA1553-301: 2 participants in the VLA1553 arm (severe coronary artery disease 119 days after vaccination, and severe COVID-19 165 days after vaccination) and 1 participant in the placebo arm (anoxic brain injury 151 days after vaccination). All were assessed as not related to vaccination.

The SAE frequency was slightly higher in pooled VLA1553 arm (1.4%, 52/3,610) vs. the placebo arm (0.8%, 8/1,033). For all SOCs, the maximum frequency differences between the 2 groups was 0.1%. The SOC for which most SAEs were documented was Infections and Infestations (0.3% in both arms).

The SOCs with the higher differences of SAE frequencies between the 2 groups were:

- Cardiac disorders: 5/3,610 (0.1%: 2 atrial fibrillations, 1 cardiac arrest, 1 cardiomyopathy, and 1 coronary artery disease) VLA1553 vs. 0/1,033 (0%) placebo
- Pregnancy, puerperium and perinatal conditions: 5/3,610 (0.1%: 5 abortions spontaneous) VLA1553 vs. 0/1,033 (0%) placebo

Cardiac events have been classified as an important potential risk in the RMP. Information on cardiac events will be collected as part of the pharmacovigilance plan, including a targeted follow-up questionnaire. For pregnancy, refer to the subsection below.

Two participants in the VLA1553 arm (in study VLA1553-301) had SAEs considered to be related to vaccination: 1 case of myalgia and 1 case of syndrome of inappropriate antidiuretic hormone (SIADH) secretion / hypovolemic hyponatremia (and with severe atrial fibrillation assessed as unlikely related to vaccine) (versus none in the placebo arm). Of note, myalgia and hypovolemic hyponatremia have been identified as ADRs.

Ten new SAEs were experienced in 9/363 (2.5%) participants during trial VLA1553-303: 7/310 [2.3%] participants of Stratum A [18 to 64 years] (gunshot wound, fatal overdose, pelvic fracture, Apallic syndrome, seizure, myocardial infarction, abdominal pain upper, and cholecystitis) and 2/53 [3.8%] participants of Stratum B [≥65 years] (intracranial aneurysm, and coronary artery disease). None of these SAEs were considered related to vaccination.

The proportion of subjects who discontinued from study due to an AE were comparable between the VLA1553 and placebo group (for both 0.1%). All cases of AEs leading to early withdrawal from study were assessed as not related to study vaccination by the investigators.

AESIs

AESIs have been captured 21 days post-vaccination (VLA1553-301, VLA1553-302, VLA1553-321) or 28 days post-vaccination (VLA1553-101). The cluster of symptoms that constitute an AESI but starting after 21 (VLA1553-301, VLA1553-302, VLA1553-321) or 28 (VLA1553-101) days post vaccination until study end is defined as late onset AESI. In study VLA1553-303, any AESI ongoing from study VLA1553-301 was followed-up. Non-specific transient muscle pain, joint pain and clusters of AEs potentially indicative of an acute stage CHIKV-associated events were registered as AESI as per protocol (sponsor definition).

AESIs (sponsor definition) were reported in 0.3% (11/3,610) of participants in the pooled VLA1553 arm and 0.1% (1/1,033) of participants in placebo arm. No AESI was reported by any participant 65 yoa or older.

Chikungunya-like adverse reactions have been retrospectively evaluated using a broader definition of AESI (broader than the one initially used in the clinical protocols): occurrence of fever (≥38° C) and at least one other symptom also reported for acute-stage chikungunya illness, including arthralgia or arthritis, myalgia, headache, back pain, rash, lymphadenopathy, and certain neurological, cardiac or ocular symptoms (i.e. optic neuritis, retinitis, and uveitis); within 30 days after vaccination, regardless of time of onset, severity or duration of the individual symptoms.

In the pooled safety dataset, adverse event combinations qualifying as chikungunya-like adverse reactions were reported in 12.1% of participants (vs. 0.6% in placebo). Among those, combinations of fever with headache, fatigue, myalgia or arthralgia were the most common, all other symptoms were reported in fewer than 10% of chikungunya-like adverse reactions. The reported symptoms were mostly mild, 1.8% of participants reported at least one severe symptom, most commonly fever or arthralgia. Median onset of chikungunya-like adverse reactions was 3 days after vaccination, and median time to resolution was 4 days. Longer-lasting symptoms ≥30 days occurred in 0.4% of participants.

In study VLA1553-321 (adolescents), the proportion of subjects with Chikungunya-like adverse reactions was 23.3% in the VLA1553 group and 4.8% in the placebo group with the broader definition (both higher percentages than in adults).

There were no Chikungunya-like adverse reactions with late onset in the pooled dataset (i.e. starting as of 21 days post-vaccination) and only one case was reported in study VLA1553-321: a seropositive at baseline >10 years old participant who had fever and myalgia with onset 30 days post-vaccination; the events were assessed as mild and not related to VLA1553 vaccination; the duration was 2 days and the subject fully recovered (viraemia not assessed).

Of note, fever, headache, fatigue, myalgia, and arthralgia have been identified as ADRs.

Risk of severe or prolonged Chikungunya-like adverse reactions has been highlighted in section 4.4 and Chikungunya-like adverse reactions have been described in a specific sub-section in section 4.8. Moreover, Chikungunya-like adverse reactions have been identified as important identified risk in the RMP.

The broad definition for Chikungunya-like adverse reactions will be used in the protocols of Post-Authorisation Safety Study VLA1553-401, Prospective Safety Cohort Study VLA1553-406 and in the Randomized Controlled Trial on Effectiveness and Safety VLA1553-404. Furthermore, the Applicant will also need to adhere to the Brazilian MoH's broad definition for study protocols VLA1553-406 and VLA1553-404. Further data will be collected via routine pharmacovigilance. A targeted questionnaire will

be used to collect follow-up information on arthralgia, arthritis cases and chikungunya like adverse reactions.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics. The frequencies are based on those for all local and systemic solicited AEs and all remaining unsolicited AEs up to 6 months post-vaccination (related and unrelated).

By age categories

Overall, in the pooled safety data, the safety VLA1553 was evaluated in 346 participants 65 yoa or older. When comparing the safety by broad age groups, solicited local and systemic AEs were less frequent in participants 65 yoa or older compared to participants 18-64 yoa. The frequency of unsolicited (all, related and severe) AEs was comparable in each category. SAEs and MAAEs were more frequent in participants 65 yoa or older (3.5% and 17.6%, respectively) compared to participants 18-64 yoa (1.2% and 11.8%, respectively).

AESIs (sponsor definition) up to 6 months were experienced by 0.3% (6/2,084), 0.4% (5/1,180), 0% (0/287), 0% (0/54) and 0% (0/5) of participants in the age groups of 18 to 45, 45 to 64, 65 to 74, 75 to 84 and 85 yoa or older respectively (i.e. VLA1553 pooled group: 0.3%; placebo group: 0.1%). However, in study VLA1553-321, up to 28 days, AESIs were reported by 3.8% (19/502) of the adolescents vaccinated by VLA1553 and by 0.8% (2/252) in the placebo arm.

In the VLA1553 group, the rate of Chikungunya-like adverse reactions (broad definition) was comparable between the younger (18-64 yoa) (12.2%) and subjects 65 yoa or older (10.7%) (i.e. VLA1553 pooled group: 12.1%; placebo group: 0.6%). However, in study VLA1553-321, up to 28 days, Chikungunya-like adverse reactions were reported by 23.3% of the adolescents in the VLA1553 group (and 4.8% in the placebo group).

For the Applicant, the increased frequency of AESI and Chikungunya-like adverse reactions in VLA1553-321 might be explained by the definition of fever in this study versus the VLA1553 studies in adults. In study VLA1553-321, fever was defined as body temperature ≥37.8°C, whereas the fever definition in the VLA1553 studies with adults was a body temperature ≥38°C. This difference is reflected in the higher proportion of subjects who experienced solicited fever in study VLA1553-321 (VLA1553 group: 24.3%, placebo group: 3.6%) compared to the proportion of subjects of the pooled dataset (pooled VLA1553 group: 13.8%, placebo group: 0.8%). However, after VLA1553 vaccination, solicited systemic AEs and solicited injection site AEs were also more frequent in adolescents in VLA1553-321 (63.5% and 32.1%, respectively) than in the pooled safety adult dataset (51.1% and 15.2%, respectively). So, the increase of AESI (sponsor definition) and Chikungunya-like adverse reactions (broad definition) could also be explained by an increased sensibility of the adolescents.

