EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

27 February 2025
EMA/116559/2025
Committee for Medicinal Products for Human Use (CHMP)

Extension of indication variation assessment report

Invented name: Ixchiq

Common name: Chikungunya vaccine (live)

Procedure No. EMEA/H/C/005797/II/0001

Marketing authorisation holder (MAH) Valneva Austria GmbH

Note

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

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List of abbreviations

µNT	micro neutralizing test
μNT_{50}	50% reduction of virus-induced cytopathic effect
µPRNT	micro plaque reduction neutralization test
$\mu PRNT_{50}$	50% plaque reduction in a micro plaque reduction neutralization test
AC/CV	Acute and convalescent visits
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BLA	Biologics License Application
BMI	body mass index
BSA	body surface area
CDC	Center for Disease Control and Prevention
CDP	clinical development plan
CHIK-like AR	Chikungunya like adverse reactions
CHIKV	Chikungunya virus
CHMP	Committee for Medicinal Products for Human
CI	confidence interval
COVID-19	Coronavirus Disease 2019
CSR	clinical study report
DSMB	Data Safety Monitoring Board
Ecrf	electronic case report form
ECSA	East Central South African
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ER	emergency room

ERA	Environmental risk assessment
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titre
i.m./IM	intramuscular
i.v.	intravenous
IAS	immunogenicity analysis set
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP	immune correlate of protection
ID	identification
IgG	immunoglobulin G
IgM	immunoglobulin M
IMM	immunogenicity (population)
IMP	investigational medicinal product
IND	Investigational New Drug
ITT	intention-to-treat (population)
LLOQ	lower limit of quantification
LOD	limit of detection
MAA	marketing authorisation application
MAAE	medically attended event
MAH	Marketing authorisation holder
Max	maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	minimum
n.a.	not applicable
n.d.	not determined
NHP	non-human primate
NSAID	non-steroidal anti-inflammatory drug
NSAID	nonsteroidal anti-inflammatory drugs

nsP3	non-structural replicase protein 3
NT_{50}	antibody titre at 50% neutralization
PAES	post-authorisation efficacy study
PASS	Post-authorisation safety study
PP	per-protocol (population)
PPAS	per-protocol analysis set
PRIME	priority medicines
PRNT	plaque reduction neutralization test
PSUR	Periodic Safety Update Reports
PT	preferred term
RCT	randomised control trial
RMP	risk management plan
RNA	ribonucleic acid
RT-qPCR	reverse transcriptase quantitative polymerase chain reaction
SA	Scientific advice
SAE	serious adverse event
SD	standard deviation
SmPC	Summary of Product Characteristics
SOC	system organ class
SRR	seroresponse rate
$TCID_{50}$	50% tissue culture infectious dose
TNCC	test negative case control
US	United States
US	United States
Vero cells	African green monkey kidney cells
VLA1553	Valneva chikungunya vaccine
WS	whole sample (population)
wt	wild-type
yoa	years of age
ZIKV	Zika virus

1. Background information on the procedure

1.1. Type II variation

Pursuant to Article 16 of Commission Regulation (EC) No 1234/2008, Valneva Austria GmbH submitted to the European Medicines Agency on 28 August 2024 an application for a variation.

The following variation was requested:

Variation requested		**Type**	**Annexes affected**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	Type II	I and IIIB

Extension of indication to include active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in adolescents 12 years and older for Ixchiq, based on interim 6 months results from study VLA1553-321; this is a randomized, double-blinded, multicentre study to evaluate the immunogenicity and safety of the adult dose of VLA1553 6 months following vaccination in adolescents from 12 years to less than 18 years of age after a single immunization. As a consequence, sections 4.1, 4.2, 4.8 and 5.1 of the SmPC are updated. The Package Leaflet is updated in accordance. Version 1.1 of the RMP has also been submitted. In addition, the MAH took the opportunity to introduce minor editorial changes to the PI.

Information on paediatric requirements

Pursuant to Article 8 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0501/2023 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0501/2023 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the MAH 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.

Scientific advice

The MAH received the following Scientific advice:

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*

Included Scientific advices on the clinical development relevant for the indication subject to the current application (and initial application):

- Concurrence that vaccine efficacy trials are not feasible and vaccine efficacy can be based on neutralising antibody titres
- Clinical development strategy for licensure of Ixchiq
- 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
- Evidence to support claims on cross protection to heterologous CHIKV strains
- 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 neutralisation assay

In general, the MAH has complied with the CHMP scientific advices. The clinical development program has been in agreement with general guidance on the clinical development of vaccines. The most relevant CHMP guidelines have been applied: "Guideline on clinical evaluation of vaccines" (EMEA/CHMP/VWP/164653/05 Rev. 1).

1.2. 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

Timetable	Actual dates
Submission date	28 August 2024
Start of procedure	14 September 2024
CHMP Rapporteur Assessment Report	8 November 2024
PRAC Rapporteur Assessment Report	14 November 2024
CHMP Co-Rapporteur Assessment	20 November 2024
PRAC members comments	20 November 2024
Updated PRAC Rapporteur Assessment Report	21 November 2024
PRAC Outcome	28 November 2024
CHMP members comments	2 December 2024
Updated CHMP Rapporteur(s) (Joint) Assessment Report	5 December 2024
Request for supplementary information (RSI)	12 December 2024
CHMP Rapporteur Assessment Report	24 January 2025
PRAC Rapporteur Assessment Report	14 January 2025
PRAC members comments	05 February 2025
Updated PRAC Rapporteur Assessment Report	06 February 2025

Timetable	Actual dates
PRAC Outcome	14 February 2025
CHMP members comments	17 February 2025
Updated CHMP Rapporteur Assessment Report	20 February 2025
Opinion	27 February 2025

2. Scientific discussion

2.1. Introduction

2.1.1. Problem statement

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 *Aedes 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.

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.

State the claimed therapeutic indication

The MAH seeks an extension of the approved indication to children and adolescents from 12 to 17 years of age. The proposed indication for Ixchiq is for active immunisation for the prevention of disease caused by Chikungunya virus (CHIKV) in individuals 12 years and older.

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 for 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 reemerged 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 2019-2023. (Source: ECDC Local transmission in mainland EU/EEA, 2007–present last update August 2024). According to Santé Publique France (update 30 October), 12 autochthonous cases have been reported in France between July and October 2024 (1 in Paris or Gennevilliers, 11 in La Réunion, see also below).

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 30 September 2024, approximately 460,000 CHIK cases and 170 deaths were reported worldwide from a total of 23 countries (Americas (15), Asia (6), Africa (1) and Europe (1)). Grenada reported CHIK cases in September and for the first time in 2024. Cases in the EU was 1 non-travel associated CHIK case from mainland France and 11 non-travel associated CHIK cases from La Réunion. All CHIK associated deaths were reported from Brazil (170). (Sources: ECDC worldwide overview, situation update September 2024, Santé Publique France update 30 October 2024).

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.

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.

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. 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 Réunion 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 timepoints. 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 reinfection 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.

Clinical presentation, 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.

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 et al. 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 et al. 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).

Management

Prophylaxis by vaccination

In the EU, Ixchiq is currently approved for active immunisation for the prevention of disease caused by Chikungunya virus (CHIKV) in individuals 18 years and older.

On 30th January 2025, the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion on another CHIK vaccine (recombinant, adsorbed) (Vimkunya, manufactured by Bavarian Nordic) intended for the prevention of chikungunya disease in individuals 12 years and older.

Currently, there's no authorised chikungunya vaccine for adolescents.

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).

2.1.2. About the product

Ixchiq (VLA1553) is a single-dose monovalent live-attenuated vaccine derived by reverse genetics from the CHIKV La Réunion 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 current indication of Ixchiq is for active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older. The MAH seeks an extension of the approved indication to children and adolescents older than 11 years of age.

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.

2.1.3. The development programme/compliance with CHMP guidance/scientific advice

Clinical development

The initial marketing authorisation (MA) was supported by four studies conducted in healthy adults in the US (VLA1553-101, VLA1553-301, VLA1553-302, VLA1553-303) and preliminary data from one Phase 3 study conducted in healthy adolescents in Brazil (VLA1553-321). Two effectiveness studies are planned post-approval (VLA1553 -402 and VLA1553-404).

Paediatric development:

The Paediatric Investigational Plan was agreed with the European Medicine Agency (EMEA-002873-PIP01-20-M01).

While the clinical development in adults has been conducted in the US, the paediatric development is/will be conducted in endemic countries.

Paediatric investigations started in adolescents (12 to <18 years old) with study **VLA1553-321**. This placebo-controlled Phase 3 study evaluated the safety and immunogenicity of VLA1553 for up to 1-year post-vaccination in adolescents residing in Brazil, an endemic country for CHIKV, including 18.4% (139/754) of participants seropositive for CHIKV at baseline due to prior exposure. Part B interim report summarizing safety and immunogenicity data up to 6 months post-vaccination was completed

and is submitted in this application. The final report (Part C) will present data up to 12 months post-vaccination.

Additional paediatric studies are planned to be conducted in a staggered approach to evaluate VLA1553, first in children (1 to <12 years old, studies VLA1553-221 and VLA1553-322) and then, if the safety profile is considered safe, in infants (<1 year old, studies VLA1553-222 and VLA1553-323), in regions where CHIKV is endemic.

2.1.4. General comments on compliance with GCP

The MAH stated that clinical studies of Ixchiq were conducted in compliance with Good Clinical Practice (GCP).

No GCP inspection is needed.

2.2. Non-clinical aspects

No new clinical data have been submitted in this application, which was considered acceptable by the CHMP.

2.2.1. Ecotoxicity/environmental risk assessment

An environmental risk assessment (ERA) was submitted and approved for the indication "active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older" during initial procedure EMEA/H/C/0005797.

The submitted type II variation concerns an extension of the indication to the adolescents 12 years and older for the same vaccine and disease. Only the target population has changed, and the approved ERA is valid as well for the indication in 12 years and above age group. With the extension of the indication, there are no major changes expected to the usage of the vaccine, hence no additional risks are expected from an ERA perspective.

2.2.2. Discussion on non-clinical aspects

The MAH submitted a justification for the absence of a new ERA. It is agreed that the conclusion on the initial environmental risk assessment remains valid for this extension of indication: 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. Putative sexual transmission of CHIKV or vaccine virus is being closely monitored via routine pharmacovigilance in the PSURs through analysis of cases, review of literature and any source of data

2.2.3. Conclusion on the non-clinical aspects

No new non-clinical data have been submitted to support this type II variation, which is acceptable.

Ixchiq is not expected to pose a risk to the environment.

2.3. Clinical aspects

2.3.1. Introduction

GCP

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

The MAH 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 is mainly supported by study VLA1553-321 conducted in adolescents,

Study VLA1553-321 is a placebo-controlled Phase 3 study that evaluated the safety and immunogenicity of VLA1553 for up to 1-year post-vaccination in adolescents residing in Brazil, an endemic country for CHIKV.

Part B interim report summarizing safety and immunogenicity data up to 6 months post-vaccination was submitted in this application.

Results of four studies conducted in healthy adults in the US (VLA1553-101, VLA1553-301, VLA1553-302, VLA1553-303) were previously submitted at initial MA and lead to the approval of Ixchiq for active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older. When needed, data in this application is compared to data generated in the adult indication.

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

Table 1: List of Clinical Studies Supporting the new indication in adolescents 12 years of age and older

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)
VLA1553-321	10, Brazil	First participant in: 14-Feb-2022 Last participant completing Day 180 : 18-Aug-2023 Final report ongoing	Double-blind, randomized placebo-controlled	**VLA1553** 1×10E4 $TCID_{50}$ per 0.5 mL	510 (500 planned) Immunogenicity subset: 335	348 M / 406 F 15.0 years (12-17)	Proportion of participants with a seroprotective CHIKV antibody level defined as $\mu PRNT_{50}$ ≥150 for baseline negative participants 28 days post-vaccination.
				Placebo: 0.5 mL	255 (250 planned) Immunogenicity subset: 57		

2.3.2. Clinical pharmacology

Different bioanalytical methods were applied to address clinical pharmacology aspects of VLA1553. These were assessed at the time of Marketing Authorisation and deemed suitable for their respective intended purposes, including assays applied for study VLA1553-321. Table 2**Error! Reference source not found.** summarizes which assays were specific for study VLA1553-321. The central laboratory, DASA, also performed CHIKV-specific IgG and IgM ELISA testing of samples collected at the Screening Visit; CHIK, Zika, and Dengue antibody detection by ELISA (IgG and IgM) for samples collected at acute and convalescent visits; and CHIK, Zika, and Dengue RT-PCR for samples collected at acute visits. Within submission of the VLA1553-321 CSR Part C, the MAH has committed to submit corresponding validation reports. In addition, details on the sequencing methodology applied to discriminate vaccine viraemia from natural CHIKV infection will be submitted within submission of the VLA1553-321 CSR Part C.

Table 2. List of Bioanalytical Methods applied in study VLA1553-321 at central laboratories

Name	Description and comments
RT-qPCR assay (nsP1)	To assess vaccine viraemia, CHIKV RNA was quantified from RNA extracted from human plasma samples. 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.

Name	Description and comments
	• 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** Validated assay for human plasma samples **Laboratory performing the assay:** Nexelis Laboratories Canada Inc.
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 VLA1553-321, seroconversion was defined as a >4-fold increase over baseline for µPRNT baseline negative and µPRNT baseline positive subjects. **STATUS** Validated assay for human plasma samples **Laboratory performing the assay:** Nexelis Laboratories Canada Inc.
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** CE marked - Validated **Laboratory performing the assay:** Laboratoire Cerba
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 levels of MAYV-specific binding IgG antibodies in human serum samples.

Name	Description and comments
	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** CE marked - Validated **Laboratory performing the assay:** Laboratoire Cerba
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** CE marked - Validated **Laboratory performing the assay:** Laboratoire Cerba
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** CE marked - Validated **Laboratory performing the assay:** Laboratoire Cerba
Additional Bioanalytical Methods applied in study VLA1553-321 at DASA and local laboratories are not included in this Table.	

2.3.3. 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.

In study VLA1553-321, vaccine viraemia was investigated in all subjects randomized to the viraemia subset (Day 1, Day 8 and Day 29) and retrospectively in some subjects if deemed clinically relevant based on safety. Vaccine shedding was not investigated in study VLA1553-321. Results of vaccine shedding were generated in study VLA1553-101. Based on obtained results, the MAH decided to not further explore shedding in the subsequent clinical studies (confer to VLA1553 Marketing Authorisation).

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 VLA1553-321 was validated for plasma samples.

The MAH submitted a validation report for the RT-qPCR assay at the time of Marketing Authorisation application. With the exception of the specificity analyses, it was 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 validated CHIKV µPRNT assay (also referred to as the MAH assay in this report), which measures in vitro neutralizing antibody responses specific to the attenuated CHIKV 181/25 clone of Asian lineage. This also applies to study VLA1553-321.

Concerning validation of the Nexelis CHIKV µPRNT assay, the MAH submitted bioanalytical method validation documentation at the time of Marketing Authorisation application, 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. Specificity analyses 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.

Additional Bioanalytical Methods

Definition of CHIKV baseline serostatus

In study VLA1553-321, baseline immunity to CHIKV was measured by ELISA assay with the aim to stratify enrolled participants by CHIKV baseline serostatus. 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 study VLA1553-321, 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.

2.3.4. Conclusions on clinical pharmacology

The main assays that were applied in study VLA1553-321 to support this extension of indication variation are the validated nsP1 RT-qPCR assay and the validated CHIKV µPRNT assay. Both were assessed in detail at the time of the initial marketing Authorisation and both 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.

2.4. 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 was discussed at initial MA.

The threshold of CHIKV µPRNT50 antibody titre ≥150 was assessed in detail during Scientific advices and at MAA. The MAH proposed this threshold of 150 µPRNT50 based on data from a non-human primate (NHP) passive transfer study using human samples from VLA1553-101, as well as supportive data from a sero-epidemiological study (Yoon et al.). . 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 immune correlate of protection (ICP).

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. 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.

2.4.1. Main study

VLA1553-321

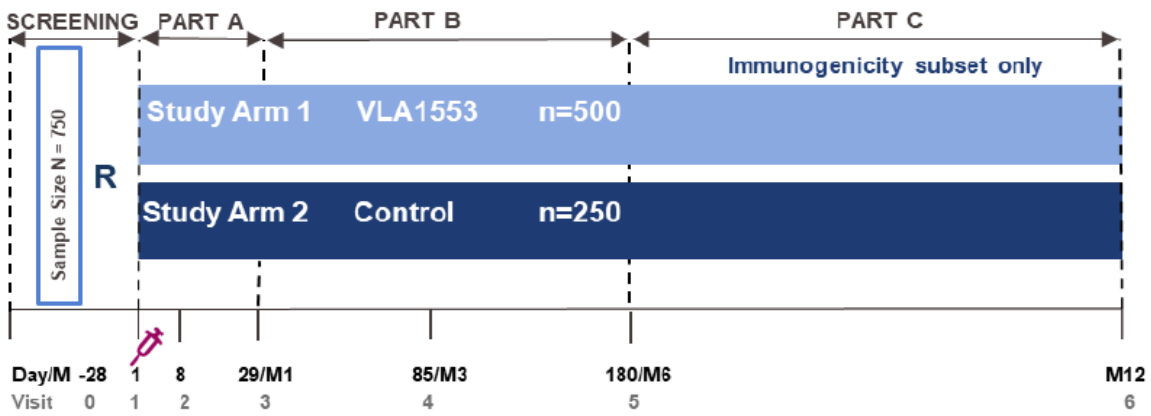
Methods

Overall study design

Study VLA1553-321 is a multicentre, prospective, randomized, double-blinded, Phase 3 clinical study evaluating the adult dose (1 x 10E4 TCID50 per 0.5 mL) of VLA1553 in comparison to control. VLA1553 and control had to be administered as single immunization on Day 1. Approximately 750 participants aged 12-17 years were planned to be enrolled in the study, with a target of 20% seropositive participants (IgM+/IgG+ or IgM-/IgG+) and 80% seronegative participants (i.e. IgM-/IgG-) for CHIKV (as defined by CHIKV ELISA). In order to meet those predefined stratum sizes, the serostatus was determined at the screening visit in a central diagnostic laboratory using a qualitative ELISA assay.

The overall study design is displayed in Figure 1. The study is still ongoing. Three analysis were planned: Part A analysis (immunogenicity and safety up to Day 29) was reported at the initial MA. The MAH now submits updated Part A analysis and Part B analysis (immunogenicity and safety data up to Month 6). Part C analysis (End of study, immunogenicity and safety up to Month 12) will be submitted in March 2025.

Figure 1: VLA1553-321: Study design



Participants were randomized in a 2:1 ratio to the VLA1553 group (n=500) or Placebo control group (n=250), stratified by baseline serostatus (see randomisation section).

Approximately 385 participants (i.e. approximately 50% of the total population) were to be included in the Immunogenicity subset. Of the Immunogenicity subset, approximately 75 participants had to constitute the Viraemia subset.

All participants received a single intramuscular administration of VLA1553 or of placebo (PBS) in the deltoid region of the arm. Participants were followed up for approximately 6 months (all subjects) or 12 months (Immunogenicity subset) following the vaccination. 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. Timepoints are the same as in the adult pivotal study VLA1553-301. An immunogenicity sample at Month 12 (Day 365) was also planned for participants from the Immunogenicity subset. The µPRNT was performed for all participants on the Day 1 sample, to allow for stratification of the statistical analysis by baseline µPRNT serostatus. For the other timepoints, the immunogenicity evaluations were only performed in the Immunogenicity subset. Overall, study procedures are similar to study VLA1553-301.

Study participants

Approximately 750 generally healthy participants 12 to 17 years of age were enrolled in this study. Adolescents were recruited in 10 sites in Brazil

Inclusion and exclusion criteria were overall standard and similar to those used in the pivotal adult study VLA1553-301.

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 hyperlipidaemia were allowed to be enrolled.

Both seropositive (i.e. IgM+/IgG+ or IgM-/IgG+ suggesting previous CHIKV infection) or seronegative (i.e. IgM-/IgG- suggesting absence of previous CHIKV infection) as determined by CHIKV-specific ELISA performed at screening could be included. However, participants who were IgM+/IgG- (suggesting ongoing or recent CHIKV infection) were excluded. Participants under treatment for unresolved symptoms attributed to a previous CHIKV infection were excluded as well.

Participants immunocompromised due to medical condition or due to immunosuppressive treatments were 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. Pregnant adolescents were excluded as well.

Treatments

- VLA1553 is present in a freeze-dried presentation and must be reconstituted with a solvent consisting of sterile water for injection in a prefilled syringe before use. One dose (0.5 mL) of the VLA1553 vaccine contains between 1.6 x 10E3 and 2.5 x 10E4 TCID50 per dose. The active ingredient is suspended in a formulation buffer of pH 7.3, before freeze drying. Two batches were used in the study. The potency of the two batches are in the commercial release specification range as the dose used in the pivotal VLA1553-301 study. All clinical lots are representative of the commercial lots.
- Placebo consists of a PBS buffer based on Dulbecco's PBS media formulation without Calcium and Magnesium.

Concomitant and rescue 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.

According to the study protocol, any of the following treatments was 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.

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

Objectives

Primary Objective

The primary objective is to evaluate the immunogenicity and safety of the adult dose of the live-attenuated CHIKV vaccine candidate (VLA1553) 28 days following vaccination in adolescents aged 12 years to <18 years after a single immunization.

Secondary Objectives

The secondary objectives are to assess the immunogenicity and safety up to Month 12 of the adult dose of VLA1553 following vaccination in adolescents aged 12 years to <18 years after a single immunization.

In addition, the immunogenicity and safety of VLA1553 in subjects previously exposed to CHIKV will be assessed.

Exploratory Objectives

The exploratory objective is to evaluate the efficacy of VLA1553 in adolescents aged 12 years to <18 years after a single immunization.

In addition, information on the CHIKV disease clinical spectrum and associated risk factors in an adolescent population will be collected.

Outcomes/endpoints

Primary Endpoint

The primary endpoint is to assess the proportion of subjects with a seroprotective CHIKV antibody level defined as µPRNT50 ≥150 for µPRNT baseline negative subjects 28 days post-vaccination.

Of note, the wording 'seroprotective level' used in the objectives (study protocol) is not deemed appropriate, the MAH should rather refer to 'µPRNT50 ≥150 for baseline negative participants. It is however noted that the term 'seroresponse' is used throughout the CSR instead of 'seroprotection' that was used in the protocol. The MAH specifies in the CSR that the term 'seroresponse' is used throughout the clinical study report and the statistical analysis plan, instead of 'seroprotection' that was used in the protocol and in previous trials in the program. This was based on a change of terminology across all studies in the VLA1553 program and implemented for consistency across studies. The wording seroresponse is considered acceptable.

Secondary Endpoints

Immunogenicity

The following secondary immunogenicity endpoints will be evaluated:

➢ Immune response as measured by CHIKV-specific neutralizing antibody titres on Day 8, Day 29, Day 85, Day 180, and Month 12 post-vaccination as determined by µPRNT assay;

➢ Proportion of subjects with seroprotective levels (defined as µPRNT50 ≥150 for baseline negative subjects)[ii] on Day 8, Day 85, Day 180 and Month 12 post-vaccination as determined by µPRNT assay;

➢ Proportion of subjects with seroconversion[iv] as compared to baseline at Day 29, Month 6 and Month 12 as determined by µPRNT assay.

➢ Fold increase of CHIKV-specific neutralizing antibody titres determined by µPRNT assay at Days 8, 29, 85, 180 and at Month 12 post-vaccination as compared to baseline.

➢ Proportion of subjects reaching an at least 4-fold, 8-fold, 16-fold or 64-fold increase in CHIKV-specific neutralizing antibody titre compared to baseline as measured by µPRNT assay.

➢ Antibody titres, seroprotection and fold increases for CHIKV-specific neutralizing antibodies, determined by µPRNT assay at Days 1, 8, 29, 85, 180, and Month 12 post-vaccination stratified by µPRNT baseline serostatus.

[ii] *Seroprotective threshold derived from animal passive transfer experiments.*

[iv] *Seroconversion defined as a >4-fold increase of µPRNT50 compared to baseline (Day 1).*

Immunogenicity analyses will be generated stratified by µPRNT baseline serostatus (Day 1): µPRNT50 > 40 for seropositive subjects and µPRNT50 ≤ 40 for seronegative subjects.

Exploratory Endpoints

➢ Incidence of CHIKV infections with onset 14 days post-vaccination as evidenced by viraemia by virus specific RT-qPCR, clinical diagnosis and seroconversion by µPRNT for the entire study period;

➢ Accumulate data of CHIKV disease signs and symptoms in adolescent population as assessed following vaccination on Day 1 for the entire study period.

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 seroprotection rate (SPR) of 80% is assumed, and 200 VLA1553-vaccinated subjects would thus be necessary for a statistical power of 90%. With an expected drop-out/major protocol deviations rate of approximately 10%, 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 enrolment at the sites only.

Randomisation

Baseline serostatus:

The randomization procedure foresees as a first step a randomization to a treatment group (stratified by serostatus at screening) in a 2:1 ratio (VLA1553 vs. control).

Considering the approach (2:1 randomization, stratification of the randomisation for CHIKV ELISA serostatus, and recruitment caps of 20% baseline CHIKV ELISA seropositive participants), a total of 150 baseline CHIKV ELISA seropositive (100 and 50 respectively in the VLA1553 and placebo groups) and 600 baseline CHIKV ELISA seronegative (400 and 200 respectively in the VLA1553 and placebo groups) were expected to be enrolled.

Immunogenicity subset:

The randomization procedure described by the MAH foresees first randomization (stratified by baseline serostatus) and second randomization to specific subgroups (Immunogenicity subset, Viremia and Immunogenicity subset) stratified by treatment group and baseline serostatus until target numbers in the corresponding subset were filled.

However, it was not clear how participants were assigned to the Immunogenicity (and Viremia) subset, as the resulting treatment group ratios (within subgroups) deviate substantially from the overall 2:1 randomization ratio. This approach is neither explained nor justified in the protocol. As the primary analysis is performed into the Immunogenicity subset, it required clarifications and justifications, which were provided by the MAH during this procedure. The MAH confirmed that randomization was developed to achieve the treatment allocation ratios described in Table 3. The MAH also explained the reasons for the treatment allocation ratios in the Immunogenicity Subset.

According to sample size calculations, 268 seronegative participants from the VLA1553 group were required in the Immunogenicity subset. Such number allows 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 µPRNT50 ≥150 (for µPRNT baseline seronegative subjects) at Day 29 (primary endpoint of the trial).

Considering the targeted proportions of CHIKV baseline seronegative and seropositive participants (80% and 20%, respectively), 67 participants were planned for the seropositive participants from the VLA1553 group.

This led to a total of 335 VLA1553 participants to be included in the Immunogenicity subset.

The number of Placebo participants in the Immunogenicity subset was not based on a specific sample size calculation but only aimed at ensuring a reasonable size for the control group. The MAH considered 50 participants for the placebo arm as reasonable, with 40 CHIKV baseline seronegative and 10 CHIKV baseline seropositive participants.

This approach is deemed acceptable and explains the substantially different allocation ratios between treatment arms for the Immunogenicity subset vs. the overall study population.

Table 3: 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)**

[a] dose used for Phase 3 trials in adults

Study site:

After being assigned to treatment arm, participants were assigned to a subset (Immunogenicity subset, Viremia and Immunogenicity subset) stratified by treatment group and baseline serostatus (i.e., seropositive/VLA1553 vs. seronegative/VLA1553 vs. seropositive/control vs. seronegative/control). A maximum number of participants (cap) was assigned to the subset groups within each of the four stratifying factors, as detailed in the table above. This second step is referred to as a 'second randomization'. However, the assignment was not made randomly. In fact, per description of the MAH, the assignment into the subsets was pursued at all sites until pre-determined target numbers in the corresponding stratum subset were filled (i.e. when the cap was reached within a stratum then that subset group was closed for that stratum).

As a consequence, the proportion of participants enrolled into the Immunogenicity/Viremia subsets varied across sites, depending on the timing/speed of recruitment at sites. The MAH clarified that study site was not a stratifying factor in either randomization (i.e. randomization to treatment group or subset group). In the opinion of the MAH, it was not feasible to stratify randomization by site and at the same time stratify randomization by serostatus with an intended CHIKV baseline seropositive stratum being 20%. This reasoning is acceptable.

Some sites are overrepresented in the Immunogenicity Subset, but this is overall not expected to influence the primary endpoint results meaningfully. The geographical location of the ten trial sites in Brazil covered regions with a history of only sporadic previous chikungunya outbreaks as well as regions with high previous chikungunya virus activity. The proportion of baseline seronegative and baseline seropositive participants varied between sites. However, the primary analysis is done in seronegative individuals (i.e. not influenced by the local variability of seroprevalence rates). The factor site is not expected to have substantially affected the immunogenicity assessments as sampling procedures and sample management procedures were standardized and as the assay was the same and performed at a central laboratory (Nexelis). In addition, as the primary analysis is conducted in the CHIKV seronegative individuals of the VLA1553 arm, it is therefore not expected to be affected by imbalances across arms. Therefore, it is agreed that the uneven distribution of participants in the Immunogenicity subset across sites is not of concern for the immunogenicity analyses.

Blinding (masking)

The study was conducted in a double-blind manner. Investigators/sites staff (apart from those designated to randomize participants and handle the investigational medicine product (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.

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

The randomization assignment is not to be revealed except in emergency cases in which unblinding is necessary for the clinical management of an SAE. No emergency unblinding occurred during the study

Statistical methods

Analysis populations:

The safety analysis population contains all subjects who entered the study and received one vaccination.

The immunogenicity analysis population (IMM) includes all randomized and vaccinated participants of the immunogenicity subset who have an evaluable µPRNT antibody titre at baseline and at least one post-baseline titre measurement after vaccination.

The per protocol analysis population (PP) contains all IMM participants who have no major protocol deviations, i.e. Protocol deviations that could impact immune responses. Some major protocol deviations leading to exclusion from the PP were pre-defined in the protocol: participant has a history of immune-mediated or clinically relevant arthritis/arthralgia; participant has a known or suspected defect of the immune system that can be expected to influence the immune response to the vaccine; participant received an immuno-suppressive therapy; participant is positive for HIV, HBsAg, or HCV; and having received the wrong (not according to randomization) or no IMP. Additional criteria could be included in the SAP during the course of the trial, based on a case-by-case evaluation by the sponsor. The following examples are provided in the SAP and were not already described in the protocol: Visit 3 missed by participant or Visit 3 immunogenicity sample missing; Visit 3 out of window (allowed window was Day29 +/- 8 days); Participant with serostatus IgM+/IgG- at screening by ELISA; Participant given an IMP kit identified as being in a temperature excursion that was not approved for use. The sponsor assessed in a blinded manner (prior to study unblinding) whether a protocol deviation could impact immune responses and thus lead to exclusion from the PP. Sample testing issues could also lead to exclusion from the PP for particular time points. This process is similar to the one used in the pivotal trial VLA1553-301 which was assessed at MA. This is further described in the results section.

In contrast with the pivotal trial VLA1553-301, the IMM and PP populations included both baseline CHIKV seronegative and seropositive participants. However, the primary immunogenicity endpoint analysis was limited to baseline µPRNT seronegative participants of the PP population.

During this trial, participants with any borderline ELISA IgG and/or IgM result at screening could be included in the trial, but borderline measurement was to be considered as positive. This implies that participants with borderline IgM ELISA results at screening (IgM borderline/IgG- results) were to be considered as IgM+/IgG- at baseline. However, these participants were considered as IgM-/IgG- by the investigators because there was no evidence of CHIKV circulation in the regions where the study was conducted at the time of enrolment. Therefore, these participants were included in the trial and considered as seronegative at screening for the purpose of stratification of the randomisation. A

modified PP population was thus used for a post hoc analysis to the primary endpoint. This modified PP population excluded participants with borderline IgM ELISA results at screening (IgM borderline/IgG-results).

Additional supplemental analyses were performed such as in seronegative participants based on the ELISA serostatus at screening.

Immunogenicity analyses were performed primarily on the PP portion of the Immunogenicity subset and secondarily on the IMM. Immunogenicity analyses were performed separately for baseline CHIKV seropositive (µPRNT50>40) and seronegative (µPRNT50 ≤40) populations. The primary analysis was performed in baseline seronegative participants (µPRNT50≤40).

The protocol indicates that participants are to be analysed according to the study arm they had been allocated to, rather than by the actual treatment they received. The SAP in not consistent with the protocol in that respect, as it states that participants are to be according to their actual treatment. Ultimately, participants having received the wrong (not according to randomization) or no IMP were excluded from the PP.

The primary hypothesis was tested using an exact binomial test comparing the observed proportion of participants reaching a CHIKV µPRNT antibody level ≥150 at Day 29 to a fixed lower bound of 70% (the Clopper-Pearson exact 95% confidence interval was used for that purpose). This is in principle acceptable, however, a comparison between groups would have been preferred. According analyses were provided based on Fisher's exact test, which is acceptable.

The cut-off to define seronegativity at baseline was more conservative in the pivotal trial VLA1553-301 (µPRNT50 <20). The MAH was recommended to provide the primary analysis for study VLA1553-321 with a definition of baseline CHIKV seronegative using the cut-off of 20 µPRNT50.

A descriptive exploratory analysis of all occurrences of definite, probable, and asymptomatic CHIKV cases conducted. As explained above, only the definite cases should be considered, given the potential bias related to the difficulty of discriminating seroconversion due to vaccination vs. wt CHIKV exposure in the VLA1553 group. The proportion of subjects with CHIKV cases was compared between the study arms by Fisher's exact test and exact 95% confidence intervals were calculated.

Overall, statistical methods planned to evaluate secondary and exploratory objectives are considered acceptable.

Planned Data Analysis of the Study:

The following data analyses will be performed:

➢ Part A includes safety and immunogenicity data after all subjects have completed Visit 3 (Day 29).

➢ Part B includes safety and immunogenicity data after all subjects have completed Visit 5 (Month 6).

➢ Part C includes safety and immunogenicity data after all subjects in the immunogenicity subset have completed Visit 6 (Month 12).

A Clinical Study Report will be compiled following each data analysis.

Study assessments

Assessment of immunogenicity:

Immunogenicity assessments are overall similar to those of the pivotal study VLA1553-301.

Neutralizing antibodies were assessed (using µPRNT) on samples collected at Day 1, 8, 29, 85, Month 6 and Month 12 after a single immunization. Day 1 samples will be analysed for all participants, whereas the other samples collected, i.e. Day 8, 29, 85, Month 6 and Month 12, will only be analysed in the immunogenicity subset.

Neutralizing antibodies are measured by using a validated micro Plaque Reduction Neutralization (µPRNT) assay (Nexelis), as for the adult VLA1553-301 and VLA1553-302 studies.

CHIKV-specific IgM and IgG were assessed by ELISA in screening samples (Day 0) from all participants. Mayaro virus-specific ELISA antibodies were assessed in Day 1 samples from all participants. Pre-existing antibodies specific for additional alphaviruses could also be performed. Assessment Dengue virus and Zika virus ELISA antibodies were also performed in Day 1 samples from all participants.

CHIKV Case Ascertainment and classification

Following the end of the study, an attempt will be made to classify CHIKV infections into acute, post-acute and chronic stage of disease. The classification of these stages will be done based on the duration of symptoms. The acute stage is defined by symptoms until the first three weeks after onset of illness. The post-acute stage is defined as having symptoms until the end of the third month. The chronic stage is defined by symptoms that persist for more than three months.

Definite CHIKV Case

Any of the cluster of clinical manifestations of CHIKV events observed:

1. Fever (≥ 37.8°C measured axillary);

AND

2. Acute (poly)arthralgia/arthritis, myalgia, neurological symptoms (e.g. meningoencephalitis, acute encephalitis, headache, seizures), retinitis/uveitis;

OR

One or more of the following signs and symptoms: macular to maculopapular rash (sometimes with cutaneous pruritus (foot plant)), pigmentary changes, bullous rash/skin blistering, purpura and ecchymosis;

AND confirmatory laboratory tests:

➢ A retrospective confirmatory CHIKV-specific quantitative RNA detection (RT-qPCR) will be performed for all samples collected at acute and if applicable at convalescent visits.

Probable CHIK case

Cases of clinical manifestations suggestive of CHIK that could not be confirmed by RT-qPCR were classified as probable CHIK cases based on CHIKV µPRNT seroconversion. Seroconversion was defined as a >4-fold increase of µPRNT50 antibody titres in acute and/or convalescent samples compared to baseline (Day 1).

The same definition was used for both µPRNT baseline negative and positive participants.

Seroconversion in the Placebo arm is likely to reflect natural infection in participants who are baseline seronegative, while it is uncertain for participants who are baseline seropositive. It is not known if

natural reinfection could induce an increase of 4-fold in antibody titres. This might depend of the baseline antibody titres.

For the VLA1553 arm, the MAH considers that after approximately 2-3 months after vaccination a more than 4-fold increase of µPRNT50 titre could indicate natural exposure to circulating virus. This reasoning is not followed, as it is not properly justified. In fact, the MAH does not provide a clear definition of probable cases for the VLA1553 arm. Overall, it is not considered possible to robustly ascertain cases based on seroconversion in the VLA1553 arm.

In addition, this definition does not allow to discriminate seroconversion due to vaccination vs wt CHIKV exposure.

In addition, antibodies specific to other alphaviruses may cross-neutralize CHIKV and be detected by the assay. In the absence of Rt-PC it might be difficult to distinguish a response to CHIKV from a seroresponse to Mayaro virus if the virus was co-circulating in the areas of Brazil where the study was conducted. Hence, for the purpose of this assessment, only the definite cases are considered, given the potential bias related to the difficulty of discriminating seroconversion due to vaccination vs. wt CHIKV exposure in the VLA1553 group.

CHIKV Infection Visits (Acute Visit and Convalescent Visit)

Participants were instructed to report any fever to the Investigator within seven days of onset. However, this is neither described in procedures corresponding to the scheduled Visits and nor in the table describing the schedule of activities. There is also no reminder (by phone for ex.) planned per protocol. Therefore, there was actually no active surveillance of potential chikungunya cases, and cases might have been largely under-detected.

The Investigator, if made aware of the event, referred participants with clinical signs/symptoms suggestive of an acute CHIKV disease to a clinical expert for clinical evaluation. An acute visit was performed (preferably within seven days of illness onset), as well as a convalescent visit three weeks after. During these visits a blood sample was collected for quantitative CHIKV RT-qPCR and for CHIKV µPRNT assessment. In addition, for diagnosis purposes, samples were collected at the acute visit for CHIKV, Zika and Dengue RT-PCR and CHIKV, Zika and Dengue antibody detection by ELISA (IgG and IgM) at the local laboratory for diagnosis purpose. At the convalescent visit, samples were collected for potential CHIKV, Zika and Dengue antibody detection by ELISA (IgG only).

All CHIKV infections and clinical manifestations of CHIK were discussed and assessed by the Sponsor and subsequently presented to an independent Data Safety Monitoring Board. The MAH does not explain why the sponsor assessed the events before the DSMB. This is not appropriate, unless properly justified, as this could have affected the independent assessment by the DSMB. Considering the low number of cases, the issue is not pursued. A proper description and justification are expected in Part C report.

Suspected cases of CHIKV infection/disease were classified into four different categories (definite, probable, asymptomatic, unconfirmed). The MAH refers to PAHO/CDC 2011 and Simon et al. 2015 for the classification.

Results

Conduct of the study

Important/not-important protocol deviations were defined according to the ICH E3 guideline, as follows: 'Important Protocol Deviations are a subset of Protocol Deviations that might significantly affect the completeness, accuracy, and/or reliability of the trial data or that might significantly affect a participant's rights, safety, or well-being.' The MAH uses the terms "protocol violation" and "protocol deviation" synonymously in the VLA1553-321 trial protocol. Both terms refer to any deviation from the protocol.

Protocol deviations were further classified into major or minor protocol deviations/violations based on their possible impact on the immune response. Major deviations were those which led to exclusion from the PP analysis set. As described in the protocol, some conditions were pre-defined as major protocol deviations (protocol deviations that were reasonably foreseeable based on previous vaccine trial experiences). However, any other (unspecified) protocol deviation identified as per the discretion of the sponsor team which may impact the immune response could be categorised as major on a case-by-case basis, and hence have led to exclusion from the PP population .In practice, only important protocol deviations could be classified as major (all not-important protocol deviations were classified as minor).

In the Safety population, any important protocol deviations were reported for 484/754 (64.2%) 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' (Visit 3 being performed out of window) and 3 participants had a deviation in the category 'inclusion criteria' (IgG-/IgM+ CHIKV specific ELISA at screening). Important minor protocol deviations were identified for 482/754 (63.9%) participants.

In the IMM population, any important protocol deviations were reported for 256/384 (66.7%) participants, i.e. a similar proportion as in the overall Safety population. Important major protocol deviations were identified for 33/384 (8.6%) participants (25 [7.6%] in the VLA1553 arm and 8 [14.3%] in the placebo arm).

The 33 participants with major protocol deviations correspond to those described in the safety population (i.e., 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 (most frequently issues related to the informed consent or diary completion) were identified for 254/384 (66.1%) participants, which is again a similar proportion as in the overall Safety population.

No EMA inspection was performed.

Participant flow and numbers analysed

The tables and figure below present the Analysis Sets and the disposition of participants, by trial arm and stratified by µPRNT baseline serostatus.

Table 4. Participant Analysis Sets (All Screened Participants) – Stratified by Trial Arm

	Stratum: Total		
Analysis Set	VLA1553	Placebo	Total
Participants Screened [n]			941
Participants Randomized [n]	510	255	765
Participants Vaccinated [n (%)[a]]	502 (98.4)	252 (98.8)	754 (98.6)
Participants Randomized but Not Vaccinated [n (%)[a]]	8 (1.6)	3 (1.2)	11 (1.4)
Included in Immunogenicity Subset [n (%)[a]]	335 (65.7)	57 (22.4)	392 (51.2)
Participants Vaccinated in Immunogenicity Subset [n (%)[a]]	328 (64.3)	56 (22.0)	384 (50.2)
Participants included in Viremia Subset [n (%)[a]]	53 (10.4)	25 (9.8)	78 (10.2)
Participants Vaccinated in Viremia Subset [n (%)[a]]	52 (10.2)	24 (9.4)	76 (9.9)
Safety Population [n][b]	502	252	754
Immunogenicity Population (IMM) [n (%)[c]]	328 (65.3)	56 (22.2)	384 (50.9)
Per Protocol Population (PP) [n (%)[c]]	303 (60.4)	48 (19.0)	351 (46.6)
Modified Per Protocol Population (mPP) [n (%)[c]]	297 (59.2)	47 (18.7)	344 (45.6)

COVID-19=coronavirus disease 19; IMM=Immunogenicity Population; µPRNT=micro plaque reduction neutralization test; mPP=Modified Per Protocol Population; n=number of participants; PP= Per Protocol Population

[a] Percentage of all randomized participants.

[b] Percentages were not included because participants were grouped according to the treatment actually received and not randomized treatment.

[c] Percentage of Safety population. Participants in PP population were grouped according to the treatment actually received.

There were 176 screen failure participants who were screened but not randomized.

The immunogenicity subset is defined as all participants who were initially enrolled into the immunogenicity evaluation group, regardless of any other factors.

The viremia subset is defined as participants from the immunogenicity subset with viremia samples analyzed from Visit 1 and Visit 2, (and Visit 3, if V2 sample was positive) who were initially enrolled into the immunogenicity and viremia evaluation group.

The safety analysis population contains all participants who entered into the trial and received one vaccination.

IMM population is defined to include all randomized and vaccinated participants of the immunogenicity subset who had evaluable µPRNT antibody titer results at baseline and at least one post-baseline titer measurement after vaccination.

The PP contains all IMM participants who had no major protocol deviations that could impact immune response.

One randomized participant (REC01-008) had no µPRNT sample collected. This impacts all stratified tables with µPRNT baseline serostatus.

One randomized participant (SAO06-001) had a delayed vaccination due to COVID-19 infection, but did not complete Visit 1 sampling on Day 1. A µPRNT result for Visit 1 was taken on the day of randomization (Day -13) but no sample collection at Day 1 due to COVID-19 infection. The sample collected on the date of randomization was used to determine the µPRNT serostatus (baseline=last non-missing result prior to or on the first dose).

Table 5. Participant Analysis Sets (All Screened Participants) – Stratified by µPRNT Baseline Serostatus

	Stratum: Seronegative by µPRNT			Stratum: Seropositive by µPRNT		
Analysis Set	VLA1553	Placebo	Total	VLA1553	Placebo	Total
Participants Screened [n]			614			139
Participants Randomized [n]	408	206	614	94	45	139
Participants Vaccinated [n (%)[a]]	408 (100)	206 (100)	614 (100)	94 (100)	45 (100)	139 (100)
Participants Randomized but Not Vaccinated [n (%)[a]]	0	0	0	0	0	0
Included in Immunogenicity Subset [n (%)[a]]	268 (65.7)	48 (23.3)	316 (51.5)	60 (63.8)	8 (17.8)	68 (48.9)
Participants Vaccinated in Immunogenicity Subset [n (%)[a]]	268 (65.7)	48 (23.3)	316 (51.5)	60 (63.8)	8 (17.8)	68 (48.9)
Participants included in Viremia Subset [n (%)[a]]	43 (10.5)	20 (9.7)	63 (10.3)	9 (9.6)	4 (8.9)	13 (9.4)
Participants Vaccinated in Viremia Subset [n (%)[a]]	43 (10.5)	20 (9.7)	63 (10.3)	9 (9.6)	4 (8.9)	13 (9.4)
Safety Population [n][b]	408	206	614	94	45	139
Immunogenicity Population (IMM) [n (%)[c]]	268 (65.7)	48 (23.3)	316 (51.5)	60 (63.8)	8 (17.8)	68 (48.9)
Per Protocol Population (PP) [n (%)[c]]	251 (61.5)	42 (20.4)	293 (47.7)	52 (55.3)	6 (13.3)	58 (41.7)
Modified Per Protocol Population (mPP) [n (%)[c]]	245 (60.0)	41 (19.9)	286 (46.6)	52 (55.3)	6 (13.3)	58 (41.7)

COVID-19=coronavirus disease 19; IMM=Immunogenicity Population; µPRNT=micro plaque reduction neutralization test; mPP=Modified Per Protocol Population; n=number of participants

[a] Percentage of all randomized participants.

[b] Percentages were not included because participants were grouped according to the treatment actually received and not randomized treatment.

[c] Percentage of Safety population. Participants in PP population were grouped according to the treatment actually received.

There were 176 screen failure participants who were screened but not randomized.

The immunogenicity subset is defined as all participants who were initially enrolled into the immunogenicity evaluation group, regardless of any other factors.

The viremia subset is defined as participants from the immunogenicity subset with viremia samples analyzed from Visit 1 and Visit 2, (and Visit 3, if V2 sample was positive) who were initially enrolled into the immunogenicity and viremia evaluation group.

The safety analysis population contains all participants who entered into the trial and received one vaccination.

IMM population is defined to include all randomized and vaccinated participants of the immunogenicity subset who had evaluable µPRNT antibody titer results at baseline and at least one post-baseline titer measurement after vaccination.

The PP contains all IMM participants who had no major protocol deviations that could impact immune response.

One randomized participant (REC01-008) had no µPRNT sample collected. This impacts all stratified tables with µPRNT baseline serostatus.

One randomized participant (SAO06-001) had a delayed vaccination due to COVID-19 infection but did not complete Visit 1 sampling on Day 1. A µPRNT result for Visit 1 was taken on the day of randomization (Day -13) but no sample collection at Day 1 due to COVID-19 infection. The sample collected on the date of randomization was used to determine the µPRNT serostatus (baseline=last non-missing result prior to or on the first dose).

Source: Table 14.1.1.1, Listing 16.2.3.1

Table 6. Participant Disposition (Safety Population)

Variable Reason [n (%)]	Stratum: Total VLA1553 (N=502)	 Placebo (N=252)	 Total (N=754)
Completed Trial (Month 6)[a]	166 (33.1)	191 (75.8)	357 (47.3)
Reached Day 29	500 (99.6)	250 (99.2)	750 (99.5)
Reached Month 6	488 (97.2)	246 (97.6)	734 (97.3)
Ongoing after Part B (Month 6)[b]	319 (63.5)	55 (21.8)	374 (49.6)
Discontinued Trial	17 (3.4)	6 (2.4)	23 (3.1)
Withdrawal by Participant	6 (1.2)	3 (1.2)	9 (1.2)
Adverse Event	0	0	0
Lost to Follow-Up	10 (2.0)	3 (1.2)	13 (1.7)
Investigator Decision	0	0	0
Trial Terminated by Sponsor	0	0	0
Protocol-Specified Withdrawal Criterion Met	0	0	0
Trial Site Terminated by Sponsor	0	0	0
Death	0	0	0
Other	1 (0.2)	0	1 (0.1)
Withdrawal By Parents	1 (0.2)	0	1 (0.1)

CRF=case report form; µPRNT=micro plaque reduction neutralization test; n=number of participants
[a] For non immunogenicity participants only
[b] For immunogenicity participants only
Note: Reasons for discontinuation are based on the End of Trial/Early Termination CRF page.
All immunogenicity participants who reached Month 6 were ongoing in the trial at their individual Part B cut-off.
Ongoing after Part B includes all participants who have completed Part B and have not discontinued at the Part B cut-off.
Discontinued trial includes all participants who discontinued prior to the Visit 5 (Month 6) cut-off for Part B.
Source: Table 14.1.1.3, Listing 16.2.1.2

Table 7. Participant Disposition (Safety Population) – Stratified by µPRNT Baseline Serostatus

Variable Reason [n (%)]	Stratum: Seronegative by µPRNT			Stratum: Seropositive by µPRNT		
	VLA1553 (N=408)	Placebo (N=206)	Total (N=614)	VLA1553 (N=94)	Placebo (N=45)	Total (N=139)
Completed Trial (Month 6)[a]	134 (32.8)	155 (75.2)	289 (47.1)	32 (34.0)	35 (77.8)	67 (48.2)
Reached Day 29	406 (99.5)	204 (99.0)	610 (99.3)	94 (100)	45 (100)	139 (100)
Reached Month 6	397 (97.3)	202 (98.1)	599 (97.6)	91 (96.8)	43 (95.6)	134 (96.4)
Ongoing after Part B (Month 6)[b]	260 (63.7)	47 (22.8)	307 (50.0)	59 (62.8)	8 (17.8)	67 (48.2)
Discontinued Trial	14 (3.4)	4 (1.9)	18 (2.9)	3 (3.2)	2 (4.4)	5 (3.6)
Withdrawal by Participant	5 (1.2)	3 (1.5)	8 (1.3)	1 (1.1)	0	1 (0.7)
Adverse Event	0	0	0	0	0	0
Lost to Follow-Up	8 (2.0)	1 (0.5)	9 (1.5)	2 (2.1)	2 (4.4)	4 (2.9)
Investigator Decision	0	0	0	0	0	0
Trial Terminated by Sponsor	0	0	0	0	0	0
Protocol-Specified Withdrawal Criterion Met	0	0	0	0	0	0
Trial Site Terminated by Sponsor	0	0	0	0	0	0
Death	0	0	0	0	0	0
Other	1 (0.2)	0	1 (0.2)	0	0	0
Withdrawal By Parents	1 (0.2)	0	1 (0.2)	0	0	0

CRF=case report form; µPRNT=micro plaque reduction neutralization test; n=number of participants
[a] For non immunogenicity participants only
[b] For immunogenicity participants only
Note: Reasons for discontinuation are based on the End of Trial/Early Termination CRF page.
All immunogenicity participants who reached Month 6 were ongoing in the trial at their individual Part B cut-off.
Ongoing after Part B includes all participants who have completed Part B and have not discontinued at the Part B cut-off.
Discontinued trial includes all participants who discontinued prior to the Visit 5 (Month 6) cut-off for Part B.
Source: Table 14.1.1.3, Listing 16.2.1.2

A total of 765 participants were randomized (versus approximately 750 planned per protocol). Of the 765 randomised participants, 11 were not vaccinated. Thus, 754 participants were vaccinated and therefore included in the Safety population (502 to VLA1553 and 252 to placebo).

A total of 753 participants from the Safety population were stratified by their µPRNT baseline serostatus: 614 participants to the CHIKV seronegative stratum (408 and 206 participants respectively to the VLA1553 and placebo arms) and 139 participants to the seropositive stratum (94 and 45 participants respectively to the VLA1553 and placebo arms). One Placebo participant (not pertaining to the Immunogenicity subset) did not have a µPRNT baseline serostatus result.

Of the 754 vaccinated participants, 750 (99.5%) reached Day 29 and 734 (97.3%) reached Month 6 (Day 180). In the Safety population, 33% vs. 76% of the participants respectively in the active vs. the placebo arms completed the trial in this part B analysis report. This imbalance may be due to the study design (as only immunogenicity subset participants continue the trial after Month 6) and does not raise concern as most of the participants in both arms completed the Day 180 Visit.