By serostatus at baseline

Preliminary safety data were also analysed by serostatus at baseline in study VLA1553-321 up to 28 days after vaccination on 753 adolescents. 614 (81.5%) participants were seronegative for CHIKV serostatus at baseline (µPRNT50 ≤40): 408 in the VLA1553 arm and 206 in the placebo arm. 139 (18.5%) participants were seropositive for CHIKV serostatus at baseline (µPRNT50 >40): 94 in the VLA1553 arm and 45 in the placebo arm.

In the VLA1553 arm, the frequency of solicited systemic AEs was higher in the seronegative stratum (67.9%) compared to the seropositive stratum (44.7%). The frequencies of solicited local AEs or unsolicited AEs were similar in both stratum.

In the VLA1553 arm, there were 2/408 (0.5%) participants of the seronegative stratum who experienced SAE (1 pyrexia and 1 hyperkalaemia) and 2/94 (2.1%) participants of the seropositive stratum (1 activated partial thromboplastin time prolonged and 1 lower limb fracture) (vs. 0% and 2%, respectively,

in the placebo arm). Only the pyrexia event was considered possibly related: fever was reported as part of an AESI in combination with mild arthralgia in arms and hands, mild myalgia, and mild headache.

In the VLA1553 arm, the proportion of participants with Chikungunya-like adverse reactions (broad definition) and MAAEs was higher for the seronegative vs. the seropositive stratum (Chikungunya-like adverse reaction rate: 11/408 seronegative participants at baseline (27.2%) compared to 6/94 seropositive at baseline (6.4%)).

Pregnancy, fertility and lactation

Pregnancy

Pregnant and lactating women have been excluded from clinical trials so far. Nevertheless, 16 pregnancies were recorded for participants vaccinated with VLA1553: 13 in study VLA1553-301 and 3 in study VLA1553-302. There were 10 healthy babies born, 1 participant lost to follow-up, and 5 spontaneous abortions (all before 20 weeks gestational age) reported as SAEs (31.3%). Taking into consideration the timely occurrence of the spontaneous abortions which occurred at least 2 months after VLA1553 vaccination, the medical history of some subjects (obesity, previous pregnancies with abnormal outcomes…), and the frequency of miscarriages in the general population, the DSMB and Sponsor could not see any safety signal, and therefore assessed all the spontaneous abortions as unrelated to VLA1553.

In VLA1553-321 (beyond Part A, data cut-off 14-Feb-2024), 3 pregnancies were reported during the study in adolescents: whereof 2 pregnancies have well progressed up to birth (2 born healthy babies), one pregnancy is currently ongoing.

In the adults, the observed rate of spontaneous abortion (31.3%) is higher than those which typically occurs in the general population (about 12-16%) (Quenby et al 2021; Magnus et al 2019) or in women vaccinated with mRNA COVID-19 vaccine (14.1%) (Zauche et al 2021). However, these data should be interpretated with caution due to the small sample size compared to the general population.

Live vaccines administered to a pregnant woman pose a theoretical risk to the foetus: it is not known whether it can be transmitted transplacental or intrapartum and/or if it causes foetal or neonatal morbidity or mortality. Therefore, live attenuated virus vaccines are generally contraindicated during pregnancy.

Vertical transmission of wild-type CHIKV from pregnant individuals with viraemia at delivery is common and can cause potentially fatal CHIKV disease in neonates or long-term neurological sequelae or myocardial disease (Torres et al. 2016, Géradin et al. 2008). However, rare maternal CHIKV infection has been associated with miscarriage (Lenglet et al. 2006). Vaccine viraemia occurs in the first week following administration of VLA1553, with resolution of viraemia by 14 days after vaccination. It is not known if the vaccine virus can be vertically transmitted and cause foetal or neonatal adverse reactions.

Because of theoretical risks and uncertainties, as a precautionary measure, a clear and careful wording on the benefits and risks has been introduced in section 4.6 of the SmPC.

Breast-feeding

It is unknown whether VLA1553 is excreted in human milk. Although CHIKV RNA has been detected in human milk (Campos, 2017), there are no reports to date of infants acquiring chikungunya through breastmilk. Due to the unknown risk of transmission of the vaccine virus strain to the infants from breastfeeding mothers, as a precautionary measure, a wording on the benefit of breast feeding for the child and the benefit of the vaccine for the woman has been introduced in section 4.6 of the SmPC.

Moreover, safety in pregnant or breastfeeding women has been recognised as an important potential risk in the RMP.

Re-vaccination

In study VLA1553-101, participants received a re-vaccination with the high dose by intramuscular injection into the musculus deltoideus: Arm L (low dose, re-vaccination at Month 12), Arm M (medium/targeted dose, re-vaccination at Month 12), and Arm H (high dose, re-vaccination at Month 12 (H1) or 6 (H2)). Overall, up to 12 months, as observed after the first vaccination in VLA1553-101, neutropenia and leukopenia were the most frequent unsolicited AEs after the second vaccination (all grade and severe). One subject in Arm H2 reported one SAE of supraventricular extrasystoles, which was considered to be not related to the vaccination.

Concomitant use

In the pooled studies, subjects considered generally healthy were included, i.e. if any chronic illness/condition was stable and well-controlled on therapy for the past 6 months, excluding infection, positive for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV), immune-mediated arthritis/arthralgia, malignancy other than squamous cell or basal cell skin cancer, and suspected defect of the immune system (such as congenital or acquired immunodeficiency, including infection with HIV). For safety evaluations, prophylactic administration of antipyretics within 4 hours prior to and during the first 72 hours after vaccination were not permitted per protocol, only for symptomatic treatment.

In the pooled safety dataset, in the VLA1553 group (3610 participants), 870 participants (24.1%) were taking non-steroidal anti-inflammatory drugs (NSAIDs), 859 antipyretics (23.8%), and 72 immunosuppressants (2%). In the placebo group (1033 participants), 159 participants (15.4%) were taking NSAIDs, 173 antipyretics (16.7%), and 18 immunosuppressants (1.7%).

In both groups, the participants who have taken NSAIDs or antipyretics or immunosuppressants were more likely to report solicited systemic AEs, solicited injection site AEs, and unsolicited AEs. In both groups, the frequency of unsolicited AEs for participants who used immunosuppressants was particularly high compared to the participant who took NSAIDs or antipyretics.

For AESI (sponsor definition), 2 AESI occurred in persons who were classified to have taken immunosuppressants, while 9 occurred in individuals who did not receive immunosuppressants. Of the subjects with Chikungunya-like adverse reactions (VLA1553 pooled group), 12 subjects used immunosuppressants as concomitant medication, while 424 Chikungunya-like adverse reactions occurred in individuals who did not receive immunosuppressants.

Overall, it is probable that these concomitant medications were used in reaction to adverse events, not as an underlying medication modifying the adverse event profile (and not for prophylaxis).

No clinical study has been carried out to evaluate the effect on safety or the immune response of other vaccines given before, after, or at the same time than Ixchiq. Co-administration with other vaccines is missing information in the RMP and will be further characterised in post-authorisation.

Clinical laboratory evaluations

In Chikungunya infection, lymphopenia (lymphocyte count <1,000 cells/mm^3) is reported as the most common laboratory abnormality in patients, severe lymphopenia (<500 cells/mm^3) is seen in only one-third of patients (Bartholomeeusen, 2023). Neutropenia is rarely seen in chikungunya while Dengue infection is more likely to cause neutropenia (Lee et al. 2012).
An increase in liver enzymes during a Chikungunya infection is observed in some patients but is usually mild (Bartholomeeusen, 2023). During the outbreak on La Reunion Island two-fold greater values than normal were seen in <10% patients (Borgherini, 2007).

In studies VLA1553-301 and VLA1553-321, no clinically relevant differences were observed between VLA1553 and placebo arms for any coagulation or urine laboratory parameters. However, leukopenia

(leukocyte decreased), neutropenia (neutrophile decreased), lymphopenia (lymphocyte decreased), ALT and AST increased were more frequently observed in the adults and adolescents vaccinated with VLA1553 than with placebo (very common vs. common, respectively).

In the pooled dataset, the proportion of subjects with leukopenia, neutropenia, lymphopenia, ALT or AST increased was comparable between the VLA1553 and placebo group pre-vaccination at Visit 1 (Day 1). The proportion of subjects with abnormal lab parameters increased by study Day 8 (i.e. 7 days postvaccination) and abnormal counts were more frequently observed in VLA1553 recipients than placebo recipients. By study Day 29 (i.e., 28 days post-vaccination) abnormal lab counts returned to pre-vaccination levels and percentages were comparable between the treatment groups (post hoc analysis).

There were five cases of neutropenia which were considered clinically significant, assessed as related to VLA1553 vaccination and severe, and were therefore reported as AE by the investigator. For those cases, the following AEs were reported:

Three cases of neutropenia had onset between 6- and 7-days post vaccination. The duration of neutropenia ranged between 8 and 13 days. In two cases, neutropenia was not co-reported with other adverse events. In one case, leukopenia was co-reported with same onset and duration than neutropenia. In 2 cases, events of arthralgia, pyrexia, headache, myalgia, oropharyngeal pain or chills were co-reported (starting 3 to 4 days after vaccination and with a duration of 1 to 3 days).

Overall, in all studies, these laboratory parameter abnormalities (leukopenia, neutropenia, lymphopenia, ALT and AST increased) were only transient, mostly mild or moderate, and normalized up to Day 29. They have been identified as ADRs in the SmPC.

Viraemia

For live-attenuated vaccines, characterisation of the kinetic and level of the vaccine virus viraemia in blood and shedding in body fluids is generally needed to address clinical and ERA aspects, such as risk of human-to-human transmission of the vaccine virus through body fluids or risks of vaccine-induced disease (e. g. CHIK-like disease).

Viraemia in serum and urinary shedding were characterised by genomic amplification in all adults participants of study VLA1553-101 at baseline and Days 3, 7, and 14 after vaccination (120 subjects randomized to 3 different dose levels of VLA1553). Viraemia was observed in most participants, with up to 90% of participants vaccinated with a dose comparable to the licensed dose that had detectable vaccine-virus in the plasma 3 days after vaccination (27/30 in Arm M). Some participants still had detectable viraemia 7 days after vaccination (5/30 corresponding to 17% for those administered a dose comparable to the targeted dose) but to levels that were comparatively lower to those detected 3 days after vaccination. No subject had detectable virus in plasma at any tested dose 14 days after vaccination.

In study VLA1553-101, a single participant shed virus in urine and no data are available from other clinical studies on shedding in urine or in bodily fluid other than urine.

Detection of viraemia by viral culture was foreseen in VLA1553-101 but was not performed. Additional analyses in infectivity assays could have been informative with respect to the potential risks associated with transmission of VLA1553 (to third parties and the environment), however considering that the RT-qPCR results support transient viraemia and no urinary shedding, additional data generated with an infectivity assay are considered not needed.

The applicant states in different documents (e.g. Clinical Study Protocol VLA1553-301 Final 6.0) that "*Viraemia was observed in some subjects in the Phase 1 study albeit short-lived and greatly reduced from levels observed after natural infection*". It is not correct to state that viraemia was observed in some subjects, as it was observed in most participants, with up to 90% of participants in arm M (hence

vaccinated with a dose comparable to the proposed licensed dose) that had detectable vaccine-virus in the plasma 3 days after vaccination. The comparison between the limited published viraemia data collected after natural infection and viraemia data collected after vaccination with VLA1553 in clinical trials indicate reduced vaccine-viraemia levels after administration of VLA1553 as compared to CHIKV levels detected after natural infection, but does not indicate comparatively greatly reduced viraemia levels. The vaccine-viraemia kinetic is comparable to the one reported after natural CHIKV infection, with high proportions of patients having detectable levels of *wt* CHIKV RNA up to 4 days after symptoms onset and reported proportions of RT-qPCR positive patients gradually declining with no *wt* CHIKV viral RNA detected in serum samples after day 7-14 of illness onset.

Based on the results of study VLA1553-101, the Applicant decided to no longer characterise shedding in urinary samples in the following trials conducted in adults and adolescents and this is considered acceptable.

The applicant was advised to also assess vaccine virus shedding in saliva samples (Scientific Technical Advice type III, FAMHP/Valneva, 7 and 26 April 2017), but shedding in saliva was not characterised in the clinical development of VLA1553. Although considered that such data would have been of interest to better characterise VLA1553 (in natural infection, wt CHIKV is detected in saliva samples in a large proportion of patients), it is also considered that the risk of vaccine-virus transmission through saliva is limited.

However, clinical studies to evaluate safety, tolerability and immunogenicity of VLA1553 in children <12 yoa is foreseen in the PIP. The applicant is invited to consider performing additional analyses in future trials to better characterise shedding of VLA1553 in the paediatric population (including also saliva samples). Indeed, pathophysiology of natural CHIKV infection is different in adults as compared to children (in particular in infants) and vaccine shedding might therefore also differ in the younger paediatric population.

It is also understood that based on the results of study VLA1553-101, the Applicant decided to no longer characterise vaccine viraemia on samples isolated 3- and 14-days post-vaccination in the following trials conducted in adults and adolescents. This is overall considered a limitation. Viraemia data in study VLA1553-101 were obtained on a very limited number of participants (n=30 adults vaccinated with a dose comparable to the targeted dose). Confirmatory data supporting viral clearance 2 weeks post-vaccination would have been of interest. In addition, there have been reports indicating an association between viral loads and clinical symptoms after natural infection (e.g. Raghavendhar 2019; Tun 2022). A more comprehensive characterisation of vaccine-viraemia at an early time-point (Day 3) could have contributed to a better characterisation of the adverse reactions. The applicant is advised to include at least these two additional time-points for vaccine viraemia assessment in future trials, in particular for the paediatric population or other populations at increased risk of atypical/severe CHIK after natural infection. The applicant has acknowledged the request to test samples from day 29 for the existence of late / delayed viraemia, even if Day 8 is tested negative, and committed to do testing in some cases (e.g. when onset of AE is after Day 8).

In the VLA1553-301 and VLA1553-302 phase 3 studies, plasma samples at baseline, Day 8 and Day 29 were collected from all subjects for clinically indicated retrospective investigation of viraemia by RT-qPCR. Day 29 samples was to be tested by RT-qPCR only if Day 8 samples result was positive.

In addition, in study VLA1553-301, viraemia was determined in a cohort of participants with severe solicited or related severe unsolicited AEs (cases, n=61) with age- and gender-matched controls (n=129). Viraemia was seen in a minority (24/190) of tested participants at Day 8, which became undetectable at Day 29.

In the adolescent VLA1553-321 study, plasma samples have been collected at Day 8 and Day 29 from all subjects for clinically indicated retrospective investigation of viraemia by RT-qPCR but also for a subset of subjects (viraemia subset of approximatively 50 randomized to VLA1553 and 25 to placebo). The preliminary data submitted for the viraemia subset, collectively do not indicate major differences with the viraemia data generated in adults in the VLA1553-101 study, when comparing results obtained 7 days post-vaccination. Indeed, samples collected 3 days and 14 days post-vaccination were only tested in study VLA1553-101. This limits the possibility to compare vaccine viraemia across these two populations.

Biological plausibility of other risks

Sexual transmission

CHIKV has been detected by RT-PCR in bodily fluids during and after an acute infection. The INOVACHIK cohort study (Martins, 2021) showed that CHIKV was detected more than 30 days after acute onset in serum, urine, saliva, semen and vaginal secretions. Additionally, persistence has been shown for more than 60 days in serum and saliva, and for more than 90 days in urine. There are case reports about the detection of CHIKV in semen (Martins 2022, Bandeira 2016) however no successful cultivation of the virus in the semen until date (Martins 2022). So, it has not been shown if the virus can be transmitted. Presence of VLA1553 in semen has not been assessed and hence cannot be ruled out, although the difficulties in generating such data are acknowledged. As specified in the RMP, putative sexual transmission of CHIKV or vaccine virus will be closely monitored in the PSURs through analysis of cases, review of literature and any source of data.

Bloodborne transmission

Bloodborne transmission of chikungunya has been described in an healthcare worker (Parola 2006) and among laboratory personnel handling infected blood. CHIKV RNA has been detected from blood donations during CHIKV outbreaks. Vaccination with VLA1553 can cause a viraemia, which can potentially cause bloodborne transmission (ex. blood transfusion). An interval of four weeks between vaccination with VLA1553 and a warning on blood donation has been proposed in the SmPC section 4.4.

Close contacts of vaccinees

With regard to risk of transmission of the vaccine virus to close contacts of vaccinees, the data suggest minimal or no risk of secondary transmission of VLA1553 via shedding. Risks related to transmission of VLA1553 during pregnancy and breastfeeding have been adequately addressed as discussed above.

Risk of transmission via mosquito

The risk of a person transmitting CHIKV to a biting mosquito or through blood is highest when the patient is viraemic which is during the first week of illness. VLA1553 causes a viraemia but the risk for transmission of the vaccine-CHIKV through mosquitos is thought to be unlikely.