Visit 2 (Day 8) was completed by 749/754 (99.3%) participants, Visit 3 (Day 29) was completed by 747/754 (99.1%) participants, Visit 4 (Day 85) was completed by 737/754 participants (97.7%), and Visit 5 (Day 180) was completed by 734/754 participants (97.3%).

A total of 385 participants were to be included into the immunogenicity subset (335 in the VLA1553 arm and 50 in the Placebo arm). A total of 392 participants were included (335 in the VLA1553 arm and 57 in the Placebo arm).

The number of participants in the different analysis sets are summarized in the Table below, overall and stratified by µPRNT status.

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 (IMM)	328	56	384
Baseline seronegative	*268*	*48*	*316*
Baseline seropositive	*60*	*8*	*68*
Per Protocol population (PP)	303	48	351
Baseline seronegative	*251*	*42*	*293*
Baseline seropositive	*52*	*6*	*58*
Modified PP population (mPP)	297	47	344
Baseline seronegative	*245*	*41*	*286*
Baseline seropositive	*52*	*6*	*58*
Viraemia subset (and vaccinated)	53 (52)	25 (24)	78 (76)
Baseline seronegative	*43*	*20*	*63*
Baseline seropositive	*9*	*4*	*13*

Of the 392 participants included in the Immunogenicity subset, 384 were included in the IMM. In total, 8 participants were excluded from the IMM (considering the definition of the IMM, this was either because participants were unvaccinated and/or do not have an evaluable µPRNT antibody titre results at baseline and/or do not have at least one post-baseline titre measurement).

A total of 351 participants were included in the PP population, corresponding to 89.5% of the Immunogenicity subset (90.4% for the VLA1553 arm and 84.2% for the Placebo arm). The percentage of participants excluded from the PP population is acceptable.

According to the protocol, participants who had major protocol deviations that could affect the assessment of immune responses were excluded from the PP population. A similar approach was applied for VLA1553-321 as for the phase 3 trials in adults (VLA1553-301 and VLA1553-302). Protocol deviations were classified into major or minor based on their possible impact on the immune response. Major deviations were those which led to exclusion from the PP analysis set. As described in the protocol, some conditions were pre-defined as major protocol deviations (protocol deviations that were

reasonably foreseeable based on previous vaccine trial experiences). However, any other (unspecified) protocol deviation identified as per the discretion of the sponsor team which may impact the immune response could be categorised as major on a case-by-case basis and hence have led to exclusion from the PP population. Given that all decisions were made while the trial was fully blinded and before database lock, this approach is deemed acceptable. In practice, the most frequent reason for post-hoc classification of a protocol deviation into major was participants with Visit 3 (Day 29) out of window. According to the SAP, participants with Visit 3 (Day 29) out of window deviations of +/- 8 days were excluded from the PP population.

The MAH was asked to describe the protocol deviations that lead to exclusion of the PP population. Overall, of the 384 participants from the IMM, there were 33 (8.6%) participants with major protocol deviations (25 [7.6%] in the VLA1553 arm and 8 [14.3%] in the placebo arm). The majority of participants with major protocol deviations had deviations from the pre-defined Visit 3 (Day 29) window (23/25 in the VLA1553 arm and 7/8 in the placebo arm). In addition, 3 participants (2/25 in the VLA1553 arm and 1/25 in the placebo arm) had major protocol deviations in the category "inclusion criteria". These participants were tested IgM+/IgG- by CHIKV specific ELISA during the screening visit and were included in the trial in the baseline CHIKV seropositive stratum. According to trial protocol, participants who are IgM+/IgG- at screening do not qualify for participation in the trial (inclusion criterion number 4; VLA1553-321, trial protocol, v6.0, Section 13.1). Therefore, these participants were excluded from the PP population.

Among the 351 participants of the PP population, 293 and 58 were baseline CHIKV seronegative (μPRNT≤40) and seropositive (μPRNT >40), respectively. The percentage of baseline seropositive participants (16.5%) is thus lower than the 20% expected for the overall study population (according to predefined caps). In the safety population, there were 139 out of 753 participants with a μPRNT >40, corresponding to 18.5%.

According to the MAH, of the 10 sites involved in the study, 4 sites enrolled 100 or more participants. At these sites, the proportion of seropositive participants varied from 2% to 37%. All 6 other sites enrolled 12-86 participants (with a proportion of seropositive varying from 0% to 43%). The unequal distribution of baseline CHIKV serostatus most probably reflects the heterogeneous epidemiological context across Brazil.

Baseline data

Demographic characteristics of the Safety population and the PP population by trial arm and stratified by μPRNT baseline serostatus are presented in the tables below.

Table 8. Demographic and Baseline Characteristics (Safety Population) – Stratified by Trial Arm

Characteristic	Stratum: Total		
	VLA1553 (N=502)	Placebo (N=252)	Total (N=754)
Sex [n (%)]			
Female	269 (53.6)	137 (54.4)	406 (53.8)
Male	233 (46.4)	115 (45.6)	348 (46.2)
Race [n (%)]			
American Indian or Alaska Native	2 (0.4)	2 (0.8)	4 (0.5)
Asian	2 (0.4)	0	2 (0.3)
Black or African American	66 (13.1)	31 (12.3)	97 (12.9)
Multiracial	120 (23.9)	72 (28.6)	192 (25.5)
White	167 (33.3)	78 (31.0)	245 (32.5)
Other	145 (28.9)	69 (27.4)	214 (28.4)
Ethnicity [n (%)]			
Hispanic or Latino	358 (71.3)	172 (68.3)	530 (70.3)
Not Hispanic or Latino	140 (27.9)	79 (31.3)	219 (29.0)
Missing	4 (0.8)	1 (0.4)	5 (0.7)
Age (years)			
n	502	252	754
Mean (std)	14.5 (1.70)	14.4 (1.66)	14.5 (1.68)
Median	15.0	14.0	15.0
Q1, Q3	13.0, 16.0	13.0, 16.0	13.0, 16.0
Min, Max	12, 17	12, 17	12, 17

Age Group [n (%)]			
12 - <15 years	244 (48.6)	129 (51.2)	373 (49.5)
15 - <18 years	258 (51.4)	123 (48.8)	381 (50.5)
Weight (kg)			
n	502	252	754
Mean (std)	57.47 (15.442)	58.22 (15.111)	57.72 (15.326)
Median	54.30	56.25	55.00
Q1, Q3	46.90, 64.10	48.15, 65.70	47.20, 64.70
Min, Max	26.3, 145.0	30.0, 121.0	26.3, 145.0
Height (cm)			
n	502	252	754
Mean (std)	162.5 (9.04)	162.9 (8.64)	162.6 (8.90)
Median	162.0	163.0	162.0
Q1, Q3	156.0, 168.0	158.0, 169.0	156.0, 169.0
Min, Max	136, 190	140, 191	136, 191
BMI (kg/m^2)[a]			
n	502	252	754
Mean (std)	21.630 (4.8094)	21.777 (4.7538)	21.679 (4.7882)
Median	20.365	20.870	20.570
Q1, Q3	18.290, 23.980	18.400, 24.165	18.330, 24.020
Min, Max	13.86, 45.19	14.01, 44.44	13.86, 45.19
Serostatus according to µPRNT assay [n (%)]			
Seronegative (µPRNT50 ≤40)	408 (81.3)	206 (81.7)	614 (81.4)
Seropositive (µPRNT50 >40)	94 (18.7)	45 (17.9)	139 (18.4)
Serostatus Strata According to ELISA from Baseline Stratification [n (%)]			
Seronegative	405 (80.7)	202 (80.2)	607 (80.5)
Seropositive/Borderline	97 (19.3)	50 (19.8)	147 (19.5)
Serostatus Strata According to ELISA Baseline Laboratory Results[b] [n (%)]			
Seronegative (IgM-/IgG-)	400 (79.7)	198 (78.6)	598 (79.3)
Seropositive	102 (20.3)	54 (21.4)	156 (20.7)
IgM+/IgG+	47 (9.4)	24 (9.5)	71 (9.4)
IgM-/IgG+	46 (9.2)	22 (8.7)	68 (9.0)
IgM+/IgG-	9 (1.8)	8 (3.2)	17 (2.3)

ELISA=enzyme-linked immunosorbent assay; Ig=immunoglobulin; µPRNT=Micro Plaque Reduction Neutralization Test; max=maximum; min=minimum; n=number of participants; Q=quartile; std=standard deviation

[a] BMI=body mass index

[b] For ELISA baseline laboratory results, a borderline IgG or IgM result is classed as positive.

Note: There were 9 participants incorrectly randomized to the seronegative stratum, although ELISA result was positive.

Explanation: 9 participants had seropositive/borderline baseline ELISA labs (i.e., they were incorrectly randomized to the seronegative stratum), of which:

1 participant was categorized as IgM+/IgG+ (ELISA IgM borderline, IgG+)

8 participants were categorized as IgM+/IgG- (ELISA IgM borderline, IgG-, who were erroneously enrolled)*

*Participants with IgM borderline/IgG- were mapped to IgM+/IgG-; however, they were allowed in the trial. Only participants with baseline IgM+/IgG- were incorrectly enrolled in the trial.

Table 9. Demographic and Baseline Characteristics (Safety Population) – Stratified by µPRNT Baseline Serostatus

Characteristic	Stratum: Seronegative by µPRNT			Stratum: Seropositive by µPRNT		
	VLA1553 (N=408)	Placebo (N=206)	Total (N=614)	VLA1553 (N=94)	Placebo (N=45)	Total (N=139)
Sex [n (%)]						
Female	230 (56.4)	111 (53.9)	341 (55.5)	39 (41.5)	25 (55.6)	64 (46.0)
Male	178 (43.6)	95 (46.1)	273 (44.5)	55 (58.5)	20 (44.4)	75 (54.0)
Race [n (%)]						
American Indian or Alaska Native	2 (0.5)	1 (0.5)	3 (0.5)	0	1 (2.2)	1 (0.7)
Asian	1 (0.2)	0	1 (0.2)	1 (1.1)	0	1 (0.7)
Black or African American	54 (13.2)	27 (13.1)	81 (13.2)	12 (12.8)	4 (8.9)	16 (11.5)
Multiracial	87 (21.3)	54 (26.2)	141 (23.0)	33 (35.1)	18 (40.0)	51 (36.7)
White	149 (36.5)	70 (34.0)	219 (35.7)	18 (19.1)	8 (17.8)	26 (18.7)
Other	115 (28.2)	54 (26.2)	169 (27.5)	30 (31.9)	14 (31.1)	44 (31.7)
Ethnicity [n (%)]						
Hispanic or Latino	295 (72.3)	138 (67.0)	433 (70.5)	63 (67.0)	33 (73.3)	96 (69.1)
Not Hispanic or Latino	109 (26.7)	67 (32.5)	176 (28.7)	31 (33.0)	12 (26.7)	43 (30.9)
Missing	4 (1.0)	1 (0.5)	5 (0.8)	0	0	0
Age (years)						
n	408	206	614	94	45	139
Mean (std)	14.4 (1.72)	14.4 (1.67)	14.4 (1.70)	15.0 (1.54)	14.5 (1.58)	14.8 (1.56)
Median	14.0	14.0	14.0	15.0	15.0	15.0
Q1, Q3	13.0, 16.0	13.0, 16.0	13.0, 16.0	14.0, 16.0	13.0, 15.0	14.0, 16.0
Min, Max	12, 17	12, 17	12, 17	12, 17	12, 17	12, 17
Age Group [n (%)]						
12 - <15 years	210 (51.5)	110 (53.4)	320 (52.1)	34 (36.2)	19 (42.2)	53 (38.1)
15 - <18 years	198 (48.5)	96 (46.6)	294 (47.9)	60 (63.8)	26 (57.8)	86 (61.9)
Weight (kg)						
n	408	206	614	94	45	139
Mean (std)	57.78 (16.071)	57.83 (14.548)	57.80 (15.565)	56.13 (12.331)	60.25 (17.546)	57.46 (14.296)
Median	54.60	56.50	55.20	53.65	55.60	54.40
Q1, Q3	46.65, 65.15	47.50, 65.40	46.80, 65.20	48.30, 61.10	49.40, 66.60	48.60, 63.00
Min, Max	26.3, 145.0	30.0, 113.0	26.3, 145.0	36.3, 121.0	31.7, 121.0	31.7, 121.0
Height (cm)						
n	408	206	614	94	45	139
Mean (std)	162.3 (9.38)	163.0 (8.68)	162.5 (9.15)	163.2 (7.37)	162.6 (8.63)	163.0 (7.78)
Median	162.0	163.0	162.0	162.0	161.0	162.0
Q1, Q3	155.0, 169.0	158.0, 169.0	156.0, 169.0	158.0, 167.0	158.0, 167.0	158.0, 167.0
Min, Max	136, 190	140, 191	136, 191	142, 190	142, 182	142, 190
BMI (kg/m^2)[a]						
n	408	206	614	94	45	139
Mean (std)	21.780 (5.0436)	21.642 (4.6387)	21.734 (4.9081)	20.976 (3.5708)	22.501 (5.2431)	21.470 (4.2273)
Median	20.520	20.830	20.580	20.145	21.190	20.550
Q1, Q3	18.175, 24.250	18.330, 24.010	18.230, 24.140	18.840, 22.510	19.910, 25.000	18.930, 23.310
Min, Max	14.04, 45.19	14.01, 40.50	14.01, 45.19	13.86, 35.35	15.31, 44.44	13.86, 44.44
Serostatus According to µPRNT Assay [n (%)]						
Seronegative (µPRNT50 ≤40)	408 (100)	206 (100)	614 (100)	0	0	0
Seropositive (µPRNT50 >40)	0	0	0	94 (100)	45 (100)	139 (100)
Serostatus Strata According to ELISA from Baseline Stratification [n (%)]						
Seronegative	400 (98.0)	202 (98.1)	602 (98.0)	5 (5.3)	0	5 (3.6)
Seropositive/Borderline	8 (2.0)	4 (1.9)	12 (2.0)	89 (94.7)	45 (100)	134 (96.4)
Serostatus Strata According to ELISA Baseline Laboratory Results[b] [n (%)]						
Seronegative (IgM-/IgG-)	396 (97.1)	198 (96.1)	594 (96.7)	4 (4.3)	0	4 (2.9)
Seropositive	12 (2.9)	8 (3.9)	20 (3.3)	90 (95.7)	45 (100)	135 (97.1)
IgM+/IgG+	1 (0.2)	0	1 (0.2)	46 (48.9)	23 (51.1)	69 (49.6)
IgM-/IgG+	2 (0.5)	0	2 (0.3)	44 (46.8)	22 (48.9)	66 (47.5)
IgM+/IgG-	9 (2.2)	8 (3.9)	17 (2.8)	0	0	0

ELISA=enzyme-linked immunosorbent assay; Ig=immunoglobulin; µPRNT=micro plaque reduction neutralization test; max=maximum; min=minimum; n=number of participants; Q=quartile; std=standard deviation

[a] BMI=body mass index

[b] For ELISA baseline laboratory results a borderline IgG or IgM result was classed as positive.

Note: There were 9 participants incorrectly randomized to seronegative strata although ELISA result is positive.

Explanation: Nine participants had seropositive/borderline baseline ELISA labs (i.e., they were incorrectly randomized to the seronegative stratum), of which:

1 participant was categorized as IgM+/IgG+ (ELISA IgM borderline, IgG+)

8 participants were categorized as IgM+/IgG- (ELISA IgM borderline, IgG-, who were erroneously enrolled). One randomized participant (REC01-008) had no µPRNT sample collected. This impacts all stratified tables with µPRNT baseline serostatus.

Table 10. Demographic and Baseline Characteristics (Per Protocol Population) – Stratified by Trial Arm

Characteristic	Stratum: Total VLA1553 (N=303)	Placebo (N=48)	Total (N=351)
Sex [n (%)]			
Female	157 (51.8)	26 (54.2)	183 (52.1)
Male	146 (48.2)	22 (45.8)	168 (47.9)
Race [n (%)]			
American Indian or Alaska Native	0	0	0
Asian	1 (0.3)	0	1 (0.3)
Black or African American	33 (10.9)	7 (14.6)	40 (11.4)
Multiracial	62 (20.5)	8 (16.7)	70 (19.9)
White	119 (39.3)	24 (50.0)	143 (40.7)
Other	88 (29.0)	9 (18.8)	97 (27.6)
Ethnicity [n (%)]			
Hispanic or Latino	231 (76.2)	36 (75.0)	267 (76.1)
Not Hispanic or Latino	68 (22.4)	11 (22.9)	79 (22.5)
Missing	4 (1.3)	1 (2.1)	5 (1.4)
Age (years)			
n	303	48	351
Mean (std)	14.5 (1.70)	14.6 (1.76)	14.5 (1.71)
Median	15.0	15.0	15.0
Q1, Q3	13.0, 16.0	13.0, 16.0	13.0, 16.0
Min, Max	12, 17	12, 17	12, 17
Age Group [n (%)]			
12 - <15 years	145 (47.9)	22 (45.8)	167 (47.6)
15 - <18 years	158 (52.1)	26 (54.2)	184 (52.4)
Weight (kg)			
n	303	48	351
Mean (std)	58.23 (16.294)	56.45 (13.371)	57.99 (15.920)
Median	55.70	55.93	55.75
Q1, Q3	48.20, 64.00	45.40, 62.35	48.00, 63.60
Min, Max	30.5, 145.0	36.8, 96.1	30.5, 145.0
Height (cm)			
n	303	48	351
Mean (std)	162.8 (9.40)	163.1 (8.56)	162.8 (9.28)
Median	162.0	163.5	162.0
Q1, Q3	156.0, 169.0	158.0, 167.5	156.0, 169.0
Min, Max	138, 190	141, 185	138, 190
BMI (kg/m^2)[a]			
n	303	48	351
Mean (std)	21.812 (4.8826)	21.078 (3.8982)	21.712 (4.7618)
Median	20.690	20.500	20.660
Q1, Q3	18.480, 23.830	18.170, 23.375	18.480, 23.820
Min, Max	13.86, 45.19	15.35, 30.12	13.86, 45.19

Serostatus according to µPRNT assay [n (%)]			
Seronegative ($\mu PRNT_{50}$ ≤40)	251 (82.8)	42 (87.5)	293 (83.5)
Seropositive ($\mu PRNT_{50}$ >40)	52 (17.2)	6 (12.5)	58 (16.5)
Serostatus strata according to ELISA from baseline stratification [n (%)]			
Seronegative	249 (82.2)	42 (87.5)	291 (82.9)
Seropositive/Borderline	54 (17.8)	6 (12.5)	60 (17.1)
Serostatus strata according to ELISA baseline laboratory results[b] [n (%)]			
Seronegative (IgM-/IgG-)	246 (81.2)	41 (85.4)	287 (81.8)
Seropositive	57 (18.8)	7 (14.6)	64 (18.2)
IgM+/IgG+	26 (8.6)	4 (8.3)	30 (8.5)
IgM-/IgG+	25 (8.3)	2 (4.2)	27 (7.7)
IgM+/IgG-	6 (2.0)	1 (2.1)	7 (2.0)

ELISA=enzyme-linked immunosorbent assay; Ig=immunoglobulin; µPRNT=Micro Plaque Reduction Neutralization Test; max=maximum; min=minimum; n=number of participants; Q=quartile; std=standard deviation

[a] BMI=Body Mass Index

[b] For ELISA baseline laboratory results, a borderline IgG or IgM result is classed as positive.

There were 4 participants incorrectly randomized to the seronegative stratum, although ELISA result was positive.

Table 11. Demographic and Baseline Characteristics (Per Protocol Population) – Stratified by Baseline µPRNT Serostatus

	Stratum: Seronegative by µPRNT			Stratum: Seropositive by µPRNT		
Characteristic	VLA1553 (N=251)	Placebo (N=42)	Total (N=293)	VLA1553 (N=52)	Placebo (N=6)	Total (N=58)
Sex [n (%)]						
Female	138 (55.0)	22 (52.4)	160 (54.6)	19 (36.5)	4 (66.7)	23 (39.7)
Male	113 (45.0)	20 (47.6)	133 (45.4)	33 (63.5)	2 (33.3)	35 (60.3)
Race [n (%)]						
American Indian or Alaska Native	0	0	0	0	0	0
Asian	0	0	0	1 (1.9)	0	1 (1.7)
Black or African American	26 (10.4)	6 (14.3)	32 (10.9)	7 (13.5)	1 (16.7)	8 (13.8)
Multiracial	49 (19.5)	7 (16.7)	56 (19.1)	13 (25.0)	1 (16.7)	14 (24.1)
White	106 (42.2)	21 (50.0)	127 (43.3)	13 (25.0)	3 (50.0)	16 (27.6)
Other	70 (27.9)	8 (19.0)	78 (26.6)	18 (34.6)	1 (16.7)	19 (32.8)
Ethnicity [n (%)]						
Hispanic or Latino	186 (74.1)	31 (73.8)	217 (74.1)	45 (86.5)	5 (83.3)	50 (86.2)
Not Hispanic or Latino	61 (24.3)	10 (23.8)	71 (24.2)	7 (13.5)	1 (16.7)	8 (13.8)
Missing	4 (1.6)	1 (2.4)	5 (1.7)	0	0	0
Age (years)						
n	251	42	293	52	6	58
Mean (std)	14.5 (1.74)	14.8 (1.79)	14.5 (1.75)	14.8 (1.50)	13.7 (1.21)	14.7 (1.50)
Median	15.0	15.0	15.0	15.0	13.5	15.0
Q1, Q3	13.0, 16.0	13.0, 16.0	13.0, 16.0	13.5, 16.0	13.0, 15.0	13.0, 16.0
Min, Max	12, 17	12, 17	12, 17	12, 17	12, 15	12, 17
Age Group [n (%)]						
12 - <15 years	125 (49.8)	18 (42.9)	143 (48.8)	20 (38.5)	4 (66.7)	24 (41.4)
15 - <18 years	126 (50.2)	24 (57.1)	150 (51.2)	32 (61.5)	2 (33.3)	34 (58.6)
Weight (kg)						
n	251	42	293	52	6	58
Mean (std)	58.79 (16.819)	57.82 (13.583)	58.65 (16.377)	55.57 (13.291)	46.88 (6.490)	54.67 (12.996)
Median	56.00	57.00	56.30	53.25	46.60	52.30
Q1, Q3	48.20, 65.10	48.90, 63.00	48.30, 64.40	48.10, 59.85	42.80, 51.70	46.90, 58.30
Min, Max	30.5, 145.0	36.8, 96.1	30.5, 145.0	36.3, 121.0	38.0, 55.6	36.3, 121.0

Height (cm)						
n	251	42	293	52	6	58
Mean (std)	162.7 (9.59)	163.9 (8.77)	162.9 (9.47)	163.1 (8.46)	157.2 (3.25)	162.5 (8.26)
Median	162.0	164.0	162.5	162.0	157.5	161.0
Q1, Q3	156.0, 170.0	159.0, 168.0	156.0, 169.0	157.5, 167.0	156.0, 158.0	157.0, 167.0
Min, Max	138, 188	141, 185	138, 188	142, 190	152, 162	142, 190
BMI (kg/m^2)[a]						
n	251	42	293	52	6	58
Mean (std)	22.035 (5.1093)	21.386 (4.0171)	21.942 (4.9667)	20.735 (3.4315)	18.927 (2.0422)	20.548 (3.3481)
Median	20.780	20.585	20.760	19.740	19.035	19.740
Q1, Q3	18.350, 24.270	18.510, 24.050	18.430, 24.230	18.915, 22.060	17.140, 20.710	18.820, 21.960
Min, Max	14.12, 45.19	15.35, 30.12	14.12, 45.19	13.86, 35.35	16.45, 21.19	13.86, 35.35
Serostatus according to µPRNT assay [n (%)]						
Seronegative ($\mu PRNT_{50} \leq 40$)	251 (100)	42 (100)	293 (100)	0	0	0
Seropositive ($\mu PRNT_{50} > 40$)	0	0	0	52 (100)	6 (100)	58 (100)
Serostatus strata according to ELISA from Baseline Stratification [n (%)]						
Seronegative	246 (98.0)	42 (100)	288 (98.3)	3 (5.8)	0	3 (5.2)
Seropositive/Borderline	5 (2.0)	0	5 (1.7)	49 (94.2)	6 (100)	55 (94.8)
Serostatus strata according to ELISA Baseline Laboratory Results[b] [n (%)]						
Seronegative (IgM-/IgG-)	243 (96.8)	41 (97.6)	284 (96.9)	3 (5.8)	0	3 (5.2)
Seropositive	8 (3.2)	1 (2.4)	9 (3.1)	49 (94.2)	6 (100)	55 (94.8)
IgM+/IgG+	0	0	0	26 (50.0)	4 (66.7)	30 (51.7)
IgM-/IgG+	2 (0.8)	0	2 (0.7)	23 (44.2)	2 (33.3)	25 (43.1)
IgM+/IgG-	6 (2.4)	1 (2.4)	7 (2.4)	0	0	0

ELISA=enzyme-linked immunosorbent assay; Ig=immunoglobulin; µPRNT=Micro Plaque Reduction Neutralization Test; max=maximum; min=minimum; n=number of participants; Q=quartile; std=standard deviation

[a] BMI=Body Mass Index

[b] For ELISA baseline laboratory results, a borderline IgG or IgM result is classed as positive.

There were 4 participants incorrectly randomized to the seronegative stratum, although ELISA result was positive.

Overall, in the Safety population, there were 53.8% females (and 46.2% males). Approximately half of the Safety population was 12-14 yeas (49.5%), and the other half was 15-17 years (50.5%). This was similar in the PP population, with 52.1% females (and 47.9% males), and 47.6% adolescents 12-14 years vs. 52.4% adolescents 15-17 years. Overall, there were no major differences in the demographic characteristics between the Safety population and the PP population.

Gender and age distributions were balanced between arms.

In the Safety and the PP populations, 139/753 (18.4%) and 58/351 (16.5%) of the participants respectively, were seropositive at baseline according to the µPRNT results (µPRNT50>40).

The protocol did not specify how the investigators had to classify participants with borderline IgM ELISA results at screening (IgM borderline/IgG- results). In general, these participants were classified as IgM-/IgG- by the investigators because there was no evidence of CHIKV circulation in the regions where the study was conducted at the time of enrolment. Therefore, these participants were considered as seronegative at screening for the purpose of stratification of the randomisation. However, according to the SAP, although participants with any borderline ELISA IgG and/or IgM result at screening could be enrolled, the result had to be classified as positive. This implies that according to the SAP, participants with IgM borderline/IgG- results at screening were to be classified as IgM+/IgG- at baseline.

According to randomization groupings, in the Safety population, 607/754 (80.5%) participants were ELISA seronegative and 147/754 (19.5%) were ELISA seropositive. However, 9 participants were incorrectly randomized as ELISA seronegative: 1 participant was IgM borderline/IgG+ (to be classified as IgM+/IgG+), and 8 participants were IgM borderline/IgG- (to be classified as IgM+/IgG-). Therefore, the actual numbers (percentages) of ELISA seronegative and seropositive participants were respectively 598/754 (79.3%) and 156/754 (20.7%). Of the 156 ELISA seropositive participants, 17 are IgM+/IgG-. Of these 17 participants, 3 were IgM+/IgG- at screening and were incorrectly enrolled into the trial. This is concordant with the 3 participants were excluded from the PP population for major

protocol deviations (category "inclusion criteria") because they were tested IgM+/IgG- by CHIKV-specific ELISA during the screening visit. The other 14 participants classified as IgM+/IgG- were IgM borderline/IgG- at baseline: 8 participants were incorrectly randomized as seronegative and each had a minor protocol deviation recorded because of this, and 6 participants were randomized as seropositive. Although IgM+/IgG-, these 6 latter participants were not excluded from the PP as the positive IgM status derived from a borderline result. This is acceptable. In addition, considering that for the statistical analyses, the baseline serostatus was defined based on µPRNT, there is no expected impact on the results.

In the PP population, the numbers (percentages) of ELISA seronegative and seropositive participants were respectively 291/351 (82.9%) and 60/351 (17.1%) according to randomisation grouping. After reclassification of the borderline results as explained above, the numbers (percentages) of ELISA seronegative and seropositive participants were respectively 287/351 (81.8%) and 64/351 (18.2%).

Of the 293 µPRNT seronegative participants (µPRNT50<40), 284 participants were also seronegative according to ELISA at screening, and 9 were seropositive according to ELISA at screening. Of these 9 participants, most (n=7) were participants with IgM+/IgG- results. Of the 58 µPRNT seropositive participants, 55 were also seropositive according to ELISA at screening, and only 3 were seronegative according to ELISA at screening (IgM-/IgG-).

In the Safety and the PP populations, as expected, the CHIKV seronegative participants (respectively n=614 and n=293) were younger than the CHIKV seropositive participants (n=139 and n=58) as evidenced by the higher proportion of participants in the age category 12-14 years in the seronegative (52.1% and 48.8% for safety and PP populations respectively), versus in the seropositive participants (38.1% and 41.4%).

An imbalance was observed for gender and age in the µPRNT CHIKV seropositive population. The proportion of female participants was lower in the VLA1553 arm compared to the placebo arm (41.5% for VLA1553 and 55.6% for placebo in the safety population). There was also a slight imbalance in terms of age, as 36.2% vs. 42.2% of the participants were in the 12-14 years category in the safety population. Similar trends were observed in the PP, however numbers of seropositive participants are very limited in the Placebo arm (n=6).

Prior and Concomitant Therapy:

In the Safety population, the use of concomitant medications (i.e. with a start or end date on or after date of vaccination) was slightly more frequent in the VLA1553 arm (73.7%) compared to the Placebo arm (64.7%).

The most common concomitant medications were analgesics (mainly metamizole and paracetamol), and those were more frequently used in the VLA1553 arm (55.2%) versus the Placebo arm (42.5%). NSAID (mainly Ibuprofen and Nimesulide) were used in respectively 17.7% and 18.7% in the VLA1553 and Placebo arms, and systemic antihistamines in respectively 12.5% and 13.9% in the VLA1553 and Placebo arms. Vaccines and antibacterials were used in approximately 8% of the participants in both arms. Overall, 4.2% of the participants received concomitant systemic steroids with suspected immunosuppressive activity (4.6% in the VLA1553 arm and 3.6% in the Placebo arm).

The frequency of use of concomitant medications was similar in the PP population compared to the Safety population. In the PP, there was an imbalance in the frequency of use of analgesics and anti-inflammatory products between study arms in the period from Day 1 post-vaccination to Day 29. For the period between day 30 and day 180, the proportions of participants who used analgesics and anti-inflammatory products were comparable between arms. The numerical imbalance was more marked

for the period between Day 1 and Day 7. Similar findings were observed in VLA1553-301 in adults. These findings reflect the medications used to treat symptoms of AEs.

Systemic corticosteroids were received as concomitant medications in 16/303 (5.3%) of the VLA1553 arm and 3/48 (6.3%) of the Placebo Arm. Immune sera/immunoglobulins (rabies antiserum) were received by two participants in the VLA1553 arm (not in the Placebo arm). It seems that in most cases these products were received in the period from Day 30 to Day 180.

No participants of the PP received immunosuppressants or antipsoriatics.

Exclusion from the PP based on use of concomitant medications:

In the protocol, it is specified in the section on concomitant medications that the following medications are not permitted, and any use of such medications was to be 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.

Despite the occurrence of protocol deviations in the category 'prohibited comedication, none of the participants were excluded from the PP population based on concomitant use of analgesics, anti-inflammatory products or suspected immunosuppressive products such as corticoids. ThreeVLA1553 participants had protocol deviations in the category 'prohibited comedication' due to the use of antipyretics as prophylactic treatment within 4 hours prior to and during the 72 hours after vaccination. However, this was not regarded as a reason for exclusion from the PP population since antipyretics were not regarded as a medication class that could impact the immune response. Similarly, anti-inflammatory products were not regarded as medications that could impact the immune response. This is considered acceptable.

Due to a lack of information, it is not fully clear why the PP population includes some participants who used systemic corticosteroids. However, considering that the numbers are low, that most of the cases took corticosteroids in the period following Day 29, and that no bias in favour of the vaccine can be generated, this issue is not pursued.

Post-hoc analysis exploring the impact of using concomitant medications on immune responses:

The MAH has submitted a post-hoc analysis exploring the impact of using concomitant medications on immune responses. The seroresponse rate (SRR) data are not considered useful, given the extremely high percentages achieved. The analysis does not suggest an impact of the use of analgesics or anti-inflammatory/anti-rheumatic products on GMTs induced by VLA1553, when drugs are used during the first week, or during the first month post-vaccination. Similar observations were made at MAA in adults (study VLA1553-301). Data were requested with respect to the impact of those medications when taken within 1 or 2 days after vaccination, for CHIKV seronegative participants from VLA1553-321, as well as from the adult studies (VLA1553-301 and VLA1553-302). Altogether the data do not suggest an influence of use anti-inflammatory/anti-rheumatic products within Day 1 and Day 3 on neutralizing antibody responses.

Vaccination History Against Relevant Traveler Diseases:

In the Safety population, 59/754 (7.8%) of the participants had an history of yellow fever vaccination. The frequency was similar in baseline CHIKV seropositive and seronegative participants. The distribution was balanced in the seronegative participants. However, there was an imbalance in the

seropositive participants (10.6% and 4.4% respectively in the VLA1553 and the Placebo arms), but numbers are limited (10/94 and 2/45).

In the PP population, 22/293 (7.5%) of the participants had an history of yellow fever vaccination.

Arbovirus antibody status at baseline:

Pre-existing antibodies to Mayaro, Dengue, and Zika viruses were assessed at baseline (Day 1) by ELISA.

In the VLA1553 and Placebo arms (Safety Population), respectively 18.6%, 43.1%, 32.3% and 18.5%, 47.0%, 28.9% of the participants were tested baseline seropositive by ELISA (IgG) for Mayaro, Dengue, and Zika viruses, respectively.

In the PP population who were CHIKV baseline seronegative (µPRNT50≤40), only 1.6% and 0.0%, tested baseline seropositive by ELISA for MAYV. In contrast, in the participants who were CHIKV baseline seropositive (µPRNT50>40), nearly all participants (94.2% and 100%) tested baseline seropositive by ELISA for MAYV.

In the PP population who were CHIKV baseline seronegative (µPRNT50≤40), 40.3% and 42.9% of the participants the VLA1553 and Placebo arms were baseline seropositive by ELISA for DENV. Those frequencies were higher in the CHIKV baseline seropositive (µPRNT50>40), as respectively 65.4% and 66.7%, tested baseline seropositive by ELISA for DENV.

In the PP population who were CHIKV baseline seronegative (µPRNT50≤40), 26.6% and 21.4% were tested baseline seropositive by ZIKV. In contrast, the frequencies were again higher in the PP population who were CHIKV baseline seropositive (µPRNT50>40), and 51.9% and 33.3% participants were also tested baseline seropositive by ELISA for ZIKV.

These observations could indicate common endemicity in some areas (as the vector is common) and/or cross-reactivities. The large overlap of seropositive results for Mayaro is consistent with the well-described cross-reactivities existing between the two alphaviruses. Cross-reactivities are less common between alphaviruses and flaviviruses.

Outcomes and estimation

Primary immunogenicity endpoint: to assess the proportion of subjects with a seroprotective CHIKV antibody level defined as µPRNT50 ≥150 for µPRNT baseline negative subjects 28 days post-vaccination.

A summary of the SRR for CHIKV-specific neutralizing antibodies at Day 29 by baseline µPRNT serostatus in the PP population is provided in the table below.

Table 12. Seroresponse Rate for CHIKV-Specific Neutralizing Antibodies at Day 29 for Baseline µPRNT Seronegative Participants (Per Protocol Population)

Statistic	Stratum: Seronegative by µPRNT VLA1553 (N=251)	Placebo (N=42)
Total[a] [n]	251	42
Participants with Seroresponse [n (%)]	248 (98.8)	1 (2.4)
95% CI for Seroresponse Rate	96.5, 99.8	0.1, 12.6
P-value[b]	<0.0001	>0.9999
Difference in Seroresponse Rate[c] (%)		
Difference	96.4	
95% CI	87.0, 99.1	
P-value[d]	<0.0001	

CHIKV=chikungunya virus; CI=confidence interval; n=number of participants; µPRNT=micro plaque reduction neutralization test; 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 H0: SRR ≤70% against the alternative H1: SRR >70% with a one-sided significance level of 2.5%.
[c] Difference, P-value, and associated confidence interval are presented for the VLA1553 arm minus the 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 $\mu PRNT_{50} \geq 150$.
Two-sided 95% exact (Clopper-Pearson) confidence interval is presented for the seroresponse rate and Chan-Zhang exact 95% confidence interval is presented for the difference in seroresponse rate.

The primary endpoint of the trial was met. At 28 days post-vaccination (Day 29), 98.8% (248/251) of the baseline CHIKV seronegative participants (µPRNT≤40) had an antibody titre of at least 150 µPRNT50 (95% CI: 96.5-99.8) in the VLA1553 arm (PP population). The LB of the 95% CI was well above the non-acceptance threshold of 70%. The proportion of participants with at least the threshold titre at Day 29 was 2.4% (1/42) in the Placebo arm (95% CI: 0.1-12.6; p<0.0001 for the difference of proportions across arms).

In total, 3 baseline CHIKV seronegative participants did not reach the threshold of 150 µPRNT50 in the VLA1553 arm. These participants did not respond at all to the vaccination.

Only 1 participant (participant 1) of the baseline CHIKV seronegative (µPRNT≤40) Placebo participants was reported as reaching the threshold of 150 µPRNT50 at Day 29.

In study VLA1553-321, baseline CHIKV seronegative participants were defined as participants with baseline µPRNT50≤40. This is not considered appropriate, especially for an endemic area. The same definition as in the pivotal trial VLA1553-301 (µPRNT50 <20) should have been used. The provided a post-hoc analysis of the primary endpoint, using the appropriate definition of baseline CHIKV seronegativity. In the PP population, 234 VLA1553 recipients and 35 placebo recipients were CHIKV baseline seronegative when defined as µPRNT50 <20, instead of 251 and 42 respectively when defined as µPRNT50≤40. In baseline CHIKV seronegative participants (defined as µPRNT50 <20) of the PP population, 98.7% (95% CI: 96.3-99.7) of the participants (231/234) in the VLA1553 group reached µPRNT50 ≥150 at Day 29. In the placebo group, only 1/35 participant reached at least the threshold. As concluded by the MAH, the results of the primary analysis are consistent for CHIKV baseline seronegative participants defined as baseline µPRNT50 <20 or baseline µPRNT50 ≤40.

Post-hoc analyses for double seronegative (µPRNT50 <20 and ELISA IgG-/IgM-) participants of the PP population (n=229 in the VLA1553 group, n=35 in the placebo group) were also performed. At Day 29, 99.1% (227/229 participants; 95% CI: 96.9-99.9) of baseline double seronegative VLA1553 participants reached at least the threshold, while 2.9% (1/35 participant; 95% CI: 0.1-14.9) of baseline double seronegative placebo participants reached at least the threshold.

The results of these post-hoc analyses of the primary endpoint with more stringent definitions of baseline seropositivity were thus similar to the results of the preplanned primary analysis.

Results were similar when the serostatus at baseline was determined by ELISA (performed at screening), instead of µPRNT. Results were also similar when participants with borderline ELISA results were excluded from the analysis population (modified PP population, excluding participants identified with borderline IgM and negative IgG ELISA results at screening).

Results obtained in the IMM population were similar, with 260/263 (98.9%; 95% CI: 96.7-99.8) of the baseline CHIKV seronegative participants reaching at least the threshold of µPRNT50 of 150 at Day 29. Of the Placebo participants 1/47 (2.1%; 95% CI: 0.1-11.3) reached the threshold.

Secondary immunogenicity endpoints:

1. *SRR for CHIKV-specific neutralizing antibodies at Day 29 by µPRNT baseline serostatus:*

A summary of the SRR for CHIKV-specific neutralizing antibodies at different timepoints by µPRNT baseline serostatus for the PP population is provided in the below table. Month 12 post-vaccination data will be provided with part C of the report.

Table 13. Seroresponse rate for CHIKV-specific neutralizing antibodies by Visit and Baseline µPRNT Serostatus (Per Protocol Population)

Table 14.2.1.5 Seroresponse rate for CHIKV-specific neutralizing antibodies by Visit and Baseline µPRNT Serostatus (Per Protocol Population)

	Stratum: Seronegative by µPRNT		Stratum: Seropositive by µPRNT		Stratum: Total	
Time Point[a] [n] Statistic	VLA1553 (N=251)	Placebo (N=42)	VLA1553 (N=52)	Placebo (N=6)	VLA1553 (N=303)	Placebo (N=48)
Visit 1 - Day 1	250	42	52	6	302	48
Subjects with Seroresponse [n (%)]	0	0	50 (96.2)	6 (100)	50 (16.6)	6 (12.5)
Difference in Seroresponse Rate[b] (%)						
Difference	0.0		-3.8		4.1	
Visit 2 - Day 8	245	42	51	5	296	47
Subjects with Seroresponse [n (%)]	14 (5.7)	0	49 (96.1)	5 (100)	63 (21.3)	5 (10.6)
95% CI for Seroresponse Rate	3.2, 9.4	0.0, 8.4	86.5, 99.5	47.8, 100.0	16.8, 26.4	3.5, 23.1
Difference in Seroresponse Rate[b] (%)						
Difference	5.7		-3.9		10.6	
95% CI	-4.3, 9.7		-16.1, 49.8		-3.4, 19.2	
p-value[c]	0.2350		>0.9999		0.1143	

CI = confidence interval; NC = non-calculable; µPRNT = Micro Plaque Reduction Neutralization Test.
a. Number of subjects with non-missing titers at the specified time point.
b. Differences, p-values and associated confidence intervals are presented for the VLA1553 arm minus Placebo treatment arm.
c. P-value from Fisher's Exact test.
Percentages are based on the number of subjects with non-missing titers at the visit.
Seroresponse is defined as µPRNT50 ≥ 150. Baseline seronegative subjects are those with baseline µPRNT50 ≤ 40, while baseline seropositive subjects are those with baseline µPRNT50 > 40.
Two-sided 95% exact (Clopper-Pearson) confidence interval is presented for the seroresponse rate and Chan-Zhang exact 95% confidence interval is presented for the difference in seroresponse rate.

Time Point[a] [n] Statistic	Stratum: Seronegative by µPRNT VLA1553 (N=251)	Stratum: Seronegative by µPRNT Placebo (N=42)	Stratum: Seropositive by µPRNT VLA1553 (N=52)	Stratum: Seropositive by µPRNT Placebo (N=6)	Stratum: Total VLA1553 (N=303)	Stratum: Total Placebo (N=48)
Visit 3 - Day 29	251	42	52	6	303	48
Subjects with Seroresponse [n (%)]	248 (98.8)	1 (2.4)	52 (100)	6 (100)	300 (99.0)	7 (14.6)
95% CI for Seroresponse Rate	96.5, 99.8	0.1, 12.6	93.2, 100.0	54.1, 100.0	97.1, 99.8	6.1, 27.8
Difference in Seroresponse Rate[b] (%)						
Difference	96.4		0.0		84.4	
95% CI	87.0, 99.1		NC		71.8, 92.5	
p-value[c]	<0.0001		NC		<0.0001	
Visit 4 - Day 85	240	41	49	6	289	47
Subjects with Seroresponse [n (%)]	238 (99.2)	0	48 (98.0)	6 (100)	286 (99.0)	6 (12.8)
95% CI for Seroresponse Rate	97.0, 99.9	0.0, 8.6	89.1, 99.9	54.1, 100.0	97.0, 99.8	4.8, 25.7
Difference in Seroresponse Rate[b] (%)						
Difference	99.2		-2.0		86.2	
95% CI	91.2, 99.9		-12.7, 44.4		73.8, 93.8	
p-value[c]	<0.0001		>0.9999		<0.0001	

CI = confidence interval; NC = non-calculable; µPRNT = Micro Plaque Reduction Neutralization Test.
a. Number of subjects with non-missing titers at the specified time point.
b. Differences, p-values and associated confidence intervals are presented for the VLA1553 arm minus Placebo treatment arm.
c. P-value from Fisher's Exact test.
Percentages are based on the number of subjects with non-missing titers at the visit.
Seroresponse is defined as µPRNT50 ≥ 150. Baseline seronegative subjects are those with baseline µPRNT50 ≤ 40, while baseline seropositive subjects are those with baseline µPRNT50 > 40.
Two-sided 95% exact (Clopper-Pearson) confidence interval is presented for the seroresponse rate and Chan-Zhang exact 95% confidence interval is presented for the difference in seroresponse rate.

Table 14.2.1.5 Seroresponse rate for CHIKV-specific neutralizing antibodies by Visit and Baseline µPRNT Serostatus (Per Protocol Population)

Time Point[a] [n] Statistic	Stratum: Seronegative by µPRNT VLA1553 (N=251)	Stratum: Seronegative by µPRNT Placebo (N=42)	Stratum: Seropositive by µPRNT VLA1553 (N=52)	Stratum: Seropositive by µPRNT Placebo (N=6)	Stratum: Total VLA1553 (N=303)	Stratum: Total Placebo (N=48)
Visit 5 - Day 180	234	39	46	6	280	45
Subjects with Seroresponse [n (%)]	232 (99.1)	0	45 (97.8)	6 (100)	277 (98.9)	6 (13.3)
95% CI for Seroresponse Rate	96.9, 99.9	0.0, 9.0	88.5, 99.9	54.1, 100.0	96.9, 99.8	5.1, 26.8
Difference in Seroresponse Rate[b] (%)						
Difference	99.1		-2.2		85.6	
95% CI	90.7, 99.9		-13.8, 45.2		72.8, 93.5	
p-value[c]	<0.0001		>0.9999		<0.0001	

CI = confidence interval; NC = non-calculable; µPRNT = Micro Plaque Reduction Neutralization Test.
a. Number of subjects with non-missing titers at the specified time point.
b. Differences, p-values and associated confidence intervals are presented for the VLA1553 arm minus Placebo treatment arm.
c. P-value from Fisher's Exact test.
Percentages are based on the number of subjects with non-missing titers at the visit.
Seroresponse is defined as µPRNT50 ≥ 150. Baseline seronegative subjects are those with baseline µPRNT50 ≤ 40, while baseline seropositive subjects are those with baseline µPRNT50 > 40.
Two-sided 95% exact (Clopper-Pearson) confidence interval is presented for the seroresponse rate and Chan-Zhang exact 95% confidence interval is presented for the difference in seroresponse rate.

2. Immune response as measured by CHIKV-specific neutralizing antibody titres post-vaccination

Table 14. GMTs for CHIKV-Specific Neutralizing Antibodies by Visit and Baseline µPRNT Serostatus (Per Protocol 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)	84.4 (496.65)	11.1 (5.43)	4517.9 (3683.45)	5211.6 (4544.17)	848.3 (2305.72)	564.3 (2103.00)
Median	10.0	10.0	3277.0	2602.0	10.0	10.0
Q1, Q3	10.0, 25.0	10.0, 10.0	2405.0, 5744.0	2570.0, 6212.0	10.0, 60.0	10.0, 10.0
Min, Max	10, 5439	10, 43	46, 19634	1916, 12758	10, 19634	10, 12758
n[a]	245	42	51	5	296	47
LS Mean (SE)[b]	17.58 (0.06)	10.56 (0.15)	3251.18 (0.14)	3993.28 (0.44)	244.49 (0.07)	159.21 (0.15)
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 (0.16)		0.81 (0.46)		1.54 (0.15)	
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	

	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 5 - Day 180						
n	234	39	46	6	280	45
Geometric Mean	1399.0	10.0	3504.7	4745.8	1626.8	22.7
95% CI for GM	1257.01, 1556.98	10.00, 10.00	2623.29, 4682.28	2837.32, 7938.13	1459.95, 1812.72	12.01, 43.06
Geometric std	2.30	1.00	2.65	1.63	2.51	8.37
Mean (std)	1892.0 (1863.23)	10.0 (0.00)	5323.8 (5988.23)	5168.3 (2053.15)	2455.8 (3210.24)	697.8 (1903.59)
Median	1447.5	10.0	3473.0	5248.5	1668.0	10.0
Q1, Q3	903.0, 2329.0	10.0, 10.0	2397.0, 5917.0	4502.0, 5869.0	950.0, 2716.5	10.0, 10.0
Min, Max	10, 21014	10, 10	123, 36679	1914, 8228	10, 36679	10, 8228
n[a]	234	39	46	6	280	45
LS Mean (SE)[b]	1398.98 (0.05)	10.00 (0.12)	3504.70 (0.14)	4745.84 (0.38)	2731.06 (0.08)	40.05 (0.16)
95% Confidence Interval[b]	1266.93, 1544.80	7.84, 12.75	2654.44, 4627.33	2198.71, 10243.75	2335.57, 3193.50	29.16, 55.00
Difference in GMT[b]						
Difference in LS Mean (SE)[c]	139.90 (0.13)		0.74 (0.41)		68.19 (0.16)	
95% Confidence Interval[c]	107.62, 181.86		0.33, 1.67		49.50, 93.95	
P-value[c]	<0.0001		0.4601		<0.0001	

CHIKV=chikungunya virus; CI=confidence interval; GM=geometric mean; GMT=geometric mean titer; LS=least squares; µPRNT=Micro Plaque Reduction Neutralization Test; NC=non-calculable; n=number of participants with available result; Q=quartile; SE=standard error; std=standard deviation

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

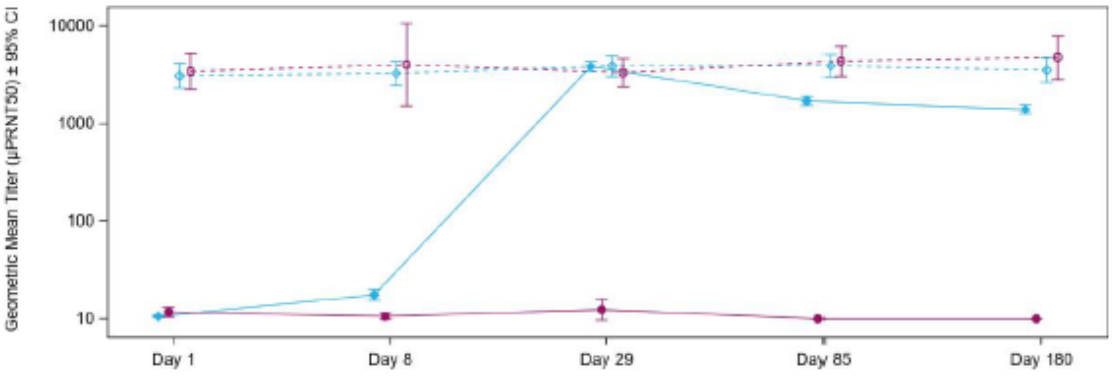
[b] LS means, standard errors, confidence intervals, and P-values are from an analysis of variance (ANOVA) model with fixed factors for trial arm and baseline $\mu PRNT_{50}$ serostatus strata. The models for the data within serostatus categories do not account for the baseline serostatus.

[c] P-values, LS mean differences, and associated confidence intervals are presented for the VLA1553 arm minus the 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. The SE value is not back-transformed and the log-transformed value is presented.

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.

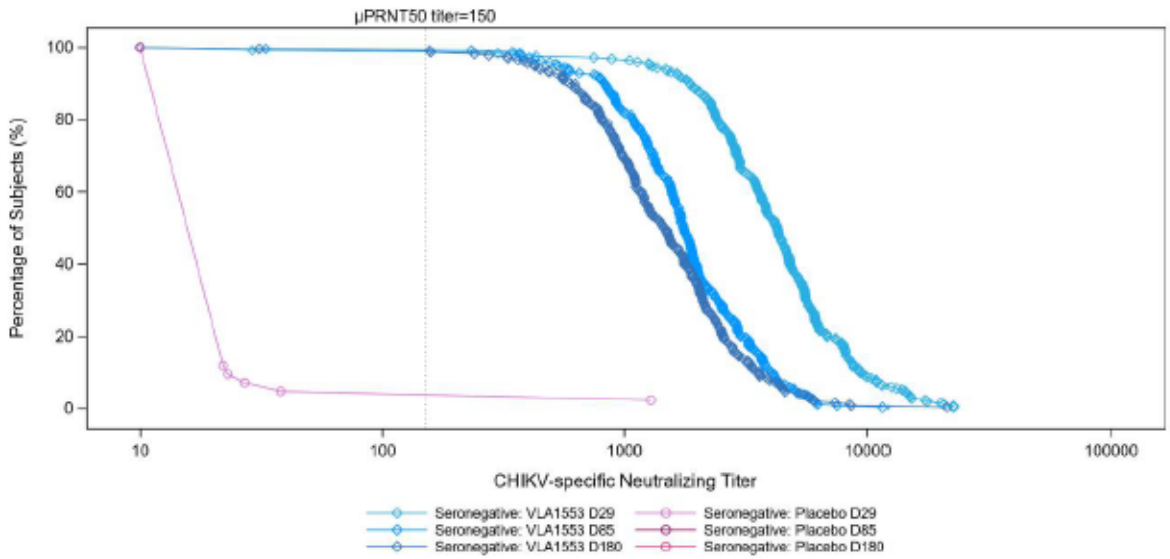
Figure 2. Line Plot of CHIKV-Specific Neutralizing Antibodies (GMT) by Trial Day, Trial Arm, and Baseline µPRNT Serostatus Strata, Using Logarithmic Scale (Per Protocol Population)



Seronegative: VLA1553	250	245	251	240	234
Seronegative: Placebo	42	42	42	41	39
Seropositive: VLA1553	52	51	52	49	46
Seropositive: Placebo	6	5	6	6	6

CI=confidence interval; GMT=geometric mean titer; µPRNT=Micro Plaque Reduction Neutralization Test.
Note: Counts below the chart show the number of participants in each arm at the time point.