Antibody-dependent Enhancement (ADE) risk

Suboptimal levels of antigen-specific antibodies or non-neutralizing antigen-specific antibodies may enhance infectivity and disease severity through antibody-dependent enhancement (ADE). ADE has been observed for other arboviruses such as dengue (Yang et al. 2017).
To our knowledge there are no reports of ADE for chikungunya in humans but antibody-mediated enhancement in CHIKV infection in vitro and in vivo mouse models (Hallengärd et al. 2014, Lum et al. 2018) suggests that low levels of antibodies against CHIKV may enhance CHIKV infection or disease severity. The clinical significance of these findings are unknown.

In addition, it is unknown if ADE can be observed in the context of infections with different alphaviruses. Co-circulation of CHIKV and Mayaro virus (MAYV) and of CHIKV and O'nyong nyong virus (ONNV) can

occur and antibody cross-reactivities have been reported. Potential ADE should be considered also in the context of heterologous infections with alphaviruses. ADE risk will be closely monitored through PSURs (analysis of the cases, review of literature and any source of data).

Pharmacovigilance study program

The RMP details the pharmacovigilance plan to further characterise the safety concerns (refer to section 2.7.).

For each study in the RMP, the Applicant should submit the final protocol and the plan for the submission of interim/progress reports. Moreover, as possible, it should be ensured that the Chikungunya-like adverse reactions broad definition is used in the protocols. (**REC**)

2.6.10. Conclusions on the clinical safety

As the population was well distributed in the pooled safety studies (with inclusion of 346 participants over 65 yoa vaccinated with VLA1553) and no major safety concern arises, the size of the safety database (4,643 adults: 3,610 vaccinated with VLA1553 and 1,033 vaccinated with placebo) is acceptable (>3000 participants).

The reactogenicity is generally similar to other live-attenuated vaccines. The most common vaccination site reaction was pain. The most common systemic adverse reactions were headache, fatigue, myalgia, arthralgia, fever and nausea.

With a 6 month follow-up, VLA1553 was generally well-tolerated across all age groups evaluated in the clinical studies (adults) and the proposed safety concerns are, overall, acceptable. Long-term safety follow-up has been recognised as a missing information in the RMP and will be addressed in study VLA1553-404 and in the ongoing clinical trials VLA1553-303 for up to 2 years post vaccination.

However, safety issues associated with VLA1553 include very common white blood cell count decreased (leukopenia, neutropenia and lymphopenia) and very common liver function test increased (ALT/AST), some serious pregnancy events (5 spontaneous abortions before 20 weeks gestational age), and chikungunya-like adverse reactions (most frequently observed: fever associated with headache, fatigue, myalgia or arthralgia).

- White blood cell count decreased (leukopenia, neutropenia and lymphopenia) and very common liver function test increased (ALT/AST) have been identified as ADRs.
- Vaccine-associated arthritis and cardiac events have been classified as an important potential risk in the Risk Management Plan and will be followed up.
- Risk of severe or prolonged Chikungunya-like adverse reactions has been highlighted in section 4.4 and Chikungunya-like adverse reactions have been described in a specific sub-section in section 4.8. Moreover, Chikungunya-like adverse reactions have been identified as important identified risks in the RMP.

Vaccine viraemia occurs in the first week following administration of VLA1553, with resolution of viraemia by 14 days after vaccination.

Overall, the safety profile of the vaccine, which has been documented in an adequate population, is very much as would be expected from the vaccine construct. Therefore, the safety profile of the Applicant-proposed dose regimen of VLA1553 is considered favorable for healthy adults.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 78. Summary of safety concerns

Important Identified Risks	Chikungunya-like adverse reactions
Important Potential Risks	Vaccine-associated arthritis
	Cardiac events
	Safety in pregnant or breastfeeding women
Missing Information	Safety in patients with autoimmune or inflammatory disorders
	Safety in frail patients with acute or progressive, unstable or uncontrolled clinical conditions, e.g. cardiovascular, respiratory, neurologic, psychiatric, or rheumatologic conditions
	Long-term safety
	Co-administration with other vaccines

2.7.2. Pharmacovigilance plan

Table 79. Summary of ongoing and planned additional pharmacovigilance activities

Study / Status	**Summary of objectives**	**Safety concerns addressed**	**Milestones / Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation			
None.			
Category 2 - Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances			
None.			
Category 3 – Required additional pharmacovigilance activities			

Study / Status	Summary of objectives	Safety concerns addressed	Milestones / Due dates
VLA1553-303 On-going	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.	Long-term safety	Clinical trial initiation: Q2/2021 The overall study duration (First Subject In – Last Subject Out) is estimated to be approximately 57 months. 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
VLA1553-304 Ongoing	Primary objective: To evaluate safety and tolerability of a live-attenuated CHIKV vaccine candidate (VLA1553) in moderately immunocompromised adult participants infected with HIV living in CHIKV endemic areas after a single immunization. Secondary objectives: To assess the immunogenicity of VLA1553 in moderately immunocompromised adult participants infected with HIV living in CHIKV endemic areas after a single immunization.	Safety in patients with autoimmune or inflammatory disorders	Study start: H1 2024. Safety data of Part A (Day 29) analysis are expected to be available mid-2025.

Study / Status	Summary of objectives	Safety concerns addressed	Milestones / Due dates
Post-Authorisation Safety Study VLA1553-401 Planned	To estimate the incidence of medically attended adverse events of special interest (AESIs), including infection with chikungunya virus as well as Chikungunya-like adverse reactions, vaccine-associated arthralgia, and cardiac events following the administration of live-attenuated chikungunya virus vaccine (VLA1553) in adults aged 18 years and above in the US planning to travel to endemic areas. To quantify the relative risk associated with VLA1553 and each medically attended AESI for which a risk window after vaccination can be defined using a self-controlled risk interval (SCRI) analysis. To compare the observed incidence rate with the expected rate in the population for each medically attended AESI. To describe the risk of medically attended AESIs following live-attenuated CHIKV vaccine (VLA1553) administration, and co-administration with other vaccines. To describe the use of the live-attenuated CHIKV vaccine (VLA1553) and the risk of medically attended AESIs in individuals aged ≥ 65 years, HIV positive participants, patients with autoimmune or inflammatory disorders, patients with acute or progressive, unstable, or uncontrolled clinical conditions, individuals with an infection in the past 3 days from the index date or with known or suspected defect of the immune system.	Chikungunya-like adverse reactions (broad definition) Vaccine-associated arthritis Cardiac events Safety in frail patients Safety in patients with autoimmune or inflammatory disorders Co-administration with other vaccines	From the date of first US participant receiving Ixchiq, the study inclusion period will be estimated to last 36 months, and data collection will last 42 months with the last participant enrolled followed for 6 months. An overall duration of 3,5 years from Ixchiq US launch early 2024 is anticipated.

Study / Status	Summary of objectives	Safety concerns addressed	Milestones / Due dates
Post-Authorisation Pregnancy Study VLA1553-403 Planned	To evaluate pregnancy and infant health up to 12 weeks post-delivery among pregnant women who received Ixchiq up to 30 days before their last menstrual period (LMP) or at any point during their pregnancy. To describe the frequency of adverse events among pregnant women exposed to Ixchiq within 30 days before their last menstrual period or anytime during their pregnancy.	Safety in pregnant women	Protocol submission to FDA: 15 Mar 2024 Start of data collection: 01 Oct 2025. Last participant in: 01 Apr 2027. End of data collection: 01 May 2028. Final report submission: 01 Nov 2029.
Post-Authorisation Pregnancy Study VLA1553-405 Planned	To monitor and evaluate the outcomes of pregnancy and infant health up to 12-weeks among women in the United States who received Ixchiq while pregnant.	Safety in pregnant women	Protocol: 22 Apr 2024 Start of data collection: 24 Jul 2024 Interim Study Report: 31 Jul 2026 Last participant in: 03 Oct 2027 End of data collection: 03 Jan 2028 Final study report: 15 Aug 2028
Prospective Safety Cohort Study VLA1553-406 Planned	To evaluate the safety of Ixchiq using primary data collection. The objectives are currently subject to re-discussion.	Chikungunya-like adverse reactions Vaccine-associated arthritis Cardiac events Safety in frail patients Safety in patients with autoimmune or inflammatory disorders Co-administration with other vaccines	Draft protocol: 30 June 2024 Start of enrolment: 01 Oct 2025 Final study report: Q1 2029

2.7.3. Risk minimisation measures

Table 80. Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Chikungunya-like adverse reactions	Routine risk minimisation measures: SmPC section 4.4 "Special warnings and precautions for use" and section 4.8. "Undesirable effects" / PL section 4. "Possible side effects". Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Targeted follow-up Questionnaire (see Annex IV). Additional pharmacovigilance activities: Prospective Safety Cohort Study VLA1553-406. Post-Authorisation Safety Study VLA1553-401.
Vaccine-associated arthritis	Routine risk minimisation measures: None. Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Targeted follow-up Questionnaire (see Annex IV). Additional pharmacovigilance activities: Prospective Safety Cohort Study VLA1553-406. Post-Authorisation Safety Study VLA1553-401.
Cardiac events	Routine risk minimisation measures: None. Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Targeted follow-up Questionnaire (see Annex IV). Additional pharmacovigilance activities: Post-Authorisation Safety Study VLA1553-401. Prospective Safety Cohort Study VLA1553-406.
Safety in pregnant or breastfeeding women	Routine risk minimisation measures: SmPC section 4.6 "Fertility, pregnancy and lactation" / PL section 2. "What you need to know before you receive Ixchiq". Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: Targeted follow-up Questionnaire (see Annex IV). Additional pharmacovigilance activities: Post-Authorisation Pregnancy Study VLA1553-405. Post-Authorisation Pregnancy Study VLA1553-403.