Figure 3. Reverse Cumulative Distribution Curve of CHIKV-Specific Neutralizing Antibodies at Day 29, Day 85, and Day 180 by Trial Arm for Baseline µPRNT Seronegative Participants (Per Protocol population)



µPRNT=Micro Plaque Reduction Neutralization Test.
Note: Seroresponse is defined as $\mu PRNT_{50} \geq 150$ for all participants.

Table 15. Geometric Mean Titres for CHIKV-Specific Neutralizing Antibodies Per Time Point by Treatment Group for Subjects with <20 µPRNT50 at Baseline (Per-Protocol Population)

Table 10: Geometric Mean Titers for CHIKV-Specific Neutralizing Antibodies Per Time Point by Treatment Group for Subjects with <20 $\mu PRNT_{50}$ at Baseline (Per-Protocol Population) *(continued)*

	Statistic	VLA1553 (N=234)	Placebo (N=35)	Total (N=269)
Visit 3 - Day 29				
	Geometric Mean	3887.9	12.4	1840.0
	[95% CI] GM	[3436.8, 4398.1]	[9.3, 16.5]	[1420.6, 2383.2]
	SD(log10)	0.42	0.37	0.94
	Mean	5186.0	47.6	4517.4
	SD	3807.90	215.17	3951.18
	Median	4310.0	10.0	3782.0
	Q1 / Q3	2764.0 / 6114.0	10.0 / 10.0	2153.0 / 5746.0
	Min / Max	10 / 22339	10 / 1284	10 / 22339
	n	234	35	269
	n(log10)	234	35	269
Visit 4 - Day 85				
	Geometric Mean	1653.7	10.0	845.7
	[95% CI] GM	[1495.6, 1828.6]	[10.0, 10.0]	[672.8, 1063.1]
	SD(log10)	0.33	0.00	0.81
	Mean	2075.8	10.0	1804.6
	SD	1442.92	0.00	1515.33
	Median	1709.0	10.0	1590.0
	Q1 / Q3	1178.0 / 2539.0	10.0 / 10.0	867.0 / 2354.0
	Min / Max	10 / 11597	10 / 10	10 / 11597
	n	225	34	259
	n(log10)	225	34	259

n...number of subjects with non-missing result

Part 2/3

CHIKV-specific neutralizing antibody GMTs in baseline CHIKV seronegative participants:

Baseline GMTs (Day 1) were 10.6 (95% CI: 10.3-10.9) and 11.7 (95% CI: 10.5-13.2) 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). As already mentioned, it is considered that only participants with a µPRNT50 of <20 should have been included in the seronegative stratum.

At Day 8, GMT slightly increased but remained low, i.e. 17.6 (95% CI: 15.4-20.1) in baseline seronegative participants of the VLA1553. In the Placebo arm GMT was 10.6 (95% CI: 9.8-11.4). In the VLA1553 arm, Q3 was of 25.0 µPRNT50 and the maximum value was 5,439 µPRNT50. In the Placebo arm, Q3 was of 10.0 µPRNT50 and the maximum value was 43 µPRNT50. The difference between VLA1553-GMT and Placebo-GMT was significant (p=0.0019) but is not deemed clinically relevant.

At Day 29, GMT was high in baseline seronegative participants of the VLA1553 arm, with GMT value of 3855.9 (95% CI: 3432.1-4332.0). The minimum GMT was 10, reflecting the data from the 3 vaccinees who did not reach the threshold of 150 µPRNT50, who in fact did not respond at all to the vaccination (2 had µPRNT50<20 and 1 had µPRNT50=29 at Day 29). Day 29-GMT of the Placebo participants remained low, i.e. 12.3 (95% CI: 9.6-15.8), with a Q3 of 10.0 µPRNT50 and a maximum value of 1,284 µPRNT50 (as described above, one placebo participant has an antibody titres of at least 150 µPRNT50 at Day 29). The p-value for the comparison between both arms was <0.0001.

At Day 180, GMT was of 1399.0 (95% CI: 1257.0-1557.0) in the VLA1553 arm while it was 10.0 (95% CI: 10.0-10.0) in the Placebo arm.

Results obtained in the IMM population were similar.

Post-hoc analyses were conducted in the PP population with more stringent definitions of baseline seronegativity, and those suggest consistent results. In the VLA1553 arm, the GMT in baseline seronegative participants defined as µPRNT50<20 was 3887.9 (95% CI: 3436.8-4398.1) at Day 29 and 1382.0 (1237.1-1543.8) at Day 180. Post-hoc analyses were performed for double seronegative (µPRNT50 <20 and ELISA IgG-/IgM-) participants (n=229 in the VLA1553 arm, n=35 in the placebo arm). In the VLA1553 arm, the Day 29-GMT were of 4005.6 (95% CI: 3572.1-4491.7) and the Day 180-GMT were of 1387.0 (95% CI: 1238.8-1553.0), which is also similar to the results for baseline seronegative participants of the PP population with the µPRNT50 cut-off ≤40 (Table 15).

CHIKV-specific neutralizing antibody GMTs in baseline CHIKV seropositive participants:

Baseline GMTs (Day 1) were 3097.1 (95% CI: 2324.9-4125.9) and 3409.0 (95% CI: 2244.4-5178.1) in baseline seropositive participants of the VLA1553 (n=52) and Placebo arm (n=6), respectively. Overall, the minimum value was 71 (as explained below, 2 participants did not have antibody titres of at least 150 µPRNT50 at baseline) 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 3251.2 (95% CI: 2458.8-4299.0).

At Day 29, GMT in the VLA1553 arm was of 3886.5 (95% CI: 3063.4-4930.8) and of 3504.7 (95% CI: 2623.3-4682.3) at Day 180, thus in the same range as at Day 1 and Day 8 (Table 14).

Results obtained in the IMM population were similar.

Seroresponse in baseline CHIKV seropositive participants:

A total of 58 participants of the PP population were baseline CHIKV seropositive (defined as µPRNT50 >40), 52 in the VLA1553 arm and 6 in the Placebo arm. Almost all those participants had an antibody titre of at least 150 µPRNT50 at baseline (50/52 [96.2%] in the VLA1553 arm and 6/6 [100%] in the placebo arm). The 2 participants who did not have antibody titres of at least 150 µPRNT50 at baseline have antibody titres of 71 and 86 µPRNT50. All 52/52 and 6/6 participants of both arms had antibody titres above the threshold of 150 µPRNT50 at Day 29. (Table 14).

The two participants with titres below 150 at baseline had increased titres at post-vaccination timepoints, although magnitude of the increase was very different (respectively 6336 at Day 29 and 165 at Day 29, i.e. 89-fold and 2-fold increase from baseline). The response peaked at Day 29 and decreased/back to the pre-vaccination level at Day 180 (respectively 46 at Day 8, 6336 at Day 29, 2760 at Day 85, and 1441 at Day 180, and 127 at Day 8, 165 at Day 29, 105 at Day 85, and 123 at Day 180).

In response to requests, the MAH presented data on immune responses induced by VLA1553 in CHIKV seropositive participants, including graphical presentation of individual antibody levels over time (Figure 2, Figure 3).

Although all 52/52 participants had antibody titres above the threshold of 150 µPRNT50 at Day 29, only 2/52 participants seroconverted (defined as >4-fold increase of µPRNT50 compared to baseline). One was a participant with baseline µPRNT50 titre <150 and had an 89-fold increase of antibody level, as mentioned above. The other one had a baseline µPRNT50 titre of 1301 that increased to 5235 at Day 29 (4-fold increase from baseline).

In these 52 baseline CHIKV seropositive participants, the geometric fold increase (GMFI) of CHIKV-neutralizing antibody titres was of 1.3 (95% CI: 0.99, 1.60), and the median fold increase was only 1.05 at Day 29 after vaccination. (see below, section on GMFIs).

It might have been hypothesized that when baseline CHIKV antibody level is low, a booster effect might be seen, although the clinical relevance of this would remain unclear. This is however not observed in this study, as the vast majority of CHIKV seropositive participants had already high antibody titres at baseline and had no substantial increase of CHIKV-neutralizing antibody titres at Day 29 after VLA1553 group administration.

As explained by the MAH, this finding suggests that the vaccine virus is neutralized in the presence of CHIKV pre-existing neutralizing antibodies. This is also in line with the findings observed in the Phase 1 trial VLA1553-101. In this 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.

In trials VLA1553-301 and VLA1553-302, numbers of CHIKV baseline seropositive adult participants were limited as the studies were conducted in non-endemic areas (US). Individual curves for participants with CHIKV baseline µPRNT50 ≥20 suggest that neutralizing antibody titres can increase following VLA1553 vaccination in participants who are seropositive, when the baseline titre is in the lower range of values. This however was based on very limited numbers. For most of the participants in these studies, the neutralizing antibody titre did not increase.

Conclusion:

Thus, overall, in the participants who have no pre-existing immunity to CHIKV, findings of this study VLA1553-321 conducted in adolescents living in Brazil, where CHIKV has circulated/is circulating, are consistent with results of both VLA1553-301 and VLA1553-302 studies, conducted in adults leaving in non-endemic areas. Day 29-GMT was of 3361.6 (95% CI: 2993.8-3774.4) and of 2643.2 (95% CI: 2354.0-2967.9) in the VLA1553 arm (PP population CHIKV baseline seronegative) of study VLA1553-301 and of study VLA1553-302 (3 Lots combined), respectively. GMT of study VLA1553-321 is thus in the same range.

Neutralizing antibody titres induced by VLA1553 are comparable to the titres at Day 0 in seropositive individuals (i.e. titres elicited by the natural infection) at Day 29, but lower at the later timepoints. The time elapsed between the infection and the sampling is unknown, and thus the antibody level reached shortly after natural infection is unknown.

No substantial increase of CHIKV neutralizing antibody levels is observed after VLA1553 vaccination in most baseline CHIKV seropositive participants. Hence, in the vast majority of participants with pre-existing immunity to CHIKV, no booster effect is induced by VLA1553. Importantly, the added value of any booster effect in terms of clinical protection would remain unknown anyway, as natural infection is believed to induce long-term protection.

CHIKV-Specific Neutralizing Antibody responses by age, gender, baseline immunity to Mayaro, Dengue and Zika virus, and history of Yellow fever vaccination:

Post-hoc analyses of SRRs and GMTs suggest similar results across age categories (12-14 years and 15-17 years) and sex (Table 16,Table 17).

Table 16. Geometric Mean Titres for CHIKV-Specific Neutralizing Antibodies Per Time Point by Age Group for CHIKV Baseline Seronegative Participants

		Statistic	VLA1553 12-<15 years (N=125)	VLA1553 15-<18 years (N=126)	Placebo 12-<15 years (N=18)	Placebo 15-<18 years (N=24)
Visit 1 - Day 1						
		Geometric Mean	10.3	10.9	10.7	12.6
		[95% CI] GM	[10.0, 10.6]	[10.5, 11.5]	[9.3, 12.3]	[10.6, 15.0]
		n	125	125	18	24
Visit 2 - Day 8						
		Geometric Mean	16.5	18.8	10.8	10.4
		[95% CI] GM	[14.2, 19.1]	[15.1, 23.4]	[9.1, 12.9]	[9.6, 11.1]
		n	123	122	18	24
Visit 3 - Day 29						
		Geometric Mean	3659.6	4061.0	10.8	13.7
		[95% CI] GM	[3020.4, 4434.0]	[3549.6, 4646.1]	[9.2, 12.6]	[8.9, 20.9]
		n	125	126	18	24
Visit 4 - Day 85						
		Geometric Mean	1397.5	2065.6	10.0	10.0
		[95% CI] GM	[1201.5, 1625.4]	[1844.4, 2313.4]	[10.0, 10.0]	[10.0, 10.0]
		n	119	121	17	24
Visit 5 - Day 180						
		Geometric Mean	1307.3	1497.1	10.0	10.0
		[95% CI] GM	[1111.0, 1538.3]	[1301.0, 1722.7]	[10.0, 10.0]	[10.0, 10.0]
		n	117	117	17	22

Table 17. Geometric mean titres for CHIKV-specific neutralizing antibodies per time point by sex for CHIKV baseline seronegative participants (Per-Protocol Population)

		Statistic	VLA1553 Male (N=113)	VLA1553 Female (N=138)	Placebo Male (N=20)	Placebo Female (N=22)
Visit 1 - Day 1						
		Geometric Mean	10.8	10.4	12.9	10.8
		[95% CI] GM	[10.3, 11.3]	[10.1, 10.8]	[10.4, 16.0]	[9.7, 12.0]
		n	113	137	20	22
Visit 2 - Day 8						
		Geometric Mean	21.3	15.0	11.2	10.0
		[95% CI] GM	[16.8, 27.0]	[13.1, 17.2]	[9.4, 13.3]	[10.0, 10.0]
		n	110	135	20	22
Visit 3 - Day 29						
		Geometric Mean	4240.1	3567.3	11.1	13.5
		[95% CI] GM	[3598.1, 4996.6]	[3026.7, 4204.4]	[9.5, 13.0]	[8.5, 21.6]
		n	113	138	20	22
Visit 4 - Day 85						
		Geometric Mean	1642.1	1752.3	10.0	10.0
		[95% CI] GM	[1411.7, 1910.0]	[1543.7, 1989.1]	[10.0, 10.0]	[10.0, 10.0]
		n	108	132	19	22
Visit 5 - Day 180						
		Geometric Mean	1222.5	1558.3	10.0	10.0
		[95% CI] GM	[1046.1, 1428.6]	[1346.6, 1803.3]	[10.0, 10.0]	[10.0, 10.0]
		n	104	130	19	20

Post-hoc analyses of SRRs and GMTs stratified by pre-existing immunity to Mayaro, Dengue and Zika (Table 18, Table 19)viruses were presented in the PP population, as well as post-hoc analyses for stratified by yellow fever (YF) vaccination history .

Considering the very high percentages of participants with seroresponse in the VLA1553 arm (nearly all participants respond), it is not possible to assess the effect of pre-existing immunity to Mayaro, Dengue and Zika viruses or YF vaccination history on the SRR.

Presence of antibodies to CHIKV is highly correlated to presence of antibodies to Mayaro virus, most probably due to cross-reactivities. There are only 4 participants who are CHIKV seronegative at baseline and have preexisting antibodies to Mayaro virus. Therefore, it is not possible to assess the independent impact of pre-existing immunity to Mayaro virus on CHIKV GMT responses.

The data suggest that pre-existing immunity to Dengue and Zika does not impact the immune responses induced by VLA1553. Within participants who are baseline CHIKV seronegative and received VLA1553, participants with and without pre-existing antibodies to Dengue or Zika have comparable responses.

Yellow fever vaccination history does not impact the antibody responses to VLA1553. In CHIKV baseline seronegative participants from the VLA1553 arm, GMTs were similar for participants with and without YF vaccination history. Of note, number of individuals with history of YF vaccination in both the VLA1553 arm and the Placebo arm was limited.

Numbers of seropositive participants are too limited to conduct stratified analyses by presence of antibodies at baseline and by YF vaccination history.

3. Proportion of Participants with Seroresponse on Day 8, Day 85, and Day 180 Post-Vaccination

A summary of the SCR for CHIKV-specific neutralizing antibodies by visit and baseline µPRNT serostatus for the PP population is provided in the below Table and Figure. Results obtained for the IMM population were highly similar to those of the PP population.

Table 18. Seroresponse Rate for CHIKV-Specific Neutralizing Antibodies by Visit and Baseline µPRNT Serostatus (Per Protocol Population)

	Stratum: Seronegative by µPRNT		Stratum: Seropositive by µPRNT		Stratum: Total	
Time Point[a] [n] Statistic	VLA1553 (N=251)	Placebo (N=42)	VLA1553 (N=52)	Placebo (N=6)	VLA1553 (N=303)	Placebo (N=48)
Visit 1 - Day 1	250	42	52	6	302	48
Participants with Seroresponse [n (%)]	0	0	50 (96.2)	6 (100)	50 (16.6)	6 (12.5)
Difference in Seroresponse Rate[b] (%)						
Difference	0.0		-3.8		4.1	
Visit 2 - Day 8	245	42	51	5	296	47
Participants with Seroresponse [n (%)]	14 (5.7)	0	49 (96.1)	5 (100)	63 (21.3)	5 (10.6)
95% CI for Seroresponse Rate	3.2, 9.4	0.0, 8.4	86.5, 99.5	47.8, 100.0	16.8, 26.4	3.5, 23.1
Difference in Seroresponse Rate[b] (%)						
Difference	5.7		-3.9		10.6	
95% CI	-4.3, 9.7		-16.1, 49.8		-3.4, 19.2	
P-value[c]	0.2350		>0.9999		0.1143	
Visit 3 - Day 29	251	42	52	6	303	48
Participants with Seroresponse [n (%)]	248 (98.8)	1 (2.4)	52 (100)	6 (100)	300 (99.0)	7 (14.6)
95% CI for Seroresponse Rate	96.5, 99.8	0.1, 12.6	93.2, 100.0	54.1, 100.0	97.1, 99.8	6.1, 27.8
Difference in Seroresponse Rate[b] (%)						
Difference	96.4		0.0		84.4	
95% CI	87.0, 99.1		NC		71.8, 92.5	
P-value[c]	<0.0001		NC		<0.0001	
Visit 4 - Day 85	240	41	49	6	289	47
Participants with Seroresponse [n (%)]	238 (99.2)	0	48 (98.0)	6 (100)	286 (99.0)	6 (12.8)
95% CI for Seroresponse Rate	97.0, 99.9	0.0, 8.6	89.1, 99.9	54.1, 100.0	97.0, 99.8	4.8, 25.7
Difference in Seroresponse Rate[b] (%)						
Difference	99.2		-2.0		86.2	
95% CI	91.2, 99.9		-12.7, 44.4		73.8, 93.8	
P-value[c]	<0.0001		>0.9999		<0.0001	
Visit 5 - Day 180	234	39	46	6	280	45
Participants with Seroresponse [n (%)]	232 (99.1)	0	45 (97.8)	6 (100)	277 (98.9)	6 (13.3)
95% CI for Seroresponse Rate	96.9, 99.9	0.0, 9.0	88.5, 99.9	54.1, 100.0	96.9, 99.8	5.1, 26.8
Difference in Seroresponse Rate[b] (%)						
Difference	99.1		-2.2		85.6	
95% CI	90.7, 99.9		-13.8, 45.2		72.8, 93.5	
P-value[c]	<0.0001		>0.9999		<0.0001	

CHIKV=chikungunya virus; CI=confidence interval; µPRNT=Micro Plaque Reduction Neutralization Test; NC=non-calculable; n=number of participants

[a] Number of participants with non-missing titers at the specified time point.

[b] Differences, P-values, and associated CIs are presented for the VLA1553 arm minus the placebo treatment arm.

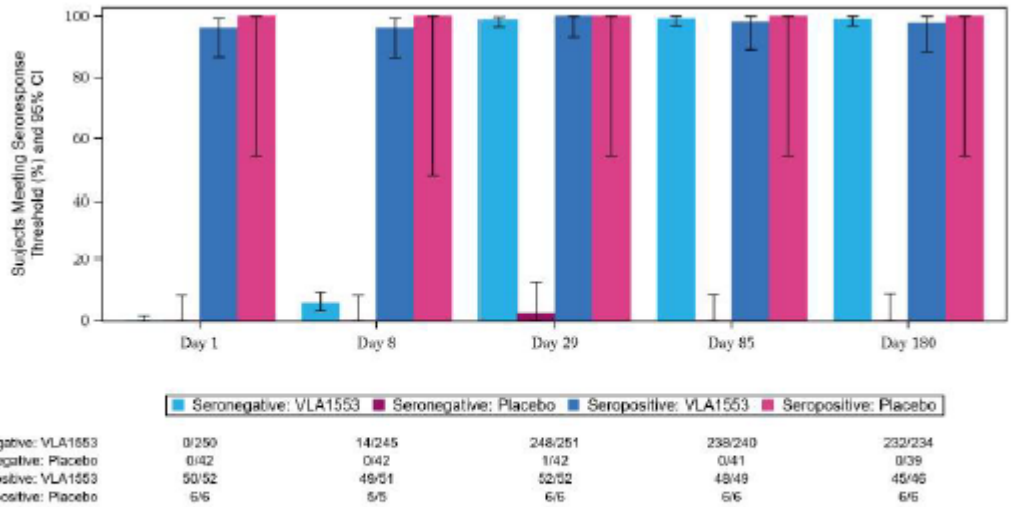
[c] P-value from Fisher's Exact test.

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

Seroresponse is defined as $\mu PRNT_{50} \geq 150$. Baseline seronegative participants are those with baseline $\mu PRNT_{50} \leq 40$, while baseline seropositive participants are those with baseline $\mu PRNT_{50} > 40$.

Two-sided 95% exact (Clopper-Pearson) CI is presented for the seroresponse rate and Chan-Zhang exact 95% CI is presented for the difference in seroresponse rate.

Figure 4. Bar Chart of Seroresponse Rate for CHIKV-Specific Neutralizing Antibodies by Trial Day and Baseline µPRNT Serostatus Strata (Per Protocol Population)



	Day 1	Day 8	Day 29	Day 85	Day 180
Seronegative: VLA1553	0/250	14/245	248/251	238/240	232/234
Seronegative: Placebo	0/42	0/42	1/42	0/41	0/39
Seropositive: VLA1553	50/52	49/51	52/52	48/49	45/46
Seropositive: Placebo	6/6	6/6	6/6	6/6	6/6

CI=confidence interval; µPRNT=Micro Plaque Reduction Neutralization Test.

Seroresponse is defined as $\mu PRNT_{50} \geq 150$ for all participants.

The counts (n/N) below the plot show the number of participants with seroresponse (n) at each time point, and number of participants with non-missing titers (N). Two-sided exact Clopper-Pearson 95% confidence intervals are presented. Bars are presented in the same order as the legend.

Within the baseline CHIKV seronegative participants vaccinated with VLA1553, 14/245 (5.7% [95% CI: 3.2-9.4]) had an antibody titre of at least 150 µPRNT50 at Day 8. None of the Placebo was seroresponder at Day 8.

The proportion of baseline seronegative participants who reached at least the threshold remains very high (232/234; 99.1% [95% CI: 96.9-99.9]) at Day 180 in the VLA1553 arm (0/39 in the placebo arm).

4. Proportion of Participants with Seroconversion on Day 29 and Day 180

Table 19. Seroconversion Rate for CHIKV-Specific Neutralizing Antibodies by Visit and Baseline µPRNT Serostatus (Per Protocol Population)

Time Point[a] [n] Statistic	Stratum: Seronegative by µPRNT VLA1553 (N=251)	Stratum: Seronegative by µPRNT Placebo (N=42)	Stratum: Seropositive by µPRNT VLA1553 (N=52)	Stratum: Seropositive by µPRNT Placebo (N=6)	Stratum: Total VLA1553 (N=303)	Stratum: Total Placebo (N=48)
Visit 3--- Day 29						
Total[a] [n]	251	42	52	6	303	48
Participants with Seroconversion [n (%)]	248 (98.8)	1 (2.4)	2 (3.8)	0	250 (82.5)	1 (2.1)
95% CI for Seroconversion Rate	96.5, 99.8	0.1, 12.6	0.5, 13.2	0.0, 45.9	77.8, 86.6	0.1, 11.1
P-value[b]	<0.0001	>0.9999	>0.9999	0.9993	<0.0001	>0.9999
Difference in Seroconversion Rate[c] (%)						
Difference	96.4		3.8		80.4	
95% CI	87.0, 99.1		-43.0, 15.1		71.3, 85.5	
P-value[d]	<0.0001		>0.9999		<0.0001	
Visit 5--- Day 180						
Total[a] [n]	234	39	46	6	280	45
Participants with Seroconversion [n (%)]	232 (99.1)	0	2 (4.3)	0	234 (83.6)	0
95% CI for Seroconversion Rate	96.9, 99.9	0.0, 9.0	0.5, 14.8	0.0, 45.9	78.7, 87.7	0.0, 7.9
P-value[b]	<0.0001	>0.9999	>0.9999	0.9993	<0.0001	>0.9999
Difference in Seroconversion Rate[c] (%)						
Difference	99.1		4.3		83.6	
95% CI	90.7, 99.9		-43.0, 16.5		75.7, 87.8	
P-value[d]	<0.0001		>0.9999		<0.0001	

CHIKV=chikungunya virus; CI=confidence interval; µPRNT=Micro Plaque Reduction Neutralization Test; n=number of participants; SCR=seroconversion rate

[a] Number of participants with non-missing titers at the specified time point.

[b] P-value from an exact binomial test for the null-hypothesis H_0: SCR $\leq$70% against the alternative H_1: SCR >70% with a one-sided significance level of 2.5%.

[c] Differences, P-values, and associated CIs are presented for the VLA1553 arm minus the placebo treatment arm.

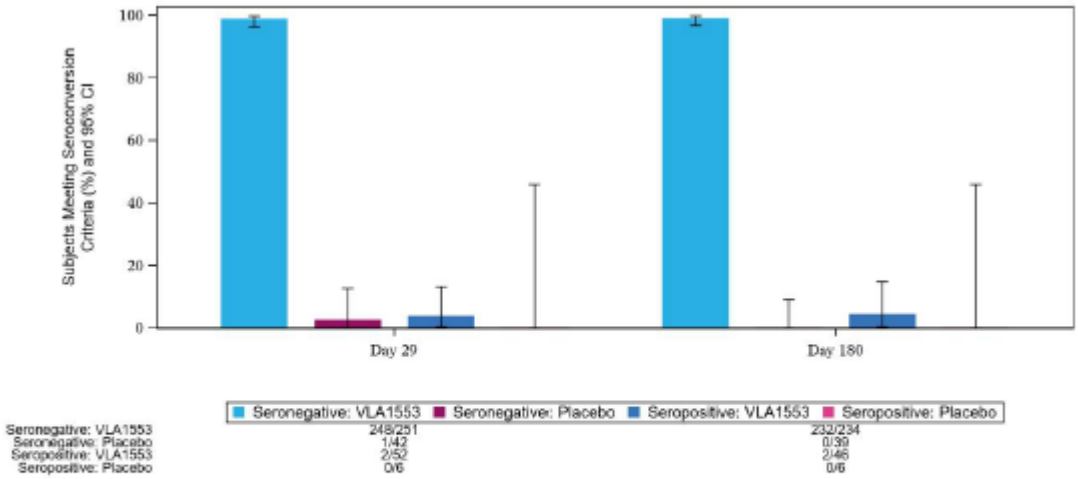
[d] P-value from Fisher's Exact test.