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Safety in patients with autoimmune or inflammatory disorders	Routine risk minimisation measures: None. Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: Post-Authorisation Safety Study VLA1553-401. Prospective Safety Cohort Study VLA1553-406. Clinical trial VLA1553-304.
Safety in frail patients with acute or progressive, unstable or uncontrolled clinical conditions, e.g. cardiovascular, respiratory, neurologic, psychiatric, or rheumatologic conditions	Routine risk minimisation measures: None. Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: Post-Authorisation Safety Study VLA1553-401. Prospective Safety Cohort Study VLA1553-406.
Long-term safety	Routine risk minimisation measures: None. Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: Clinical Trial VLA1553-303.
Co-administration with other vaccines	Routine risk minimisation measures: SmPC section 4.5 "Interaction with other medicinal products and other forms of interaction" / PL section 2. "What you need to know before you receive Ixchiq". Additional risk minimisation measures beyond the Product Information: None.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: Post-Authorisation Safety Study VLA1553-401. Prospective Safety Cohort Study VLA1553-406.

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.0 is acceptable.

The applicant is reminded that in case of a Positive Opinion, the body of the RMP and Annexes 4 and 6 (as applicable) will be published on the EMA website at the time of the EPAR publication, so considerations should be given on the retention/removal of Personal Data (PD) and identification of Commercially Confidential Information (CCI) in any updated RMP submitted throughout this procedure.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 09 November 2023. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Ixchiq (chikungunya virus, strain CHIKV LR2006-OPY1, live attenuated) is included in the additional monitoring list as it contains a new active substance, which on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The applicant seeks approval of Ixchiq (VLA1553) for active immunisation to prevent disease caused by Chikungunya virus (CHIKV) in individuals 18 years and older.

Chikungunya (CHIK) (also called CHIK fever) is a mosquito-borne viral disease caused by infection with Chikungunya virus (CHIKV). CHIKV is an arthritogenic alphavirus essentially transmitted to humans by the bites of infected female mosquitoes (*Aedes aegypti* and *Aedes albopictus*). Human-to-human transmission (vertical and blood-borne transmission) of CHIKV has been described.

Once exposed, approximately 50-97% of infected individuals will become symptomatic, with an incubation period that can range from 1 to 12 days (average of 3–7 days).

Acute disease is typically characterised by a rapid onset of high fever, debilitating polyarthralgia, rash and myalgia. Other common signs and symptoms include joint swelling, headache, nausea, fatigue, eye complications, lymphadenopathy, pruritus and gastrointestinal symptoms. Severe CHIK can manifest as encephalopathy and encephalitis, myocarditis, hepatitis, and multiorgan failure. Patients at extremes of the age spectrum are at higher risk for severe disease and risk factors for more severe CHIK are intrapartum exposure for neonates, older age (>65 YoA) and co-morbidities. Newborns infected during delivery and older people with underlying medical conditions may become severely ill and are at increased risk of death.

Acute CHIK is typically self-limiting and >50% of patients reports resolution after 1 month. However, a significant proportion of patients will progress to chronic CHIK following the acute stage, which may lead to significant, long-term disability. Estimates of progression to chronic disease ranges from ~14% to ~87%, with an average prevalence of approximately 48% among infected patients that has been estimated. Chronic CHIK is characterised predominantly by persistence of arthritic conditions for >3 months. Risk factors that have been associated to progression to chronic CHIK include patient age (>45 years), preexisting chronic inflammatory arthropathy, CHIKV genotype, increased severity of symptoms during the acute phase (arthralgias, body aches and weakness) and increased viral loads during the acute stage.

3.1.2. Available therapies and unmet medical need

Since 2004, CHIKV is responsible of major emerging and re-emerging outbreaks of disease in the Indian Ocean islands, South east Asia, and the Americas. It is estimated that during the sudden and large outbreaks caused by CHIKV, one third to three quarters of the population is affected in the areas where the virus is circulating. An attack rate of 35% was estimated for the 2005-2006 CHIKV outbreak that occurred in La Réunion (French oversea department).

CHIKV circulation has been reported in more than 100 countries and more than 10 million cumulative CHIK cases have been reported so far. At the EU level, small outbreaks with autochthonous transmission originating from imported cases have been reported in continental Europe from 2007 to 2017. In view of this autochthonous outbreaks of CHIKV infections in continental Europe, of the widespread presence of competent vectors (*Aedes albopictus*) in the Mediterranean basin, and the return of travellers from endemic areas, in the EU CHIK is included in the list of communicable diseases

threatening public health that have emerged or re-emerged to be covered by epidemiological surveillance. In addition, further geographical expansion of CHIKV beyond the tropics and neotropics is to be expected due to viral adaptation, climate change and globalization. Climate change models generally anticipate an expansion of the global distribution of *Ae. albopictus* and *Ae. aegypti* and thereby increasing the risk of CHIKV transmission including to parts of China, sub-Saharan Africa, Europe and the Americas.

There are no specific approved therapeutics for CHIK. Supportive symptomatic treatments are applied, which differ according to the disease phase. Supportive treatments include hydration during the acute phase; relief of pain during the acute, subacute/post-acute, and chronic phases of CHIK disease; corticosteroid therapy (not recommended during the acute phase) administered during the post-acute and chronic phases of infection; administration of antirheumatic drugs to act on the rheumatological symptoms during the chronic phase.

VLA1553 was granted approval from the U.S. Food and Drug Administration (FDA) in November 2023 for active immunization for the prevention of disease caused by CHIKV in individuals ≥18 yoa who are at increased risk of exposure to CHIKV.

In the EU, no vaccine is approved for the prevention of disease caused by CHIKV infection.

Given the absence of authorised products for prevention or treatment of CHIK in the EU, and taking into consideration the risk for travellers and outbreaks to occur in EU territories, the serious complications which may be exceptionally fatal and the debilitating long-term sequelae in a large proportion of infected individuals, an unmet medical need for a vaccine to prevent disease caused by CHIKV is acknowledged.

3.1.3. Main clinical studies

The current application is supported mainly by data from 2 completed clinical Phase 3 studies (VLA1553-301 and VLA1553-302). Data from a Phase 1 dose finding study (VLA1553-101) and from an ongoing long-term extension study (VLA1553-303) were also submitted. All the studies were conducted in generally healthy adults in the US. There are no efficacy data.

VLA1553-301 (pivotal trial) is a randomised, placebo-controlled, double-blind, multicentre, Phase 3 trial designed to assess the immunogenicity and safety of VLA1553 in adults for 6 months after vaccination. Participants were randomized in a 3:1 ratio to receive a single dose of VLA1553 (n= 3,093) or placebo (n= 1,035), intramuscularly. Immunogenicity was assessed in a subset of 362 participants (PP population, 266 in the VLA1553 arm and 96 in the placebo arm), including 280 adults 18-64 years (207 and 73 in the VLA1553 and in the placebo arm, respectively) and 82 adults ≥65 years (59 and 23 in the VLA1553 and in the placebo arm, respectively).

VLA1553-302 is a randomised, double-blinded, multicentre, Phase 3 trial investigating 3 Lots of VLA1553 in adults of 18-45 years, for 6 months after vaccination. A total of 409 participants were randomized in a 1:1:1 ratio to each of the 3 Lot arms. Of them, 362 constituted the PP population.

VLA1553-303 is an ongoing open-label, single arm, study evaluating antibody persistence (up to 5 years post-vaccination) in a subset of participants of study VLA1553-301 (n=363). The PP population for the Year 1 immunogenicity data is composed of 157 and 27 adults of 18-64 years and ≥65 years, respectively. Year 2 immunogenicity data were available for 234 and 42 adults of 18-64 years and ≥65 years, respectively.