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

Seroconversion is defined as >4-fold increase of $\mu PRNT_{50}$ compared to baseline. Note that any $\mu PRNT_{50}$ value <20 is imputed as 10 for analysis. Baseline seronegative participants are those with baseline $\mu PRNT_{50} \leq 40$, while baseline seropositive participants are those with baseline $\mu PRNT_{50}$ >40.

Two-sided 95% exact (Clopper-Pearson) CI is presented for the seroconversion rate and Chan-Zhang exact 95% CI is presented for the difference in seroconversion rate.

Figure 5. Bar Chart of Seroconversion Rate for CHIKV-Specific Neutralizing Antibodies by Trial Day and Baseline µPRNT Serostatus Strata (Per Protocol Population)



CI=confidence interval; µPRNT=Micro Plaque Reduction Neutralization Test.
Seroconversion is defined as >4-fold increase of µPRNT$_{50}$ compared to baseline for baseline seronegative and seropositive participants.
The counts (n/N) below the plot show the number of participants with seroconversion (n) at each time point, and the number of participants with non-missing titers (N). Two-sided exact Clopper-Pearson 95% CIs are presented. Bars are presented in the same order as the legend.

Seroconversion was defined as a >4-fold increase of µPRNT50 compared to baseline (Day 1) for both baseline seronegative and seropositive participants.

The MAH clarified the baseline values imputed to µPRNT50 results <40. µPRNT50 baseline titre <20 (lower limit of quantification [LLOQ]) was imputed as 10 [LLOQ/2]) and µPRNT50 baseline titre from 20-40 were not imputed (reported values were used).

In the baseline CHIKV seronegative participants 248/251, 98.8% (95% CI: 96.5-99.8) of the participants seroconverted in the VLA1553 arm (PP population) at Day 29. The proportion remained high at Day 180, with 232/234, 99.1% (95% CI: 96.9-99.9) of the participants still meeting the definition of seroconversion.

Seroconversion in the baseline seropositive participants is presented in above sections.

5. Fold Increase of CHIKV-Specific NTs Determined by µPRNT Assay post-vaccination

Table 20. GMFIs for CHIKV-Specific Neutralizing Antibodies by Visit and Baseline µPRNT Serostatus (Per Protocol Population)

Time point / Statistic	Stratum: Seronegative by µPRNT VLA1553 (N=251)	Stratum: Seronegative by µPRNT Placebo (N=42)	Stratum: Seropositive by µPRNT VLA1553 (N=52)	Stratum: Seropositive by µPRNT Placebo (N=6)	Stratum: Total VLA1553 (N=303)	Stratum: Total Placebo (N=48)
Visit 2 - Day 8						
n	245	42	51	5	296	47
GMFI	1.7	0.9	1.1	1.2	1.5	0.9
95% CI for GMFI	1.46, 1.90	0.82, 0.99	0.88, 1.26	0.50, 2.98	1.37, 1.73	0.84, 1.03
Geometric std	2.89	1.34	1.88	2.06	2.76	1.44
Mean (std)	8.2 (49.48)	0.9 (0.20)	1.3 (0.83)	1.5 (1.00)	7.0 (45.08)	1.0 (0.39)
Median	1.0	1.0	1.1	1.3	1.0	1.0
Q1, Q3	1.0, 2.3	1.0, 1.0	0.7, 1.5	0.8, 1.8	1.0, 2.2	1.0, 1.0
Min, Max	0, 544	0, 1	0, 4	0, 3	0, 544	0, 3
n[a]	245	42	51	5	296	47
LS Mean (SE)[b]	1.66 (0.06)	0.90 (0.15)	1.05 (0.09)	1.22 (0.29)	1.36 (0.07)	0.80 (0.15)
95% Confidence Interval[b]	1.47, 1.88	0.67, 1.21	0.88, 1.26	0.69, 2.16	1.18, 1.56	0.60, 1.07
Difference in GMFI[b]						
Difference in LS Mean (SE)[c]	1.85 (0.16)		0.86 (0.30)		1.70 (0.15)	
95% Confidence Interval[c]	1.34, 2.56		0.47, 1.58		1.27, 2.27	
P-value[c]	0.0002		0.6303		0.0004	
Visit 3 - Day 29						
n	251	42	52	6	303	48
GMFI	364.0	1.1	1.3	1.0	137.6	1.0
95% CI for GMFI	322.72, 410.56	0.80, 1.38	0.99, 1.60	0.56, 1.72	105.56, 179.27	0.82, 1.33
Geometric std	2.63	2.39	2.37	1.71	10.41	2.30
Mean (std)	495.6 (379.06)	4.1 (19.66)	3.1 (12.22)	1.1 (0.51)	411.1 (391.88)	3.7 (18.39)
Median	413.2	1.0	1.1	1.0	345.2	1.0
Q1, Q3	251.5, 607.9	1.0, 1.0	0.7, 1.8	0.8, 1.4	130.3, 557.8	1.0, 1.0
Min, Max	1, 2234	0, 128	0, 89	0, 2	0, 2234	0, 128
n[a]	251	42	52	6	303	48
LS Mean (SE)[b]	364.00 (0.06)	1.05 (0.15)	1.25 (0.12)	0.98 (0.34)	26.10 (0.08)	0.16 (0.18)

Time point / Statistic	Stratum: Seronegative by µPRNT VLA1553 (N=251)	Stratum: Seronegative by µPRNT Placebo (N=42)	Stratum: Seropositive by µPRNT VLA1553 (N=52)	Stratum: Seropositive by µPRNT Placebo (N=6)	Stratum: Total VLA1553 (N=303)	Stratum: Total Placebo (N=48)
95% Confidence Interval[b]	323.28, 409.86	0.79, 1.40	0.99, 1.58	0.49, 1.94	22.09, 30.83	0.11, 0.22
Difference in GMFI[b]						
Difference in LS Mean (SE)[c]	346.45 (0.16)		1.28 (0.36)		167.23 (0.18)	
95% Confidence Interval[c]	253.24, 473.96		0.62, 2.64		118.04, 236.93	
P-value[c]	<0.0001		0.4960		<0.0001	
Visit 4 - Day 85						
n	240	41	49	6	289	47
GMFI	161.3	0.8	1.3	1.3	70.9	0.9
95% CI for GMFI	146.40, 177.61	0.75, 0.95	0.96, 1.66	0.73, 2.21	56.30, 89.21	0.79, 1.01
Geometric std	2.14	1.45	2.59	1.69	7.30	1.51
Mean (std)	202.6 (141.93)	0.9 (0.23)	2.3 (5.43)	1.4 (0.64)	168.6 (149.63)	1.0 (0.35)
Median	170.2	1.0	1.2	1.4	151.3	1.0
Q1, Q3	115.3, 251.2	1.0, 1.0	0.7, 2.2	0.9, 1.8	74.6, 213.0	1.0, 1.0
Min, Max	1, 1160	0, 1	0, 39	1, 2	0, 1160	0, 2
n[a]	240	41	49	6	289	47
LS Mean (SE)[b]	161.25 (0.05)	0.85 (0.11)	1.26 (0.13)	1.27 (0.38)	17.40 (0.07)	0.18 (0.15)
95% Confidence Interval[b]	147.20, 176.64	0.68, 1.06	0.97, 1.65	0.60, 2.71	15.03, 20.15	0.14, 0.25
Difference in GMFI[b]						
Difference in LS Mean (SE)[c]	190.01 (0.12)		0.99 (0.40)		94.74 (0.15)	
95% Confidence Interval[c]	149.67, 241.23		0.45, 2.21		70.05, 128.13	
P-value[c]	<0.0001		0.9841		<0.0001	
Visit 5 - Day 180						
n	234	39	46	6	280	45
GMFI	132.4	0.8	1.1	1.4	60.3	0.9
95% CI for GMFI	118.76, 147.67	0.74, 0.95	0.82, 1.48	0.75, 2.60	47.76, 76.09	0.79, 1.03
Geometric std	2.33	1.46	2.71	1.81	7.24	1.56
Mean (std)	181.0 (182.33)	0.9 (0.24)	1.8 (2.98)	1.6 (0.71)	151.5 (179.40)	1.0 (0.40)
Median	129.7	1.0	1.1	1.4	110.7	1.0
Q1, Q3	86.6, 218.2	1.0, 1.0	0.6, 2.0	1.4, 2.3	55.7, 203.4	1.0, 1.0
Min, Max	1, 2101	0, 1	0, 20	0, 2	0, 2101	0, 2

Time point Statistic	Stratum: Seronegative by µPRNT VLA1553 (N=251)	 Placebo (N=42)	Stratum: Seropositive by µPRNT VLA1553 (N=52)	 Placebo (N=6)	Stratum: Total VLA1553 (N=303)	 Placebo (N=48)
n[a]	234	39	46	6	280	45
LS Mean (SE)[b]	132.43 (0.05)	0.84 (0.13)	1.10 (0.14)	1.39 (0.39)	14.92 (0.08)	0.20 (0.17)
95% Confidence Interval[b]	119.52, 146.73	0.65, 1.08	0.83, 1.46	0.63, 3.07	12.71, 17.51	0.14, 0.27
Difference in GMFI[b]						
Difference in LS Mean (SE)[c]	157.37 (0.14)		0.79 (0.42)		76.19 (0.17)	
95% Confidence Interval[c]	119.96, 206.43		0.34, 1.83		54.88, 105.78	
P-value[c]	<0.0001		0.5770		<0.0001	

CHIKV=chikungunya virus; CI=confidence interval; GMFI=geometric mean fold increase; LS=least squares; max=maximum; µPRNT=Micro Plaque Reduction Neutralization Test; min=minimum; n=number of participants with available result; Q=quartile; SE=standard error; std=standard deviation

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

[b] LS means, SEs, CIs, and P-values are from an analysis of variance (ANOVA) model with fixed factors for trial arm and baseline $\mu PRNT_{50}$ 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 confidence intervals are presented for the VLA1553 arm minus the placebo treatment arm.

Note: The ANOVA model is applied to the log-transformed fold increase, and back-transformed results are displayed for the LS mean and difference. The difference in GMFI is a ratio of the LS means. The SE value is not back-transformed and the log-transformed value is presented.

Baseline seronegative participants are those with baseline $\mu PRNT_{50} \leq 40$, while baseline seropositive participants are those with baseline $\mu PRNT_{50} > 40$.

When compared with baseline, a mean 8-fold increase in CHIKV-specific neutralizing antibody titres was seen in the VLA1553 arm of the seronegative participants at Day 8, and 496-fold, 203-fold, and 181-fold increases were observed at Day 29, Day 85, and Day 180, respectively (PP population).

Limited fold increases were seen in the VLA1553 arm of the seropositive participants (1-fold on Day 8, 3-fold on Day 29, 2-fold on Day 85, and 2-fold on Day 180).

In the seronegative participants of the VLA1553 arm, a GMFI of 1.7 (95% CI: 1.5-1.9) was observed on Day 8. On Day 29, the GMFI increased to 364.0 (95% CI: 322.7-410.6). GMFIs of 161.3 (95% CI: 146.4-177.6) and 132.4 (95% CI: 118.8-147.7) were observed on Day 85 and Day 180, respectively, in the VLA1553 arm of the seronegative stratum.

In the seropositive participants of the VLA1553 arm, GMFIs close to 1 were observed at each time point, including at Day 29 (1.3-fold [95%CI: 1.0-1.6]; 1.1-fold on Day 8, 1.3-fold on Day 85; and 1.1-fold on Day 180).

Exploratory endpoints

Predefined criteria from the Protocol version 6.0 were applied to identify potential CHIKV infections with onset at least 14 days post-vaccination, and classification of potential CHIKV infections into 3 different categories (definite, probable, and asymptomatic) based on viraemia, clinical diagnosis, and seroconversion.

The data presented in this Part B CSR are preliminary. CHIKV case ascertainment and classification is not finalized. Nevertheless, the MAH presents data based on a preliminary classification of the cases performed by the Sponsor and discussed with an independent DSMB. The process of classification is not described in sufficient detail to be properly assessed. However, this issue is not pursued, and the process will be assessed with reporting of Part C, as the MAH proposes to present data based on final CHIKV case ascertainment when the complete 1-year follow up in Part C.

Definite CHIKV cases were defined as cases with clinical manifestations suggestive of CHIKV and confirmed viraemia as assessed by CHIKV-specific quantitative RNA detection (RT-qPCR). No definite CHIKV cases were identified up to Month 6.

Cases with clinical manifestations suggestive of CHIKV that could not be confirmed by RT-qPCR were classified as probable CHIKV cases based on CHIKV µPRNT seroconversion. As discussed in the methods section, it is however not considered possible to robustly ascertain the probable cases. One probable CHIKV case was identified up to Month 6 in the VLA1553 arm (1/502 [0.2%]) and none in the Placebo arm. The probable CHIKV case was identified based on a >4-fold titre increase between Day 85 and Month 6, associated with migraine.

Overall, 28.1% and 15.8% of participants in the VLA1553 group and placebo group of the Immunogenicity (IMM) subset, respectively, underwent for an acute visit and/or convalescent visit. Viraemia results from all samples collected at acute visits and mapped to Day 8, 29, 85, or 188 are available and discussed below.

In baseline seronegative VLA1553 participants of the PP population who had an acute visit at or close to Day 8, 7 participants had positive viremia results, including 6 participants with quantifiable viremia levels (>LOD). To discriminate vaccine viraemia from CHIKV wild-type infection, sequencing analysis were performed on positive viraemia samples (i.e., samples with results <LLOQ and quantifiable results). Complete sequencing data will be submitted within Part C (only partial data submitted in this procedure), no methodological details submitted so far.

For 2/7 participants, the sequence was confirmed to match the vaccine virus VLA1553 delta5nsP3 strain, for 5/7 participants, the sequencing failed. Insufficient details were provided on the applied sequencing methodology, , it is however acknowledged that with the applied assay, sequencing of samples with a low viral load will not be conclusive. It is not known but considered unlikely, that those cases correspond to a wild-type CHIKV infection since all cases are clustered in the immediate post-vaccination period, and no case is described later. In addition, it is understood that there were no CHIKV circulation during the study.

One baseline seropositive VLA1553 participant of the PP population had a quantifiable viraemia result at an acute visit close to Day 8 and experienced symptoms complying with CHIK-like AR definition. Sequencing failed for this subject. Due to uncertainties for this subject (inconsistent µPRNT results at different time-points), an investigation is ongoing for this case, which will be presented in the Part C CSR.

No viraemia was detected in any subject that underwent acute/convalescents visits mapped at later timepoints.

Summary of main study

The following table summarise the immunogenicity results from the main study supporting the present application. This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 21. Summary of immunogenicity for trial VLA1553-321

Table 1. **Title: VLA1553-321** **Table 2.** A multicentre, randomized, controlled, double-blinded pivotal study to evaluate safety and immunogenicity of a live-attenuated chikungunya virus vaccine candidate in adolescents aged 12 years to <18 years		
Study identifier	Clinical Trial No.: Not provided IND NUMBER: 17854 Study code: VLA1553	
Design	Prospective, randomized, double-blinded, placebo-controlled, multicentre pivotal clinical study	
	Duration of part B (Month 6)	First Participant Consented: 14 Feb 2022. Last Participant Last Visit (Month 6 [Part B]): 18 Aug 2023.
Hypothesis	Proportion of baseline CHIV seronegative participants 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%.	

Table 1. Title: VLA1553-321

Table 2. A multicentre, randomized, controlled, double-blinded pivotal study to evaluate safety and immunogenicity of a live-attenuated chikungunya virus vaccine candidate in adolescents aged 12 years to <18 years

Study identifier	Clinical Trial No.: Not provided IND NUMBER: 17854 Study code: VLA1553		
Treatments groups	VLA1553		VLA1553 (1x10E4 TCID50 / 0.5 mL) Single intramuscular immunization on Day 1 Number randomized: 510 Number in the immunogenicity subset: 335
	Placebo		Placebo Single intramuscular immunization on Day 1 Number randomized: 255 number in the immunogenicity subset: 57
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	Data Cut-off Date: 27 Feb 2024 (Note that data cut-off corresponds to data extraction date).		

Results and Analysis

Analysis description	**Primary Analysis** Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving at least a Day 29 CHIKV neutralizing antibody titre μPRNT50 ≥150		
Analysis population and time point description	The Per Protocol (PP) population includes all randomized and vaccinated participants of the immunogenicity subset who have an evaluable μPRNT antibody titre at baseline, have at least one post-baseline titre measurement after vaccination and who have no major protocol deviations, i.e. protocol deviations that could impact immune responses. The primary immunogenicity endpoint analysis was limited to baseline μPRNT seronegative participants (μPRNT50 ≤40). Among the 351 participants of the PP population (303 and 48 in the VLA1553 and Placebo arms respectively), 251 and 42 were baseline CHIKV seronegative (μPRNT≤40) respectively in the VLA1553 and Placebo arms.		
Descriptive Statistics and estimate variability	Treatment group	VLA1553	Placebo
	Number of participants	251	42
	Immunogenicity (Day 29) – proportion above threshold [%, n]	98.8% (248)	2.4% (1)
	95% CI on proportion above threshold	96.5% - 99.8%	0.1% - 12.6%
Notes	The lower bound of the 95% CI around the proportion exceeds the non-acceptance threshold of 70%.		
Analysis description	**Secondary analysis** Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving at least a Day 8 CHIKV neutralizing antibody titre μPRNT50 ≥150		

Table 1. Title: VLA1553-321 **Table 2.** A multicentre, randomized, controlled, double-blinded pivotal study to evaluate safety and immunogenicity of a live-attenuated chikungunya virus vaccine candidate in adolescents aged 12 years to <18 years			
Study identifier	Clinical Trial No.: Not provided IND NUMBER: 17854 Study code: VLA1553		
Analysis population and time point description	Same population as for the primary analysis.		
Statistics and estimate variability	Treatment group	VLA1553	Placebo
	Number of participants	245	42
	Immunogenicity (Day 8) – proportion above threshold [%, n]	5.7% (14)	0.0% (0)
	95% CI on proportion above threshold	3.2% - 9.4%	0.0% - 8.4%
Analysis description	**Secondary analysis** Proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving at least a Day 180 CHIKV neutralizing antibody titre μPRNT50 ≥150		
Analysis population and time point description	Same population as for the primary analysis.		
Descriptive statistics and estimate variability	Treatment group	VLA1553	Placebo
	Number of participants	234	39
	Immunogenicity (Day 180) – proportion above threshold [%, n]	99.1% (232)	0.0% (0)
	95% CI on proportion above threshold	96.9% - 99.9%	0.0% - 9.0%

2.4.2. Discussion on clinical efficacy

Study design

VLA1553-321 is a randomised, placebo-controlled, double-blind, multicentre, Phase 3 clinical study evaluating the safety and immunogenicity of a single dose of VLA1553 in adolescents aged 12-17 years. The study was conducted at 10 study sites in Brazil which is an endemic country for chikungunya. VLA1553 was administered at the final dose selected for adults (1x10E4 TCID50 per 0.5 mL). Approximately 750 generally healthy participants aged 12-17 years were planned to be randomly allocated in a 2:1 ratio to the VLA1553 group (n=500) or Placebo control group (n=250), with a target of 20% seropositive participants (i.e. IgM+/IgG+ or IgM-/IgG+) and 80% seronegative participants (i.e. IgM-/IgG-) for CHIKV (as defined by CHIKV ELISA). In order to meet those predefined stratum sizes, the serostatus was determined at the screening visit. The sample size was driven by the need to accumulate a sufficient number of participants in the safety database.

The first approximately 385 participants (i.e. approximately 50% of the total population) were to be included in the Immunogenicity subset. The size of the Immunogenicity subset is based on the primary endpoint. According to sample size calculations 268 CHIKV seronegative participants from the VLA1553 group were required in the Immunogenicity subset. Considering the targeted proportions of CHIKV baseline seronegative and seropositive participants (80% and 20%, respectively), 67 participants were planned for the seropositive participants from the VLA1553 group. This led to a total of 335 VLA1553

participants to be included in the Immunogenicity subset. The number of Placebo participants (n=50) in the Immunogenicity subset was not based on a specific sample size calculation but only aimed at ensuring a reasonable size for the control group. This approach is deemed acceptable and explains the substantially different allocation ratios between treatment arms for the Immunogenicity subset vs. the overall study population.

Of the Immunogenicity subset, approximately 75 participants had to constitute the Viraemia subset.

The randomization to study arm was stratified according to CHIKV ELISA serostatus at screening. Randomization was not stratified by site.

Assignment to the subsets was not made randomly as enrolment into the subsets was pursued at all sites until pre-determined target numbers were met. The process resulted in vastly different proportions of participants included in the Immunogenicity subset between sites, depending on the timing/speed of recruitment at sites. However, it is agreed that the factor site is not expected to have substantially affected the immunogenicity findings.

Investigators/sites staff (apart from those designated to randomize participants and handle the IMP, the DSMB voting members and the biostatistician involved in the DSMB), study participants, and sponsor staff were blinded to the assignment into study arms.

All participants received a single intramuscular administration of VLA1553 or of placebo (PBS) in the deltoid region of the arm. Participants were followed up for approximately 6 months (all subjects) or 12 months (Immunogenicity subset) following the vaccination. 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. An immunogenicity sample at Month 12 (Day 365) was also planned for participants from the Immunogenicity subset. Neutralizing antibodies were assessed (using µPRNT) for all participants on the Day 1 sample, to allow for stratification of the statistical analysis by baseline µPRNT serostatus. For the other timepoints, the immunogenicity evaluations were only performed in the Immunogenicity subset.

Overall, study procedure, timepoints and immunogenicity assessments are similar to study VLA1553-301 and are acceptable.

The study is still ongoing. Part A analysis (data up to Day 29) was reported at MAA. The MAH now submits updated Part A analysis and Part B analysis (Data up to Month 6). Part C analysis (up to Month 12) will be reported in March 2025.

Apart from age, the inclusion/exclusion criteria are similar to those used in study VLA1553-301 and acceptable.

The primary immunogenicity endpoint is the proportion of baseline CHIKV seronegative participants in the VLA1553 arm achieving a Day 29 µPRNT50 CHIKV neutralizing antibody titre ≥150. The primary endpoint is identical to the one used in the pivotal trial VLA1553-301 which led to the approval of Ixchiq in adults.

It has been agreed by CHMP before MA that efficacy trials are currently not feasible pre-authorization due to unpredictable and short-lived outbreaks. There is no established immune correlate of protection (ICP) for Chikungunya. Hence, an alternative approach was followed to establish the effect of VLA1553 based on neutralizing antibodies which are known to have a major role in protecting against CHIKV infection and/or disease. The threshold of CHIKV µPRNT50 antibody titre ≥150 was assessed in detail during Scientific advice and at MAA. It was agreed that it can be considered as reasonably likely to predict protection. The MAH proposed this threshold of 150 µPRNT50 based on data from a NHP passive transfer study, as well as supportive data from a sero-epidemiological study (Yoon et al.). Yoon

et al also have shown correlation between presence of neutralising antibodies against and a lower risk of disease in children and adolescents.

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.

Of note, the wording 'seroprotective level' used in the objectives is not deemed appropriate, the MAH should rather refer to 'µPRNT50 ≥150 for baseline negative participants. It is however noted that the term 'seroresponse' is used throughout the CSR instead of 'seroprotection' that was used in the protocol. This is considered acceptable.

As part of the secondary objectives, CHIKV-specific neutralizing antibody responses up to 1-year post-vaccination were characterized in terms of GMT, proportion of participants with antibody titre ≥150, proportion of participants with seroconversion (defined as a >4-fold increase of CHIKV-specific neutralization antibody titre compared to baseline), and fold increase compared to baseline. Immune responses were characterized stratifying by baseline CHIKV serostatus (used as a surrogate for previous exposure to CHIKV).

The incidence of natural CHIKV infections with onset at least 14 days post-vaccination was to be described in in the VLA1553 and Placebo arms, as part of an exploratory assessment of efficacy.

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). VLA1553 is a live-attenuated vaccine based on the La Réunion strain (LR2006-OPY1) of East Central South African genotype. The primary immunogenicity µPRNT assay is thus based on a heterologous strain, which is the same as the strain used in the sero-epidemiological study from Yoon et al.

CHIKV-specific IgM and IgG were assessed by ELISA in screening samples (Day 0) from all participants. Mayaro virus as well as Dengue virus and Zika virus ELISA antibodies were assessed in Day 1 samples from all participants.

The immunogenicity population (IMM) includes all randomized and vaccinated participants of the immunogenicity subset who have an evaluable µPRNT antibody titre at baseline and at least one post-baseline titre measurement after vaccination. The per protocol population (PP) contains all IMM participants who have no major protocol deviations, i.e. protocol deviations that could impact immune responses. Some major protocol deviations leading to exclusion from the PP were pre-defined in the protocol. Additional protocol deviations could be included in the SAP during the course of the trial, based on a case-by-case evaluation by the sponsor. Given that all decisions were made in a blinded manner (prior to study unblinding), this approach is considered reasonable. This process is similar to the one used in the pivotal trial VLA1553-301 which was assessed at MA.

Immunogenicity analyses were performed primarily on the PP population and secondarily on the IMM population. In contrast with the pivotal trial VLA1553-301, the IMM and PP populations included both baseline CHIKV seronegative and seropositive participants. However, the primary immunogenicity endpoint analysis was limited to baseline µPRNT seronegative participants, which is deemed appropriate.

CHIKV seronegativity was defined as µPRNT50 ≤40, which is not consistent with the more stringent and more appropriate definition used in the VLA1553-301 pivotal trial (µPRNT50 was <20). The MAH conducted a post-hoc analysis with for baseline CHIKV seronegative participants defined as µPRNT50

<20 for the primary immunogenicity analysis. Additional supplemental analyses were performed such as in seronegative participants based on the ELISA serostatus at screening, and in a modified PP population excluding participants with borderline IgM ELISA results at screening (IgM borderline/IgG-results).

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 hypothesis was tested using an exact binomial test comparing the observed proportion of participants reaching a CHIKV µPRNT antibody level ≥150 at Day 29 to a fixed lower bound of 70% (the Clopper-Pearson exact 95% confidence interval was used for that purpose). 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 nearly 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 MAH provides corresponding between group comparisons as a secondary endpoints which support the immunogenicity conclusions based on the primary endpoint.

The clinical development plan (CDP) was aligned with the Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1)

Results

Participant flow and numbers analysed:

A total of 765 participants were randomized (versus approximately 750 planned per protocol). Of the 765 randomised participants, 754 were vaccinated and therefore included in the Safety population (502 to VLA1553 and 252 to Placebo). A total of 753 participants from the Safety population were stratified by their µPRNT baseline serostatus: 614 participants to the CHIKV seronegative stratum (408 and 206 participants respectively to the VLA1553 and Placebo arms) and 139 participants to the seropositive stratum (94 and 45 participants respectively to the VLA1553 and placebo arms). One Placebo participant (not pertaining to the Immunogenicity subset) did not have a µPRNT baseline serostatus result. Of the 754 vaccinated participants, Visit 3 (Day 29) was completed by 747 (99.1%) participants, and Visit 5 (Day 180) was completed by 734 participants (97.3%).

A total of 392 participants were actually included into the Immunogenicity subset (335 in the VLA1553 arm and 57 in the Placebo arm). Of the 392 participants included in the Immunogenicity subset, 384 were included in the IMM population, and 351 participants were included in the PP population, corresponding to 89.5% of the Immunogenicity subset (90.4% for the VLA1553 arm and 84.2% for the Placebo arm). Among the 351 participants of the PP population, 293 and 58 were baseline CHIKV seronegative (µPRNT≤40) and seropositive (µPRNT >40), respectively.

According to the protocol, participants who had major protocol deviations (defined as those that could affect the assessment of immune responses) were excluded from the PP population. Of the 384 participants from the IMM population, there were 33 (8.6%) participants with major protocol deviations (25 [7.6%] in the VLA1553 arm and 8 [14.3%] in the placebo arm). The majority of participants with major protocol deviations had deviations from the pre-defined Visit 3 (Day 29) window (23/25 in the VLA1553 arm and 7/8 in the placebo arm). According to the SAP, participants with Visit 3 (Day 29) out of window deviations of +/- 8 days were excluded from the PP population.

Baseline data:

The PP population included 52.1% females (vs. 47.9% males), and 47.6% adolescents 12-14 yeas (vs. 52.4% adolescents 15-17 years). Overall, there were no major differences in the demographic characteristics between the Safety population and the PP population.

In the Safety and the PP populations, 139/753 (18.4%) and 58/351 (16.5%) of the participants respectively, were CHIKV seropositive at baseline (µPRNT50>40). The percentage of baseline seropositive participants was thus slightly lower than the 20% expected according to predefined caps. In the Safety and the PP populations, as expected, the CHIKV seronegative participants (respectively n=614 and n=293) were younger than the CHIKV seropositive participants (n=139 and n=58) as evidenced by the higher proportion of participants in the age category 12-14 years in the seronegative (52.1% and 48.8% for Safety and PP populations respectively), versus in the seropositive participants (38.1% and 41.4%).

In the Safety population, 59/754 (7.8%) of the participants had an history of yellow fever vaccination. The frequency was similar in baseline CHIKV seropositive and seronegative participants.

In the VLA1553 and Placebo arms (Safety Population), respectively 18.6%, 43.1%, 32.3% and 18.5%, 47.0%, 28.9% of the participants were tested baseline seropositive by ELISA (IgG) for Mayaro, Dengue, and Zika viruses. The presence of Mayaro virus antibodies was highly associated to the presence of CHIKV antibodies at baseline. In the PP population who were CHIKV baseline seronegative (µPRNT50≤40), only 1.6% and 0.0% of respectively the VLA1553 arm and the Placebo arm, tested baseline seropositive by ELISA for Mayaro virus. In contrast, in the participants who were CHIKV baseline seropositive (µPRNT50>40), nearly all participants (94.2% and 100% of respectively the VLA1553 arm and the Placebo arm) tested baseline seropositive by ELISA for Mayaro virus. The presence of Dengue and Zika antibodies was associated to the presence of CHIKV antibodies at baseline, although to a lesser extent. Of the CHIKV baseline seronegative participants (PP population), 40.3% and 42.9% were baseline seropositive for Dengue virus respectively in the VLA1553 and Placebo arms. Those frequencies were higher in the CHIKV baseline seropositive, as respectively 65.4% and 66.7%, tested baseline seropositive for dengue virus. The same pattern was observed for Zika virus, as in the PP population who were CHIKV baseline seronegative, 26.6% and 21.4% of respectively the VLA1553 arm and the Placebo arm were tested baseline seropositive for Zika virus. In contrast, the frequencies were again higher in the PP population who were CHIKV baseline seropositive as 51.9% and 33.3% participants were also tested baseline seropositive for Zika virus. These observations could indicate common endemicity in some areas (as the vector is common) and/or cross-reactivities. The large overlap of seropositive results for Mayaro is consistent with the well-described cross-reactivities existing between the two alphaviruses. Cross-reactivities are less common between alphaviruses and flaviviruses.

Gender, age and history of Yellow fever vaccination and presence of antibodies for Mayaro, Dengue, and Zika viruses, distributions were balanced between arms in the seronegative participant. An imbalance was observed for these characteristics in the µPRNT CHIKV seropositive population (both in the Safety population and in the PP population), but numbers of CHIKV seropositive participants are limited in the PP population.

The most common concomitant medications (i.e. with a start or end date on or after date of vaccination) were analgesics (mainly metamizole and paracetamol) and anti-inflammatory products (mainly Ibuprofen and Nimesulide). Overall, the frequency of their use was similar in the PP population compared to the Safety population. In the PP population, analgesics were used more frequently in the VLA1553 arm vs. the Placebo arm (57.4% vs. 45.8%). The imbalance between arms was observed for the period from Day 1 postvaccination to Day 29 (45.5% vs. 22.9% in the VLA1553 and Placebo arms respectively) and was particularly marked during the 7 days following vaccination (38.3% vs. 8.3% in the VLA1553 and Placebo arms respectively). NSAID were used in 17.8% and 22.9% participants in

the VLA1553 and Placebo arms respectively (PP population). Use of anti-inflammatory products in the period Day 1 to Day 29 was slightly more frequent in the VLA1553 arm vs. the Placebo arm (7.9%% vs. 4.2%, respectively). As for the analgesics, the imbalance was more marked for the period between Day 1 and Day 7 (4.6% vs. 0%, respectively). The proportions of participants who used analgesics or anti-inflammatory products between Day 30 and Day 180 were comparable between arms. Similar findings were observed in VLA1553-301 in adults. These findings reflect the medications used to treat symptoms of AEs. Analgesics or anti-inflammatory products were not regarded as medications that could impact the immune response. Hence those did not constitute a reason for exclusion from the PP population.

A post-hoc analysis explored the impact of using those concomitant medications on immune responses in seronegative participants. This analysis does not suggest an impact of the use of analgesics or anti-inflammatory products on GMTs induced by VLA1553, when drugs are used during the first week, or during the first month post-vaccination. It is not possible to study the impact of medication use on SRR, due to the very high percentages of participants reaching the threshold. Similar observations were made at MAA in adults (studies VLA1553-301 and VLA1553-302). Upon request, data were also provided with respect to the impact of those medications when taken in the early post-vaccination period. Data were requested from studies VLA1553-301 and VLA1553-302 as well. Altogether, these data do not suggest an impact of the use of analgesics or anti-inflammatory/anti-rheumatic products during the period Day 1 - Day 3 on neutralizing antibody responses.

Systemic corticosteroids were received as concomitant medications in 16/303 (5.3%) of the VLA1553 arm and 3/48 (6.3%) of the Placebo Arm. Immune sera/immunoglobulins (rabies antiserum) were received by two participants in the VLA1553 arm (not in the Placebo arm). No participants of the PP population received immunosuppressants. None of the participants were excluded from the PP population for those reasons. Due to a lack of information, the reasons for this are not fully clear. However, considering that the numbers are low, that most of the cases took corticosteroids in the period from Day 30 to Day 180, and that no bias in favour of the vaccine can be generated, this issue is not pursued.

Primary endpoint results:

The primary endpoint of the trial was met. At Day 29, 98.8% (248/251) of the baseline CHIKV seronegative participants (µPRNT≤40) had an antibody titre of at least 150 µPRNT50 (95% CI: 96.5-99.8) in the VLA1553 arm (PP population). The LB of the 95% CI was well above the non-acceptance threshold of 70%. The proportion of participants with at least the threshold titre at Day 29 was 2.4% (1/42) in the Placebo arm (95% CI: 0.1-12.6; p<0.0001 for the difference of proportions across arms). In total, 3 baseline CHIKV seronegative participants did not reach the threshold of 150 µPRNT50 in the VLA1553 arm.

In the PP population, 234 VLA1553 recipients and 35 placebo recipients were CHIKV baseline seronegative when defined as µPRNT50 <20, instead of 251 and 42 respectively when defined as µPRNT50≤40. In baseline CHIKV seronegative participants (defined as µPRNT50 <20) of the PP population, 98.7% (95% CI: 96.3-99.7) of the participants (231/234) in the VLA1553 group reached µPRNT50 ≥150 at Day 29. In contrast, in the Placebo group, only 1/35 participant reached at least the threshold. Post-hoc analyses for double seronegative (µPRNT50 <20 and ELISA IgG-/IgM-) participants of the PP population (n=229 in the VLA1553 group, n=35 in the Placebo group) were also performed and were consistent. The results of these post-hoc analyses of the primary endpoint with more stringent definitions of baseline seropositivity were thus similar to the results of the preplanned primary analysis. Results were similar when the serostatus at baseline was determined by ELISA (performed at screening), instead of µPRNT. Results were also similar when participants with

borderline ELISA results were excluded from the analysis (modified PP population). Finally, results obtained in the IMM population were similar as well.

Secondary endpoint results:

Neutralizing antibody responses in the CHIKV seronegative participants:

Seroconversion was defined as a >4-fold increase of µPRNT50 compared to baseline (Day 1) for both baseline seronegative (µPRNT50 results <40) and seropositive participants. µPRNT50 baseline titre <20 (lower limit of quantification [LLOQ]) was imputed as 10 [LLOQ/2]) and µPRNT50 baseline titre from 20-40 were not imputed (reported values were used).

Of the baseline CHIKV seronegative participants from the VLA1553 arm (PP population) 248/251, 98.8% (95% CI: 96.5, 99.8) seroconverted at Day 29. The proportion remained high at Day 180, with 232/234, 99.1% (95% CI: 96.9, 99.9) of the participants still meeting the definition of seroconversion.

Overall, in the CHIKV seronegative participants of the VLA1553 arm, GMTs increased slightly at Day 8 (from 10.6 [95% CI: 10.3-10.9] to 17.6 [95% CI: 15.4-20.1]). The difference between VLA1553-GMT and Placebo-GMT (10.6 [95% CI: 9.8-11.4]) was statistically significant (p=0.0019) at Day 8 but is not deemed clinically relevant. GMTs reached their peak at Day 29 (3855.9 [95% CI: 3432.1-4332.0]). At Day 29, the minimum GMT was 10, reflecting the data from the 3 vaccinees who did not reach the threshold of 150 µPRNT50, who in fact did not respond at all to the vaccination (2 had µPRNT<20 and 1 had µPRNT=29 at Day 29). Day 29-GMT of the Placebo participants remained low, i.e. 12.3 (95% CI: 9.6-15.8. The p-value for the comparison between both arms was <0.0001. GMTs decreased markedly at Day 85 (1701.8 [95% CI: 1544.8-1874.7]) and continued to decrease slightly up to Day 180 (1399.0 [95% CI: 1257.0-1557.0]). In the Placebo arm, the GMTs were stable. Results obtained in the IMM population were similar. Post-hoc analyses were conducted in the PP population with more stringent definitions of baseline seronegativity, and those suggest consistent results. Results were also similar across age categories and gender.

When compared with baseline, a mean 496-fold increase in CHIKV-specific neutralizing antibody titres was seen in the VLA1553 arm of the seronegative participants at Day 29, with a geometric mean fold increase (GMFI) of 364.0 (95% CI: 322.7-410.6). GMFI of 132.4 (95% CI: 118.8-147.7) was observed on Day 180.

Thus, overall, in the participants who have no pre-existing immunity to CHIKV, findings of this study VLA1553-321 conducted in adolescents leaving in Brazil, where CHIKV has circulated/is circulating, are consistent with results of both VLA1553-301 and VLA1553-302 studies, conducted in adults leaving in non-endemic areas. Day 29-GMT was of 3361.6 (95% CI: 2993.8-3774.4) and of 2643.2 (95% CI: 2354.0-2967.9) in the VLA1553 arm (PP population CHIKV baseline seronegative) of study VLA1553-301 and of study VLA1553-302 (3 Lots combined), respectively. GMT of study VLA1553-321 is thus in the same range.

Day 29 GMTs induced by VLA1553 in the baseline CHIKV seronegative participants are similar to the baseline GMTs of the CHIKV seropositive participants (i.e. titres elicited by the natural infection). However at the two later timepoints (Day 85 and Day 180), GMTs are lower compared to the baseline GMTs of the CHIKV seropositive participants (3097.1 [95%CI: 2324.9-4125.9] in the VLA1553 arm). The time elapsed between the infection and the sampling is unknown, and thus the antibody level reached shortly after natural infection is unknown.

Persistence of antibodies in the CHIKV seronegative participants:

As in study VLA1553-301, the proportion of baseline seronegative participants who reached at least the threshold remains very high at Day 180 in the VLA1553 arm (232/234; 99.1% [95% CI: 96.9-

99.9] in VLA1553-321 and 233/242; 96.3% [95% CI of 93.1-98.3,] in VLA1553-301), while this proportion was 0/39 in the placebo arm (study VLA1553-321). Study VLA1553-303 (follow up study VLA1553-301) indicates 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 still of 97.1% (95% CI:94.4, 98.7). Participants from VLA1553-303 will be followed for 5 years post-vaccination.

Neutralizing antibody responses in baseline CHIKV seropositive participants:

Almost all the CHIKV seropositive participants of the VLA1553 arm already had an antibody titre of at least 150 µPRNT50 at baseline (50/52), and all of them (52/52) had a titre above the threshold at Day 29. The 2 participants who did not have antibody titres ≥150 µPRNT50 at baseline (antibody titres of 71 and 86 µPRNT50) had increased titres at post-vaccination timepoints, although magnitude of the increase was very different (respectively 6336 and 165 at Day 29, i.e. 89-fold and 2-fold increase from baseline).

GMT tended to increase slightly at Day 29 (3886.5 [95%CI: 3063.4-4930.9]) compared to Day 0 (3097.1 [95%CI: 2324.9-4126.0]). However, 95% CI overlapped, and overall, it is considered that the GMTs are stable over the study period in VLA1553 recipients who are CHIKV seropositive at baseline. This is confirmed by GMFI which are at 1.3 (95% CI: 1.0-1.6) at Day 29 after vaccination. In addition, although all 52/52 participants had antibody titres above the threshold of 150 µPRNT50 at Day 29, only 2/52 participants seroconverted (defined as >4-fold increase of µPRNT50 compared to baseline). In the Placebo arm, the GMTs were also stable (variations are observed, but numbers are very limited, n=6).

VLA1553 thus did not induce an increase of CHIKV-neutralizing antibody titres at Day 29 in individuals with pre-existing immunity to CHIKV. This finding suggests that the vaccine virus is neutralized in the presence of CHIKV pre-existing neutralizing antibodies. This is also in line with the findings observed in the Phase 1 trial VLA1553-101. In this 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.

Data in CHIKV seropositive adult participants involved in the trials supporting MA were scarce, as these trials were conducted in the US. Data from study VLA1553-321 in endemic area, in which the vast majority of participants with pre-existing immunity to CHIKV present antibody titres above the predefined threshold of 150 µPRNT50 at baseline (50/52) thus show that VLA1553 does not induce a booster effect. Whether a booster effect might be observed in individuals with low neutralising antibody titres is not known. However, since natural infection is believed to induce long-term (even maybe life-long) protection, and hence the risk of re-infection is likely absent/very low, the added value of any booster effect in terms of clinical protection would probably be lacking/very limited anyway. Therefore, individuals who have been previously infected by CHIKV might not benefit from the vaccine.

Analyses stratified by pre-existing immunity to Mayaro, Dengue and Zika viruses and Yellow fever vaccination history:

A clinical study evaluated immunological interference from sequential administration of vaccines against heterologous alphaviruses (i.e., Venezuelan equine encephalitis virus and CHIKV) (McClain D J, Pittman PR, et al. Immunologic interference from sequential administration of live attenuated alphavirus vaccines. J Infect Dis. 1998;177(3):634-641). In this study, pre-existing alphavirus immunity interfered with subsequent neutralizing antibody responses to a live-attenuated heterologous vaccine. The MAH conducted a post-hoc analysis stratified by pre-existing immunity to Mayaro.

Presence of antibodies to CHIKV is highly correlated to presence of antibodies to Mayaro virus, most probably due to cross-reactivities, and only 4 participants who are CHIKV seronegative at baseline and have preexisting antibodies to Mayaro virus. Therefore, it is not possible to assess the independent impact of pre-existing immunity to Mayaro virus on CHIKV GMTs responses.

Post-hoc analyses stratified by pre-existing immunity to Dengue and Zika viruses (which are flaviviruses) were presented in the PP population, as well as post-hoc analyses for stratified by Yellow Fever (a flavivirus as well) vaccination history. The data suggest that pre-existing immunity to Dengue and Zika as well as Yellow fever vaccination history does not impact the immune responses induced by VLA1553.

Exploratory objectives and endpoints, CHIK Case Ascertainment and Classification:

The incidence of natural CHIKV infections with onset at least 14 days post-vaccination was to be described in the VLA1553 and Placebo arms, as part of an exploratory assessment of efficacy. Participants reporting an event of fever and presenting clinical signs/symptoms suggestive of an acute CHIK (i.e. Chikungunya) were to be referred to a clinical expert. An acute visit was performed (preferably within 7 days of illness onset), as well as a convalescent visit 3 weeks after. Blood samples were collected for quantitative CHIKV RT-qPCR and for CHIKV µPRNT assessment. In addition, for diagnosis purposes, samples were collected for CHIKV, Zika and Dengue RT-PCR and CHIKV, Zika and Dengue antibody detection by ELISA.

Suspected cases of CHIKV infection/disease were to be classified into four different categories (definite, probable, asymptomatic, unconfirmed). The MAH refers to PAHO/CDC 2011 and Simon et al. 2015 for the classification. Definite CHIK cases were defined as cases with clinical manifestations suggestive of CHIK and confirmed viraemia as assessed by CHIKV-specific quantitative RNA detection (RT-qPCR).

CHIK case ascertainment and classification is not finalized. Nevertheless, in this Part B CSR, the MAH presents data based on a preliminary classification of the cases performed by the Sponsor and discussed with an independent DSMB (although it seems that findings still require confirmation). The process of classification is not described in sufficient detail to be properly assessed. However, this issue is not pursued, and the process will be assessed with reporting of Part C, as the MAH proposes to present data based on final CHIKV case ascertainment when the complete 1-year follow up.

No definite CHIK cases were identified up to Month 6. Cases of clinical manifestations suggestive of CHIK that could not be confirmed by RT-qPCR were classified as probable CHIK cases based on CHIKV µPRNT seroconversion. One probable CHIK case was identified up to Month 6 in the VLA1553 arm (1/502 [0.2%]) and none in the Placebo arm. The probable CHIK case was identified based on a >4-fold titre increase between Day 85 and Month 6, associated with migraine. Seroconversion in the Placebo arm is likely to reflect natural infection in participants who are baseline CHIKV seronegative, while no conclusion can be drawn for participants who are baseline CHIKV seropositive. In the VLA1553 arm, it is impossible to discriminate seroconversion due to natural infection from seroconversion induced by VLA1553. Probable CHIK cases will therefore not be considered in the assessments of Parts B and C.

2.4.3. Conclusions on the clinical efficacy

Ixchiq (VLA1553) is a live-attenuated vaccine based on the La Réunion strain (LR2006-OPY1) of East Central South African genotype of CHIKV. Ixchiq has recently been approved for adults ≥18 years based on two immunogenicity and safety Phase 3 studies conducted in the US (VLA1553-301 and VLA1553-302). With the aim to extend the indication to adolescents ≥12 years, the MAH submits data

from study VLA1553-321 which assessed the immunogenicity and safety of a single dose of VLA1553 at the final adult dose level in adolescents aged 12-17 years in Brazil.

The primary endpoint of trial VLA1553-321 was met, with 99% (248/251) 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%. In the placebo arm, 1/42 of the participants reached the threshold at Day 29. The same point estimate and LB 95% CI were observed in the pivotal study VLA1553-301 conducted in adults.

At early timepoint (Day 8 post-vaccination), no relevant neutralizing antibody response was detected, as in study VLA1553-301.

The proportion of baseline CHIKV seronegative participants achieving the threshold was still very high up to 6 months post-vaccination (99% [95% CI: 97-100]), similarly to the proportion observed in adults up to 2 years post-vaccination (97% [95% CI: 94-99], study VLA1553-303).

As in adults, neutralizing antibody GMTs peak at Day 29 and decrease up to Day 180 in CHIKV baseline seronegative participants.

Overall, in the participants 12-17 years who have no pre-existing immunity to CHIKV (seronegative at baseline), findings of this study VLA1553-321 conducted in adolescents leaving in Brazil, where CHIKV has circulated/is circulating, are consistent with results of both VLA1553-301 and VLA1553-302 studies, conducted in adults leaving in non-endemic areas.

Day 29 GMTs induced by VLA1553 in the baseline CHIKV seronegative are similar to the titres observed following a past natural infection (i.e. in baseline GMTs of the CHIKV seropositive participants). Of note, the baseline GMTs of the CHIKV seropositive participants does not reflect the peak antibody level reached shortly after natural infection. The time elapsed between the past infection and the study baseline sampling is unknown. The peak of antibody level post-acute infection is likely to be much higher than the peak antibody level induced by VLA1553. At the two later timepoints (Day 85 and Day 180), GMTs are lower compared to the baseline GMTs of the CHIKV seropositive participants in the VLA1553 arm, suggesting that antibody titres induced by VLA1553 are lower than those induced by a natural infection over the longer term. The clinical relevance of these observations is not known.

Data in CHIKV seropositive adult participants involved in the trials supporting MA were scarce, as these trials were conducted in the US. Data from study VLA1553-321 in endemic area, in which the vast majority of participants with pre-existing immunity to CHIKV present high antibody titres at baseline (50/52 at level above the predefined threshold of 150 μPRNT50), show that VLA1553 does not induce a boost of natural immunity. This suggests that the vaccine virus is neutralized in the presence of CHIKV pre-existing neutralizing antibodies. Importantly, the added value of any booster effect of natural immunity in terms of clinical protection would be lacking/remain limited anyway, as natural infection is believed to induce long-term protection.

Results are consistent across the populations of analyses, and in the sensitivity analyses.

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. Therefore, as in the pivotal trials in adults, the primary endpoint of VLA1553-321 is based on a CHIKV-specific neutralizing antibody titre threshold considered reasonably likely to predict protection. This 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 this trial in adolescents are compelling (as were the results of the pivotal trials in adults) 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.

At the time of the initial MA, the CHMP considered 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

The MAH is revising the protocol of the agreed PAES in order to reflect that adolescents (12-17 yrs at the time of vaccination) will be added to the trial population. Therefore, the measure is amended as follows:

Post-authorisation efficacy study (PAES): In order to confirm the efficacy of Ixchiq in individuals 12 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.

2.5. Clinical safety

2.5.1. Introduction

The present report focuses on study VLA1553-321: a placebo-controlled Phase 3 study in adolescents residing in an endemic country for CHIKV (including data from seronegative and seropositive subjects), with interim data up to 6 months post vaccination. In the initial MAA, safety data for all participants up to 28 days after vaccination (Part A analysis) was provided as supportive data. In this application, safety data is available up to six months post-vaccination (part B analysis).

When needed, comparison is done with adult data (from initial MAA procedure EMA/CHMP/40090/2024).

Safety data collection

VLA1553-321 is a multicentre, prospective, randomized, double-blinded, pivotal clinical study in adolescents in Brazil, an endemic country, evaluated the adult dose) of VLA1553 in comparison to a placebo control. VLA1553 and control were administered as single immunization on Day 1.

Study safety population: All subjects who received one vaccination on Day 1 were included in the safety analysis. Solicited injection site AEs and systemic AEs were reported within 10 days post-vaccination and unsolicited AEs up to 28 days (Part A) and up to 6 months (Part A + B) after each vaccination.

Only SAEs, AESI, medically attended AEs (MAAEs) and AEs leading to withdrawal will be recorded until the end of the study (total of 12-month follow-up on the immunogenicity - IMM subset only).

Beyond trial end, SAEs that were fatal, life-threatening, or suspected to be related to the trial treatment will be reported until 6 months after the last trial visit of the subject (i.e. Visit 6 for the IMM subset or Visit 5 for the other subjects).

A DSMB met to review accumulating safety data on a regular basis until all subjects had completed Visit 3 (Day 29). During these meetings the DSMB reviewed listings of SAEs, AESI, deaths and severe (Grade 3) solicited AEs. The DSMB periodically reviewed accruing safety information throughout the study, as applicable.

A subject eDiary was distributed to all subjects for the collection of solicited safety information from vaccination to 10 days post-vaccination. The following information was collected:

- Measurement of oral body temperature
- Solicited injection site reactions (injection site pain, tenderness, erythema/redness, induration and swelling). All solicited injection site AEs were considered related to trial treatment.
- Solicited systemic reactions (fever, fatigue, headache, nausea, vomiting, muscle pain/myalgia, joint pain/arthralgia, and rash)
- Other adverse events (AEs)
- Any new concomitant medication or changes in medication taken after vaccination
- Information on travel to geographical regions where CHIKV is endemic

Adverse Events of Special Interest (protocol definition)

The following cluster of symptoms suggestive of CHIKV infection with or without remissions or exacerbations received particular consideration:

1. Fever (≥37.8°C [100.0°F], measured axillary);

AND

2. Acute (poly)arthralgia/arthritis, myalgia, neurological symptoms (e.g., meningoencephalitis, acute encephalitis, headache, seizures), retinitis/uveitis;

OR

3. One or more of the following signs and symptoms: macular to maculopapular rash (sometimes with cutaneous pruritus [foot plant]), pigmentary changes, bullous rash/ skin blistering, purpura and ecchymosis;

AND

4. Onset of symptoms 2 to 21 days after vaccination (i.e., Day 3 to Day 22);

AND

5. Duration of event ≥3 days.

Note: The cluster of symptoms defined above but starting 22 days after vaccination (i.e., Day 23) until study end is defined as late onset AESI.