3.2. Favourable effects

The primary endpoint of the pivotal Phase 3 study VLA1553-301 was met. At 28 days post-vaccination, 98.9% (263/266, 95% CI of 96.7-99.8) of the baseline CHIKV seronegative participants had an antibody titre equal or greater than the threshold accepted as reasonably likely to predict protection (≥ 150 µPRNT50) in the VLA1553 arm (PP population). The lower bound of the 95% CI around the proportion exceeds the non-acceptance threshold of 70%. None of the placebo participants reached the threshold. Results were similar in both age categories (18-64 years and ≥65 years).

Results of VLA1553-302 are in line with VLA1553-301, with proportion of baseline CHIKV seronegative participants with an antibody titre ≥150 µPTN50 of 97.8% (348/356, 95% CI: 95.6%-99.0%) 28 days post-vaccination.

Proportions remain high up to Day 180 in studies VLA1553-301 and VLA1553-302 (respectively 96.3% [95% CI: 93.1-98.3] and 96.0% [95% CI: 93.3-97.9]), and at Year 2 in study VLA1553-303 (97.1% [95%CI: 94.4-98.7]).

3.3. Uncertainties and limitations about favourable effects

There are no efficacy data from clinical trials. Efficacy trials were considered not feasible pre-authorization due to unpredictable and short-lived outbreaks of CHIKV. There is no established immune correlate of protection (ICP) for Chikungunya. Hence, in alignment with the Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1), an alternative approach was followed to establish the effect of VLA1553 based on the identification of the immune parameter of greatest importance for protection and its evaluation in clinical trials. The indication of this immune parameter was obtained from non-clinical studies in animals and sero-epidemiological data and was subsequently evaluated in the clinical trials with VLA1553.

The primary endpoint of the pivotal trial is based on a neutralizing antibody titre threshold considered reasonably likely to predict protection. Even if the exact mechanisms of protection are unknown, neutralizing antibodies have a major role in protecting against CHIKV infection and/or disease. The threshold is based on both animal and sero-epidemiological data. Non-human primates with human (transferred) VLA1553-antibody titres above the threshold of 150 µPRNT50 were shown to be protected from viraemia after a challenge with the wild-type La Reunion strain. Yoon et al. showed in a prospective study in the Philippines that individuals with PRNT80 titre ≥10 (reflecting a prior natural infection) experienced a lower frequency of symptomatic PCR confirmed CHIKV infections for the two-years study period. Data also suggest that they were protected from subclinical/asymptomatic infection (based on a rise in neutralizing antibody titres from baseline). A PRNT80 of 10 in the sero-epidemiological study of Yoon corresponds to a value of approximately 50 µPRNT50 in the Applicant assay. Despite several limitations for both the animal and the human data, the use of a threshold of 150 µPRNT50 is considered conservative. Nevertheless, uncertainties remain around its clinical relevance, and around how this threshold actually translates into protection.

Uncertainty remains on whether the vaccine will protect against CHIKV infection and/or disease, including chronic Chikungunya, which greatly contributes to the burden of disease. Consequently, effectiveness data are needed. Two effectiveness studies are planned post-approval, a test-negative case-control effectiveness study (VLA1553-402) planned to be conducted in Brazil and a randomized, controlled trial with pragmatic elements to estimate the VE and safety of VLA1553 (study VLA1553-404) planned to be conducted in different countries/regions. No feasibility evaluations were completed yet.

Since VLA1553 is a live-attenuated vaccine, induced immune responses might resemble those resulting from natural infection. There are however no data to support this assumption. Immune responses after vaccination should be more broadly characterized and compared to those induced after natural infection.

Vaccine viraemia was undetectable within 14 days after re-vaccination at Month 12 with the high dose level (3.2x105 TCID50) in the 23 participants of VLA1553-101 who received primary vaccination with the final dose level, while vaccine viraemia was detected in up to 90% (27/30) of participants after a primary vaccination, with a resolution by 14 days after vaccination. The absence of viraemia after re-vaccination suggests protection against a challenge with the vaccine virus.

It remains uncertain if protection would differ according to the infecting CHIKV strain (homologous and/or heterologous strains/genotypes). Limited data generated with VLA1553 suggest that VLA1553-induced antibodies are able to cross-neutralize wild-type CHIKV strains from 3 CHIKV genotypes (IOL/ECSA, West African and Asian) but at variable level in *in vitro* assays. More data are expected with respect to cross-neutralization of wild-type CHIKV strains of different circulating genotypes.

No neutralizing antibody responses are observed 7 days after vaccination. Early protection via other mechanisms is possible.

Whether a booster would be needed at a certain time is not known. Persistence of CHIKV-specific neutralizing antibodies at a higher level than baseline has been shown up to 2 years post-vaccination in study VLA1553-303. 97.1% (268/276) of the participants had neutralizing antibody titres above the threshold of 150 µPRNT50. Data up to 5 years post-vaccination will be obtained post-marketing.

Concomitant administration with other vaccines has not been studied but is planned in the risk management plan.

Immune responses induced by VLA1553 do not appear to be impacted by concomitant use of anti-inflammatory and anti-rheumatic products or analgesics (VLA1553-301 and VLA1553-302).

Natural infection is believed to induce long-term (even maybe life-long) protection. Therefore, individuals who have been previously infected by CHIKV might not benefit from the vaccine. Limited immunogenicity data were generated in adults with pre-existing immunity to CHIKV living in the US, which is not an endemic country for Chikungunya, and in adolescents living in Brazil, which is an endemic country (VLA1553-321). Overall, no booster response is observed. It is not known if a booster effect might be seen in subgroups of participants (such as those with low baseline antibody titres). Whether participants with low neutralising antibody titres are not able to neutralise the attenuated 181/25 CHIKV strain, and might not be able to protect from re-infection, remains uncertain.

It is unknown whether vaccination with VLA1553 could lead to cross-protection against other alphaviruses. Results obtained on a limited number of participants suggest that VLA1553-induced antibodies are capable of neutralising O´nyong-nyong virus and Mayaro virus in *in vitro* assays.

Only generally healthy adults were included in the clinical trials. No immunogenicity data have been obtained in frail participants and participants immunocompromised due to medical condition or due to immunosuppressive treatments. These populations will be further characterised as part of the risk management plan.

3.4. Unfavourable effects

The Pooled Safety Population consisted of 4,643 adult participants from the 3 completed Phase 1 and 3 clinical VLA1553 studies after 1 vaccination: VLA1553-101, VLA1553-301, and VLA1553-302. 3,610 participants were randomized and vaccinated with VLA1553 (120, 3,082, and 408 participants,

respectively) and 1,033 participants randomized and vaccinated with placebo (VLA1553-301). The initial duration was 6 months after vaccination (with 6 additional month follow-up for 363 participants of VLA1553-301 in VLA1553-303).

The reactogenicity is generally similar to other live-attenuated vaccines. The most common vaccination site reactions were tenderness and pain. The most common systemic adverse reactions were headache, fatigue, myalgia, arthralgia, fever and nausea. Some common unsolicited AEs have been observed such as rash, chills, lymphadenopathy, diarrhoea, vomiting and back pain.

Safety issues associated with VLA1553 include very common white blood cell count decreased (leukopenia, neutropenia and lymphopenia), very common liver function test increased (ALT/AST), and chikungunya-like adverse reactions, such as fever associated with headache, fatigue, myalgia or arthralgia. Chikungunya-like adverse reactions has been classified as important identified risk in the RMP and will be followed-up.

In the Pooled Safety Population, there were 346 participants 65 yoa or older vaccinated with VLA1553. Preliminary safety data were also analysed by serostatus at baseline in study VLA1553-321 up to 28 days after vaccination on 753 adolescents. 139 (18.5%) participants were seropositive for CHIKV serostatus at baseline (μPRNT): 94 in the VLA1553 arm and 45 in the placebo arm. In the VLA1553 arm, no higher reactogenicity was observed in older adults compared to younger adults, or in adolescents CHIKV seropositive at baseline compared to seronegative.

3.5. Uncertainties and limitations about unfavourable effects

Several missing information have been identified and will be further studied in post-authorisation such as: long-term safety follow-up, safety in patients with autoimmune or inflammatory disorders, safety in frail patients, and co-administration with other vaccines.

Currently, only 2-year follow-up data are available for 363 adults in US (6 months in VLA321-301, followed by 18 months in VLA1553-303) and 28 days follow-up data for adolescents in Brazil (VLA1553-321).

As for other live attenuated vaccines, VLA1553 is contraindicated for immunodeficient or immunosuppressed individuals due to disease or medical therapy.

Vaccine associated arthritis and cardiac events have been classified as an important potential risk in the Risk Management Plan.

Pregnant women were excluded from clinical trials, but some pregnancies were reported. In the pooled studies, the observed rate of spontaneous abortion (31.3%) is higher than those which typically occurs in the general population (about 12-16%). However, these data should be interpretated with caution due to the very limited number of pregnancies and sample size. Live vaccines administered to a pregnant woman pose a theoretical risk to the foetus: it is not known whether it can be transmitted transplacental or intrapartum and/or if it causes foetal or neonatal morbidity or mortality. Therefore, live attenuated virus vaccines are generally contraindicated during pregnancy. Nonetheless, vertical transmission of wild-type CHIKV from pregnant individuals with viraemia at delivery is common and can cause potentially fatal CHIKV disease in neonates or long term neurological sequelae or myocardial disease (Torres et al. 2016, Géradin et al. 2008). However, rare maternal CHIKV infection has been associated with miscarriage (Lenglet et al. 2006). It is also unknown whether VLA1553 is excreted in human milk. Because of theoretical risks and uncertainties, as a precautionary measure, a clear and careful wording on the benefits and risks has been introduced in section 4.6 of the SmPC, and the safety concern for pregnant and breastfeeding woman has been classified as an important potential risk in the risk management plan, with post-authorisation safety studies planned to further characterise it.

Vaccine virus was demonstrated to be present in blood and urine and might be present in other body fluids. Viraemia has been characterized in a rather limited number of healthy adults and healthy adolescent participants and investigated retrospectively when considered clinically relevant by DSMB in all phase 3 studies. Vaccine viraemia occurs in the first week following administration of VLA1553, with resolution of viraemia by 14 days after vaccination. Available clinical data do not allow to conclude on the impact or absence of impact of vaccine viraemia on the safety of VLA1553. Recent data indicate an association between clinical symptoms and viral loads during natural CHIKV infection, the clinical studies conducted so far with VLA1553 were not adequately designed and powered to establish if such an association also applies to VLA1553 and the adverse reactions.

Bloodborne transmission of CHIKV has been documented, transmission of the vaccine virus through blood donation is considered possible. An interval of four weeks between vaccination with VLA1553 and blood donation has been proposed in the SmPC.

Concerning potential risk of transmission of the vaccine virus to any population who may be at increased risk of severe CHIK (e.g. severely immunosuppressed persons, pregnant women) who may be close contacts of vaccinees, it is considered that secondary transmission via shedding or via a vector (through infection of mosquitoes feeding on vaccinated subjects) is negligible.

Presence of VLA1553 in semen has not been assessed. However, Martins et al., 2022 reported the presence of CHIKV RNA in 14,3% of semen samples up to 56 days after symptom onset and suggested, yet without proof of presence of infective particles, the potential for sexual transmission. Without data on the presence of Ixchiq in semen, uncertainty remains as to whether there is a possibility of sexual transmission of VLA1553. As specified in the RMP, putative sexual transmission of CHIKV or vaccine virus will be closely monitored via routine pharmacovigilance in the PSURs through analysis of cases, review of literature and any source of data.

To our knowledge, there are no reports of ADE for chikungunya in humans but antibody-mediated enhancement in CHIKV infection in vitro and in vivo mouse models suggests that low levels of antibodies against CHIKV may enhance CHIKV infection or disease severity. The clinical significance of these findings are unknown. In addition, it is unknown if ADE can be observed in the context of infections with different alphaviruses. Co-circulation of CHIKV and Mayaro virus and of CHIKV and O'nyong nyong virus can occur and antibody cross-reactivities have been reported. Potential ADE should be considered also in the context of heterologous infections with alphaviruses. The risk of ADE will be monitored through PSURs (analysis of the cases, review of literature and any source of data).

Uncertainty remains also as to whether recombination events could occur *in vivo* upon simultaneous presence in one cell of VLA1553 and replicons harbouring replicase encoding genes originating from alphaviruses, such as self-amplifying mRNA (samRNA) replicons (e. g. candidate COVID-19 vaccine (ARCT-154) or cancer therapies in clinical development). The theoretical concern is that potential in vivo recombination and/or complementation events could possibly lead to (partial) complementation of the attenuated phenotype of VLA1553 and/or the formation of new and uncharacterized viral particles with unknown properties. The biodistribution or persistence of VLA1553 or samRNA is not fully characterised in humans, hence co-localization in one cell (like for example in lymph nodes or spleen) can currently not be ruled out if both are administered in one single host. If the administration of samRNA based (investigational) medicinal products to receivers of VLA1553 becomes likely (for example if several medicinal products based on samRNA technology get authorized on the market), it should prompt a reconsideration of the environmental risk assessment focusing on biodistribution and persistence properties and likelihood of interaction between both VLA1553 and (investigational) medicinal products using samRNA technology. This reconsideration could lead to a precautionary measure such as the implementation of a safety time interval between administration of VLA1553 and the samRNA replicon in order to minimize the likelihood of having both entities present in one cell.

3.6. Effects Table

Table 81. Effects Table for VLA1553 - active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older.

Effect	Short Description	Unit	VLA1553	Placebo	Uncertainties / Strength of evidence	References
Favourable Effects						
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 29 CHIKV neutralizing antibody titre µPRNT50 ⩾ 150	% [95% CI] (n of subjects)	98.9% [96.7, 99.8] (266)	0% [0.0, 3.8] (96)	**SoE:** At Day 29 post-vaccination, proportion of baseline VLA1553 vaccinated subjects with a CHIKV µPRNT50≥150 exceeds the non-acceptance threshold of 70% for the lower bound of the 95% CI required. **Unc:** uncertainties around clinical relevance of the CHIKV µPRNT50≥150 in inferring efficacy against CHIK or CHIKV infection	VLA1553-301 - Clinical Study Report (V 4.0)

Effect	Short Description	Unit	VLA1553	Placebo	Uncertainties / Strength of evidence	References
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 180 CHIKV neutralizing antibody titre µPRNT50 ⩾ 150	% [95% CI] (n of subjects)	96.3% [93.1; 98.3] (242)	0% [0.0, 4.0] (91)	**SoE:** At Day 180 post-vaccination, proportion of baseline VLA1553 vaccinated subjects with a CHIKV µPRNT50≥150 comparable to the one at Day 29 (lower bound of the 95% CI exceeds 70%) **Unc:** uncertainties around clinical relevance of the CHIKV µPRNT50≥150 in inferring efficacy against CHIK or CHIKV infection	VLA1553-301 - Clinical Study Report (V 4.0)
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 29 CHIKV neutralizing antibody titre µPRNT50 ⩾ 150	% [95% CI] (n of subjects)	97.8% [95.6, 99.0] (356)	N/A	**SoE:** At Day 29 post-vaccination, proportion of baseline VLA1553 vaccinated subjects with a CHIKV µPRNT50≥150 has a lower bound of the 95% CI that exceeds 70% **Unc:** uncertainties around clinical relevance of the CHIKV µPRNT50≥150 in inferring efficacy against CHIK or CHIKV infection	VLA1553-302 - Clinical Study Report (V 2.0)

Effect	Short Description	Unit	VLA1553	Placebo	Uncertainties / Strength of evidence	References
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 180 CHIKV neutralizing antibody titre µPRNT50 ⩾ 150	% [95% CI] (n of subjects)	96.0% [93.3, 97.9] (329)	N/A	**SoE:** At Day 180 post-vaccination, proportion of baseline VLA1553 vaccinated subjects with a CHIKV µPRNT50≥150 comparable to the one at Day 29 (lower bound of the 95% CI exceeds 70%) **Unc:** uncertainties around clinical relevance of the CHIKV µPRNT50≥150 in inferring efficacy against CHIK or CHIKV infection	VLA1553-302 - Clinical Study Report (V 2.0)
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Year 1 CHIKV neutralizing antibody titre µPRNT50 ⩾ 150	% [95% CI] (n of subjects)	99.5% [97.0, 100,0] (184)	N/A	**SoE:** At Year 1 post-vaccination, proportion of baseline VLA1553 vaccinated subjects with a CHIKV µPRNT50≥150 comparable to the one at Day 29 of study VLA1553-301 (lower bound of the 95% CI exceed 70%) **Unc:** uncertainties around clinical relevance of the CHIKV µPRNT50≥150 in inferring efficacy against CHIK or CHIKV infection	VLA1553-303 - Clinical Study Report

Effect	Short Description	Unit	VLA1553	Placebo	Uncertainties / Strength of evidence	References
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Year 2 CHIKV neutralizing antibody titre µPRNT50 ⩾ 150	% [95% CI] (n of subjects)	97.1% [94.4, 98.7] (276)	N/A	**SoE:** At Year 2 post-vaccination, proportion of baseline VLA1553 vaccinated subjects with a CHIKV µPRNT50≥150 comparable to the one at Day 29 of study VLA1553-301 (lower bound of the 95% CI exceed 70%) **Unc:** uncertainties around clinical relevance of the CHIKV µPRNT50≥150 in inferring efficacy against CHIK or CHIKV infection	VLA1553-303 - Clinical Study Report
Unfavourable Effects (pooled safety population completed studies VLA1553-301/302/101)						
Solicited systemic AEs	Headache	%	32.0	14.6	Total of 4,643 vaccinated participants	P3.10
	Fatigue	%	29.4	12.6	Idem	P.3.10
	Myalgia	%	23.7	7.4	Idem	P.3.10
	Arthralgia	%	16.6	4.8	Idem	P.3.10
	Fever	%	13.8	0.8	Idem	P.3.10
	Nausea	%	11.4	5.6	Idem	P.3.10
	Rash	%	2.4	0.5	Idem	P.3.10
	Vomiting	%	2.0	1.0	Idem	P.3.10
Solicited local AEs	Tenderness	%	10.8	8.1	Idem	P3.8.
	Vaccination site pain	%	6.1	3.7	Idem	P3.8.
	Erythema	%	1.6	1.5	Idem	P3.8.

Effect	Short Description	Unit	VLA1553	Placebo	Uncertainties / Strength of evidence	References
	Induration	%	1.4	0.8	Idem	P3.8.
	Swelling	%	0.7	0.8	Idem	P3.8.
Unsolicited AEs	Chills	%	2.0	0.3	Idem	P.3.20
	Diarrhoea	%	1.6	0.4	Idem	P.3.20
	Back pain	%	1.5	0.7	Idem	P.3.20
	Lymphadenopathy	%	1.1	0.2	Idem	P.3.20
	Dizziness	%	0.7	0.4	Idem	P.3.20
	Paraesthesia	%	0.4	0.1	Idem	P.3.20
	Eye pain	%	0.4	0.1	Idem	P.3.20
	Oedema peripheral Peripheral swelling	%	0.2 0.3	0 0.3	Idem	P.3.20 P.3.20
	Tinnitus	%	0.2	0.0	Idem	P.3.20
	Dyspnoea	%	0.2	0.0	Idem	P.3.20
	Hyperhidrosis Night sweats	%	0.2 0.1	0.1 0.0	Idem	P.3.20 P.3.20
	Asthenia Muscular weakness	%	0.1 0.1	0 0.1	Idem	P.3.20 P.3.20
	Hypovolaemic hyponatraemia	%	0 (1 part.)	0	Idem	P.3.20
White blood cell count decreased	Neutropenia (neutrophile decreased)	%	42.3 42.7 (41.8)*	12.4	301: 497 vac. part. 302: 273 vac. part.	301 T14.3.3.1.2 302 T14.3.3.1.2
	Leukopenia (leukocyte decreased)	%	32.0 31.4 (31.2)*	5.8	301: 497 vac. part. 302: 408 vac. part.	301 T14.3.3.1.2 302 T14.3.3.1.2
	Lymphopenia (lymphocyte decreased)	%	23.5 22.0 (22.3)*	7.4	301: 497 vac. part. 302: 273 vac. part.	301 T14.3.3.1.2 302 T14.3.3.1.2

Effect	Short Description	Unit	VLA1553	Placebo	Uncertainties / Strength of evidence	References
Liver function test increased	Alanine aminotransferase (ALT)	%	16.9 14.9 (15.5)*	9.9	301: 497 vac. part. 302: 273 vac. part.	301 T14.3.3.2.2 302 T14.3.3.2.2
	Aspartate aminotransferase (AST)	%	13.0 10.9 (11.7)*	7.4	301: 497 vac. part. 302: 273 vac. part.	301 T14.3.3.2.2 302 T14.3.3.2.2
Chikungunya-like adverse reactions (broad definition)	Combinations of fever with headache, fatigue, myalgia, arthralgia, or other symptoms also reported for acute-stage chikungunya illness	%	12.1	0.6	Total of 4,643 vaccinated participants	Post Hoc analysis

Abbreviations: vac. part.: vaccinated participants
Notes: *Calculated based on studies 301 and 302

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Chikungunya constitutes an important disease burden worldwide. No autochthonous cases were detected in continental Europe between 2019-2023. However local outbreaks with autochthonous transmission have occurred between 2007 and 2017 in regions colonized by *Aedes albopictus*. An expansion of the global distribution of *Ae. albopictus* and *Ae. Aegypti* is anticipated by climate change models, thereby increasing the risk of CHIKV transmission including among others in new regions of continental Europe. Currently, it is considered that a CHIK vaccine will be of advantage for the European population, mainly for travellers to endemic regions.

To date, no prophylactic vaccine is licensed in EU and no specific treatment, other than supportive, exists. Ixchiq (VLA1553) is intended for active immunization for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older. Ixchiq could potentially address the unmet medical need.

There are no efficacy data from clinical trials. The conduct of efficacy clinical trials was considered not feasible pre-authorization due to the unpredictable and short-lived outbreaks of CHIKV. In addition, there is no established immune correlate of protection (ICP). Hence, in alignment with the Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1), an alternative approach was followed to establish the effect of VLA1553 based on the identification of the immune parameter of greatest importance for protection and its evaluation in clinical trials. The indication of this immune parameter was obtained from non-clinical studies in animals and sero-epidemiological data and was subsequently evaluated in the clinical trials with VLA1553. This approach to rely on a threshold value of neutralizing antibodies to infer efficacy has been endorsed by the CHMP.

VLA1553 has been shown to induce robust CHIKV-specific neutralizing antibody responses, with titres above the threshold reasonably likely to predict protection in most participants, up to 2 year post-vaccination.

Although results of the VLAA553-301 are compelling, they are based on a neutralizing antibody titre threshold reasonably likely to predict protection (supported by animal and sero-epidemiological data) and not on an established immune correlate of protection (ICP). Uncertainties remain around how this threshold actually translates into protection against disease and/or infection. Therefore, the actual protection of VLA1553 remains to be confirmed.

Two post-approval effectiveness studies are currently planned. The plan for conducting two studies, with 2 different designs and intended to be conducted at multiple sites in different countries, increase the likelihood to generate effectiveness data to confirm and characterize the clinical protection offered by the vaccine. No feasibility evaluations were completed yet.

The reactogenicity is generally similar to other live-attenuated vaccines. VLA1553 was generally well tolerated across all age groups evaluated in the clinical studies (adults) and the proposed safety concerns are, overall, acceptable. However, safety issues associated with VLA1553 include very common white blood cell count decreased (leukopenia, neutropenia and lymphopenia) and very common liver function test increased (ALT/AST), some serious pregnancy events, and chikungunya-like adverse reactions (such as fever associated with headache, fatigue, myalgia or arthralgia).

3.7.2. Balance of benefits and risks

VLA1553 has been shown to induce robust CHIKV-specific neutralizing antibody responses, with titres above the threshold reasonably likely to predict protection in most participants, up to 2 year post-vaccination. Uncertainties remain around how this threshold actually translates into protection against disease and/or infection.

The actual protection of VLA1553 remains to be confirmed. Two post-approval efficacy studies are currently planned. In depth feasibility assessment are needed to ensure the successful of study execution.

Overall, the safety profile of VLA1553 is considered favourable in healthy adults. However, Chikungunya-like adverse reactions is an important identified risk to be further characterised with post authorisation safety studies.

3.8. Conclusions

The overall benefit/risk balance of Ixchiq is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Ixchiq is favourable in the following indication(s):

Ixchiq is indicated for active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older.

The use of this vaccine should be in accordance with official recommendations.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Description	**Due date**
Post-authorisation efficacy study (PAES): In order to confirm the efficacy of Ixchiq in individuals 18 years and older, the MAH should conduct, according to an agreed protocol, and submit the results of, a randomized, controlled trial with pragmatic elements to assess the effectiveness of Ixchiq vaccination in the prevention of symptomatic, laboratory confirmed chikungunya after a single vaccination with Ixchiq in adults in endemic areas.	Final report due date: 31 Dec 2029

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that 'chikungunya virus (CHIKV) Δ5nsP3 strain (live, attenuated)'is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.