Chikungunya-like Adverse Reactions (Post-hoc Analysis) (abbreviated: CHIK-like ARs)

In consideration of the feedback received from regulatory agencies after review of the clinical trial data of adults vaccinated with VLA1553, to alleviate any concerns of underestimation of AESI during the trial, a post-hoc analysis was performed with a broader definition:

a) Fever (≥37.8°C / ≥100.0°F)

AND

b) any single symptom (from the list initially defined in the trial protocol for AESI; VLA1553-321, see above)

AND

c) occurring within 30 days post-vaccination (regardless of the order of their onset and duration).

These will also be named early onset CHIK-like ARs hereafter.

A medically attended AE (MAAE) was defined as any AE where subjects sought medical care (i.e., doctor's office, emergency service, hospital, but not including use of self-medication). MAAEs were identified by the Investigator and recorded on the eCRF.

Safety laboratory samples were taken only from the IMM subset.

Patient exposure

A total of 765 adolescents aged 12 years to <18 years were enrolled in study VLA1553-321 from 10 investigative sites in Brazil. Eleven participants were randomized but not vaccinated, thus 754 participants were vaccinated and included in the Safety Population (502 participants to VLA1553 and 252 participants to placebo).

750 (99.5%) subjects (500 in the VLA1553 arm and 250 in the placebo arm) reached Day 29, including 610 seronegative subjects (406 in the VLA1553 arm and 204 in the placebo arm) and 139 seropositive subjects (94 in the VLA1553 arm and 45 in the placebo arm).

734 (97.3%) subjects (488 in the VLA1553 arm and 246 in the placebo arm) reached Day 180 (Month 6), including 599 seronegative subjects (397 in the VLA1553 arm and 202 in the placebo arm) and 134 seropositive subjects (91 in the VLA1553 arm and 43 in the placebo arm).

23/754 (3.1%) of subjects (17 in the VLA1553 arm and 6 in the placebo arm) discontinued the study; 18 were seronegative subjects and 5 were seropositive subjects.

Table 22. Study VLA1553-321: Subjects Overview

	Seronegative by µPRNT			Seropositive by µPRNT			Stratum: Total	
	VLA1553	Placebo	Total	VLA1553	Placebo	Total	VLA1553	Placebo
Participants Randomized	408	206	614	94	45	139	510	255
Participants Vaccinated[a], n (%)	408 (100)	206 (100)	614 (100)	94 (100)	45 (100)	139 (100)	502 (98.4)	252 (98.8)
Safety Population	408	206	614	94	45	139	502	252[b]
Reached Day 29[c], n (%)	406 (99.5)	204 (99.0)	610 (99.3)	94 (100)	45 (100)	139 (100)	500 (99.6)	250 (99.2)
Reached Month 6, n (%)	397 (97.3)	202 (98.1)	599 (97.6)	91 (96.8)	43 (95.6)	134 (96.4)	488 (97.2)	246 (97.6)
Ongoing after Part B (Month 6)[d]	260 (63.7)	47 (22.8)	307 (50.0)	59 (62.8)	8 (17.8)	67 (48.2)	319 (63.5)	55 (21.8)
Discontinued Study, n (%)	14 (3.4)	4 (1.9)	18 (2.9)	3 (3.2)	2 (4.4)	5 (3.6)	17 (3.4)	6 (2.4)

Abbreviations: eCRF=electronic case report form; µPRNT=micro plaque reduction neutralization test; $TCID_{50}$=50% tissue culture infectious dose.
a. Percentage of all randomized and sentinel participants who received a vaccination.
b. One participant was randomized to and vaccinated with placebo and included in the Safety Population, but had no µPRNT sample collected, and so was not included in either the seronegative or seropositive stratum.
c. 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.
d. For Immunogenicity subjects only.

For demographic and baseline characteristics, please refer to Table 8.

2.5.2. Adverse events per trial arm

2.5.2.1. Solicited adverse events

Table 23. VLA1553-321: All and Related Solicited Systemic and Injection Site Adverse Events Within 10 Days Post-Vaccination (Study Safety Population)

	VLA1553-321			
	Adolescents			
Adverse Event Category	VLA1553 (N=502) n (%)		Placebo (N=252) n (%)	
	All	Related	All	Related
Any Solicited Adverse Events	**353 (70.3)**	**348 (69.3)**	**123 (48.8)**	**119 (47.2)**
Solicited Systemic Adverse Events	**319 (63.5)**	**313 (62.4)**	**107 (42.5)**	**102 (40.5)**
Headache	256 (51.0)	250 (49.8)	87 (34.5)	83 (32.9)
Fatigue	112 (22.3)	111 (22.1)	24 (9.5)	22 (8.7)
Myalgia/Muscle Pain	135 (26.9)	129 (25.7)	31 (12.3)	29 (11.5)
Arthralgia/Joint Pain	65 (12.9)	62 (12.4)	14 (5.6)	13 (5.2)
Fever	121 (24.1)	119 (23.7)	9 (3.6)	8 (3.2)
Nausea	80 (15.9)	78 (15.5)	31 (12.3)	29 (11.5)
Rash	18 (3.6)	17 (3.4)	2 (0.8)	2 (0.8)
Vomiting	13 (2.6)	13 (2.6)	9 (3.6)	7 (2.8)
Solicited Injection Site Adverse Events[a]	**160 (31.9)**	**160 (31.9)**	**62 (24.6)**	**62 (24.6)**
Tenderness	100 (19.9)	100 (19.9)	37 (14.7)	37 (14.7)
Pain	97 (19.3)	97 (19.3)	34 (13.5)	34 (13.5)
Erythema/Redness	11 (2.2)	11 (2.2)	3 (1.2)	3 (1.2)
Induration	21 (4.2)	21 (4.2)	11 (4.4)	11 (4.4)
Swelling	7 (1.4)	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.
n is the number of participants with event; percentages are based on N.
a. Injection site reactions are considered to be related to vaccination.

In the VLA1553 arm, solicited AEs were mainly reported on Day 1, Day 4, and Day 5 (183/502 [36.5%], 84/502 [16.7%], and 97/502 [19.3%] subjects, respectively). Frequencies were lower on Day 2, Day 3, and decreased from Day 6 to Day 11, with only 5/502 (1.0%) subjects reporting solicited AEs on Day 11. In the placebo arm, 79/252 (31.3%) subjects reported solicited AEs on Day 1, and decreased from Day 2 to Day 7, and remained low thereafter, with only 2/252 (0.8%) subjects reporting a solicited AE on Day 11.

Mean duration of solicited AEs ranged from 3.6 days (headache) to 1.5 days (swelling), with most solicited AEs lasting 2 to 3 days.

Solicited local AEs

Solicited local AEs included injection site pain, tenderness, erythema/redness, induration, and swelling.

Significantly more solicited injection site AEs were reported in the VLA1553 arm compared to placebo (31.9% vs. 24.6%, p<0.0421). 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.

Overall, most solicited injection site AEs were graded as mild (30.9% in VLA1553 vs. 23.4% in placebo).

Five of 754 (0.7%) subjects experienced a moderate solicited injection site AE (3/502 [0.6%] in the VLA1553 arm and 2/252 [0.8%] in the placebo arm). Only 3/754 (0.4%) subjects experienced a severe solicited injection site AE (2/502 [0.4%] in the VLA1553 arm [1 event of induration and 1 of erythema/redness] and 1/252 [0.4%] in the placebo arm).

All solicited injection site AEs were considered related to trial treatment.

Solicited general AEs

Solicited general AEs included fever, fatigue, headache, nausea, vomiting, muscle pain/myalgia, joint pain/arthralgia, and rash.

Significantly more solicited systemic AEs were reported in the VLA1553 arm (63.5%) compared to the placebo arm (42.5%) ($p<0.0001$).

Headache was the most observed solicited system AEs: 51.0% in VLA1553 vs. 34.5% in placebo. The median duration was 2.0 days for both study arms. This was followed by myalgia (26.9% vs. 12.3%), fever (24.1% vs. 3.6%) and fatigue (22.3% vs. 9.5%). 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).

Most cases were mild (45.4% in VLA1533 versus 32.1% in placebo) or moderate (14.7% vs. 9.9%, respectively). Eighteen of 754 (2.4%) subjects experienced at least one AE that was graded severe (17/502 [3.4%] in the VLA1553 arm and 1/252 [0.4%] in the placebo arm). Severe solicited systemic AEs reported in the VLA1553 arm were fever (14 subjects), headache (four subjects), arthralgia (one subject), and myalgia (one subject) compared with headache (one subject) in the placebo arm. Some subjects in the VLA1553 experienced several severe solicited systemic AEs.

Most of the solicited systemic AEs were assessed as related to the vaccine (62.4% vs. 40.5%, in each arm respectively).

Viraemia

Of the subjects with severe solicited local and systemic AEs, viraemia data are available for 7/21 subjects. Some of these subjects had an acute visit sample tested and some were tested as they complied with the AESI-sponsor definition. In Day 8 samples, 4 had detectable viraemia), 1 had an inconclusive result and 2 had non-detectable levels. The subject with the highest detected viraemia experienced Grade 3 CHIKV-associated event, 2 days after study drug administration. This was classified as an AESI with OSEs of Grade 3 arthralgia (described as arthralgia), Grade 3 pyrexia (described as fever), and Grade 3 headache (described as headache). Sequencing results are available and confirmed match to vaccine strain. Viraemia was not detectable in Day 29 samples from any of these subjects. In total, 14 subjects of the VLA1553 arm experienced severe (grade 3, ≥39°C) solicited fever in the VLA1553 arm, which for some participants was accompanied by additional symptoms. Viraemia data are available for these 14 subjects (either from an acute visit sample or from retrospective analysis of samples isolated at visit 2 – Day 8). For 7/14 subjects, viraemia was not detectable; 2/14 had inconclusive viraemia test

results, and 5/14 subjects had quantifiable viremia levels ranging from 12,517.9 GCE/ml to 138,491.9 GCE/ml. The subject with the highest viremia level was seronegative at baseline and experienced multiple solicited and unsolicited severe AE meeting the CHIK-like AR criterion.

Within submission of the VLA1553-321 CSR Part C, the MAH has committed to submit viraemia assessment data for all subjects administered VLA1553 that experienced severe solicited adverse events. Results will have to be compiled in an additional separate table also summarizing preferred term, severity, if the subjects underwent AC/CV visits and if AESI criteria were met (both definition separately).

2.5.2.2. Unsolicited AE

Unsolicited AE up to 28 days post vaccination

Table 24. VLA1553-321: All and Related Unsolicited Adverse Events up to 28 Days After Single Vaccination Occurring at a Frequency of ≥2% in the VLA1553 Arm (Study Safety Population)

	Adolescents			
Adverse Event Category	**VLA1553-321**			
	VLA1553 (N=502) n (%)		**Placebo (N=252) n (%)**	
	All	**Related**	**All**	**Related**
Any Unsolicited Adverse Events	**197 (39.2)**	**40 (8.0)**	**81 (32.1)**	**3 (1.2)**
Influenza	17 (3.4)	0 (0.0)	6 (2.4)	0 (0.0)
Odynophagia	19 (3.8)	0 (0.0)	9 (3.6)	0 (0.0)
Abdominal pain	13 (2.6)	0 (0.0)	5 (2.0)	0 (0.0)
Headache	24 (4.8)	4 (0.8)	17 (6.7)	1 (0.4)
Cough	13 (2.6)	0 (0.0)	9 (3.6)	0 (0.0)
Pyrexia	20 (4.0)	5 (1.0)	8 (3.2)	0 (0.0)
Eye pain	15 (3.0)	6 (1.2)	0 (0.0)	0 (0.0)
Neutropenia	10 (2.0)	4 (0.8)	0 (0.0)	0 (0.0)

n is the number of participants with event; percentages are based on N.
Note: Adverse events were coded using Medical Dictionary for Regulatory Activities version 24.1. For each preferred term, subjects were included only once, even if they experienced multiple events in that system organ class or preferred term.

The overall incidence of unsolicited AEs was not significantly different between the VLA1553 arm and the placebo arm (39.2% versus 32.1%; p=0.0656).

Overall, headache was the most frequently reported unsolicited AEs in VLA1553 (4.8% vs. 6.7% in placebo), followed by pyrexia (4.0% vs. 3.2%, respectively), odynophagia (3.8% vs. 3.6%), influenza (3.4% 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 2.0% (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 3.0% in VLA1553.

The median onset day of unsolicited AE in the VLA1553 group varied depending on the SOC:

- 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 (29.3% in VLA1553 vs. 22.6% in placebo) or moderate (8% in VLA1553 vs. 8.3% in placebo).

Thirteen of 754 (1.7%) subjects (10/502 [2.0%] in the VLA1553 arm and 3/252 [1.2%] 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 general disorders and administration site conditions (5/754 [0.7%] subjects, 3/502 [0.6%] in the VLA1553 arm and 2/252 [0.8%] in the placebo arm). The most common severe unsolicited AEs were pyrexia (5/754 [0.7%] subjects, 3/502 [0.6%] in the VLA1553 arm and 2/252 [0.8%] in the placebo arm) and neutropenia (3/754 [0.4%] subjects, all in the VLA1553 arm).

A significantly higher frequency of related unsolicited AEs was reported in the VLA1553 arm compared with the placebo arm (8.0% versus 1.2%; $p<0.0001$).

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%).

Overall, most related unsolicited AEs were graded as mild or moderate. Only 2/502 (0.4%) subjects in the VLA1553 arm experienced at least one related unsolicited AE that was graded severe (headache), both in the seronegative stratum (versus none in the placebo arm).

Unsolicited AE up to 6 months after single vaccination

Table 25. VLA1553-321: All and Related Unsolicited Adverse Events 6 Months After Single Vaccination Occurring at a Frequency of ≥2% in the VLA1553 Arm (Study Safety Population)

	Adolescents			
Adverse Event Category	**VLA1553-321**			
	VLA1553 (N=502) n (%)		**Placebo (N=252) n (%)**	
	All	**Related**	**All**	**Related**
Any Unsolicited Adverse Events	**309 (61.6)**	**42 (8.4)**	**148 (58.7)**	**4 (1.6)**
Pyrexia	97 (19.3)	5 (1.0)	60 (23.8)	0
Headache	76 (15.1)	4 (0.8)	50 (19.8)	1 (0.4)
Cough	50 (10.0)	0	26 (10.3)	0
Odynophagia	45 (9.0)	1 (0.2)	26 (10.3)	0
Influenza	40 (8.0)	0	18 (7.1)	0
Myalgia	37 (7.4)	1 (0.2)	24 (9.5)	0
Nausea	26 (5.2)	0	15 (6.0)	0
Oropharyngeal pain	24 (4.8)	2 (0.4)	17 (6.7)	0
Abdominal pain	23 (4.6)	2 (0.4)	9 (3.6)	0
Rhinitis	19 (3.8)	1 (0.2)	14 (5.6)	0
Rhinorrhoea	19 (3.8)	0	16 (6.3)	0
Vomiting	18 (3.6)	0	17 (6.7)	0
Upper respiratory tract infection	17 (3.4)	0	2 (0.8)	0
Diarrhoea	17 (3.4)	2 (0.4)	11 (4.4)	0
Eye pain	17 (3.4)	6 (1.2)	2 (0.8)	0
Nasal congestion	15 (3.0)	0	9 (3.6)	0
Neutropenia	15 (3.0)	5 (1.0)	0	0
COVID-19	14 (2.8)	0	3 (1.2)	0
Gastroenteritis	13 (2.6)	0	11 (4.4)	0
Dysmenorrhoea	12 (2.4)	0	10 (4.0)	0
Fatigue	10 (2.0)	1 (0.2)	8 (3.2)	0
Dizziness	10 (2.0)	2 (0.4)	3 (1.2)	0

n is the number of participants with event; percentages are based on N.
Note: Adverse events were coded using Medical Dictionary for Regulatory Activities version 24.1. For each preferred term, subjects were included only once, even if they experienced multiple events in that system organ class or preferred term.

The overall incidence of unsolicited AEs was not significantly different between the VLA1553 arm and the placebo arm (61.6% versus 58.7%; p=0.4774); much higher percentage than those observed after 28 days in both arms.

Overall, pyrexia was the most frequently reported unsolicited AEs in VLA1553 (19.3% vs. 23.8%), followed by headache (15.1% vs. 19.8%), and cough (10% vs. 10.3%).

Most unsolicited AEs after vaccination were graded as mild (35.3% in VLA1553 vs. 34.1% in placebo) or moderate (19.7% in VLA1553 vs. 19.0% in placebo). Only 33/502 (6.6%) and 14/252 (5.6%) subjects in the VLA1553 and placebo arms, respectively, experienced at least one severe unsolicited AE (not significant, p=0.6353). Related severe unsolicited AEs were reported in only 2/502 (0.4%) subjects in the VLA1553 arm.

Overall, the SOC for which most severe unsolicited AEs were documented was general disorders and administration site conditions (16/502 [3.2%] in the VLA1553 arm and 9/252 [3.6%] in the placebo arm). The most common severe unsolicited AEs were pyrexia (16/502 [3.2%] in the VLA1553 arm and 8/252 [3.2%] in the placebo arm) and neutropenia (6/754 [0.8%] subjects, all in the VLA1553 arm).

A significantly higher frequency of related unsolicited AEs was reported in the VLA1553 arm compared with the placebo arm (8.4% versus 1.6%; p<0.0001); similar percentage than those observed after 28 days in both arms. 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%), and neutropenia (1% vs. 0%).

Overall, most related unsolicited AEs were graded as mild or moderate. Only 2/502 (0.4%) subjects in the VLA1553 arm experienced at least one related unsolicited AE that was graded severe (headache).

By day 180, 33 participants reported a total of 46 severe unsolicited AEs. Overall, they were reported in both, baseline seropositive (17 severe unsolicited AEs by 12 subjects) and baseline seronegative (29 severe unsolicited AEs by 21 subjects) subjects. They were considered as unrelated to VLA1553, except for two CHIK-like adverse reactions that is discussed in the AESI section.

For unsolicited SAE or AESI, refer to the respective sections.

2.5.2.3. Serious adverse event/deaths/other significant events

Deaths

There were no deaths in study VLA3221-321 up t0 6 months post-vaccination.

Other Serious Adverse Events

Table 26. Study VLA1553-321: Serious Adverse Events by System Organ Class and Preferred Term (Safety Population)

System Organ Class Preferred Term	VLA1553 (N=502) n (%) / Events	Placebo (N=252) n (%) / Events	Total (N=754) n (%) / Events
Any Serious Adverse Events	7 (1.4) / 9	4 (1.6) / 8	11 (1.5) / 17
Infections and Infestations	2 (0.4) / 2	2 (0.8) / 2	4 (0.5) / 4
Pneumonia	1 (0.2) / 1	1 (0.4) / 1	2 (0.3) / 2
Appendicitis	1 (0.2) / 1	0	1 (0.1) / 1
Pharyngitis	0	1 (0.4) / 1	1 (0.1) / 1
General Disorders and Administration Site Conditions	2 (0.4) / 2	1 (0.4) / 2	3 (0.4) / 4
Pyrexia	2 (0.4) / 2	1 (0.4) / 1	3 (0.4) / 3
Fatigue	0	1 (0.4) / 1	1 (0.1) / 1
Investigations	0	2 (0.8) / 2	2 (0.3) / 2
Prothrombin time prolonged	0	1 (0.4) / 1	1 (0.1) / 1
Transaminases increased	0	1 (0.4) / 1	1 (0.1) / 1
Nervous System Disorders	1 (0.2) / 1	1 (0.4) / 1	2 (0.3) / 2
Headache	0	1 (0.4) / 1	1 (0.1) / 1
Juvenile myoclonic epilepsy	1 (0.2) / 1	0	1 (0.1) / 1
Blood and Lymphatic System Disorders	1 (0.2) / 1	0	1 (0.1) / 1
Neutropenia	1 (0.2) / 1	0	1 (0.1) / 1
Gastrointestinal Disorders	1 (0.2) / 1	0	1 (0.1) / 1
Abdominal pain	1 (0.2) / 1	0	1 (0.1) / 1
Injury, Poisoning and Procedural Complications	1 (0.2) / 1	0	1 (0.1) / 1
Lower limb fracture	1 (0.2) / 1	0	1 (0.1) / 1
Metabolism and Nutrition Disorders	1 (0.2) / 1	0	1 (0.1) / 1
Hyperkalaemia	1 (0.2) / 1	0	1 (0.1) / 1
Psychiatric Disorders	0	1 (0.4) / 1	1 (0.1) / 1
Suicidal ideation	0	1 (0.4) / 1	1 (0.1) / 1

n is the number of participants with event; percentages are based on N.
Note: Serious adverse events were coded using Medical Dictionary for Regulatory Activities 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.

1.4% (7/502) of subjects vaccinated with VLA1553 versus 1.6% (4/252) of subjects vaccinated with placebo had a total of 9 and 8 SAEs, respectively.

The SOCs for which most SAEs were documented were:

- infections and infestations (2/502 [0.4%] subjects in the VLA1553 arm and 2/252 [0.8%] subjects in the placebo arm)
- general disorders and administration site conditions (2/502 [0.4%] subjects in the VLA1553 arm and 1/252 [0.4%] subjects in the placebo arm).

The most frequently reported SAE was pyrexia (2/502 [0.4%] subjects in the VLA1553 arm and 1/252 [0.4%] subjects in the placebo arm), followed by pneumonia (1/502 [0.2%] subjects in the VLA1553 arm and 1/252 [0.4%] subjects in the placebo arm). All other SAEs were reported once only.

In the VLA1553 arm, the following SAEs were reported: 1 pneumonia, 1 appendicitis, 2 pyrexia, 1 juvenile myoclonic epilepsy, 1 neutropenia, 1 abdominal pain, 1 lower limb fracture and 1 hyperkalaemia.

Only one subject had an SAE considered possibly related (study VLA1553-321-Part B, VLA1553 arm).

A >10 years subject 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 temperature was normothermic 5 days post-vaccination. 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, but retrospective analysis showed that viraemia was not detected at Day 8 nor at Day 29 for this subject. Out of the 7 of subjects vaccinated with VLA1553 who had 9 SAEs, 1 SAE was considered as related to the vaccine (described before), and 2 SAEs were described in the initial MAA application: lower limb fracture 17 days after vaccination, grade 4 hyperkalaemia 8 days after vaccination.

The remaining new SAEs in the VLA1553 arm (1 pneumonia, 1 pyrexia), 1 juvenile myoclonic epilepsy, 1 neutropenia, and 1 abdominal pain) were all assessed as non-related because of the absence of temporal association between vaccination and symptom onset, alternative aetiology, or absence of biological plausibility.

Finally, as described in the initial MAA application, a >12 year seropositive at baseline participant experienced activated partial thromboplastin time (APTT) prolonged 7 days after VLA1553 vaccination. This AE was initially reported as serious. Later on, it was completely removed from the AE page of the eCRF. The subject had a prolonged APTT already at Visit 0 (Grade 1), so it was assessed as not clinically relevant.

2.5.2.4. Adverse events of special interest (AESI)

2.5.2.4.1. AESI (protocol definition)

AESIs (protocol definition) for VLA1553 include fever in combination with signs and symptoms potentially indicative of an acute stage CHIKV-associated event (with a duration ≥ 3 days). They have been captured 21 days post-vaccination. The cluster of symptoms that constitute an AESI but starting after 21 days post vaccination until study end was defined as late onset AESI.

By Day 180, AESIs (early and late onset) were reported in 42/502 (8.4%) subjects in the VLA1553 arm and 19/252 (7.5%) subjects in the placebo arm (i.e. total of 61 subjects). AESI symptoms were pyrexia (8.4% vs. 7.5%, respectively), and a combination of pyrexia and headache (7.2% vs. 7.1%), myalgia

(5.2% vs. 5.2%), arthralgia (1.8% vs. 1.6%), rash (0.4% vs.0), or maculo-papular rash (0.2% vs. 0.8%).

17/502 (3.4%) in VLA1533 and 2/252 (0.8%) in placebo had an early onset AESI, and 29/502 (5.8%) in VLA1533 and 17/252 (6.7%) in placebo had a late onset AESI (i.e. total of 65 subjects).

The early onset AESI symptoms were pyrexia (17/502 [3.4%] in VLA1553 vs. 2/252 [0.8%] in placebo), and a combination of pyrexia with headache (16/502 [3.2%] in VLA1553 vs. 2/252 [0.8%] in placebo), myalgia (10/502 [2.0%] in VLA1553 vs. 2/252 [0.8%] in placebo), arthralgia (7/502 [1.4%] in VLA1553 vs. 1/252 [0.4%] in placebo), rash (2/502 [0.4%] in VLA1553 vs. none in placebo), or maculo-papular rash (1/502 [0.2%] in VLA1553 vs. none in placebo).

The late onset AESI symptoms were pyrexia (29/502 [5.8%] in VLA1553 vs. 17/252 [6.7%] in placebo), and a combination of pyrexia with headache (21/502 [4.2%] in VLA1553 vs. 16/252 [6.3%] in placebo), myalgia (18/502 [3.6%] in VLA1553 vs. 11/252 [4.4%] in placebo), arthralgia (2/502 [0.4%] in VLA1553 vs. 3/252 [1.2%] in placebo), rash (none in VLA1553 vs. 2/252 [0.8%] in placebo), or maculo-papular rash (none in VLA1553 vs. 2/252 [0.8%] in placebo).

Most AESIs (early and late onset) were graded as mild (3.4% in VLA1553 vs. 4.0% in placebo) or moderate (3.6% in VLA1553 vs. 2.4% in placebo). Ten of 754 (1.3%) participants (7/502 [1.4%] in the VLA1553 arm and 3/252 [1.2%] in the placebo arm) experienced an AESI that was graded severe. Severe AESIs of pyrexia (6/502 [1.2%] in VLA1553 vs. 3/252 [1.2%] in placebo), and a combination of severe pyrexia with severe headache (2/502 [0.4%] in VLA1553 vs. none in placebo), or severe arthralgia (1/502 [0.2%] in VLA1553 vs. none in placebo) were reported.

Most early onset AESIs were graded as mild (1.4% in VLA1553 vs. none in placebo) or moderate (1.2% in VLA1553 vs. 0.8% in placebo). Four of 754 (0.5%) participants (4/502 [0.8%] in the VLA1553 arm and none in the placebo arm) experienced an early onset AESI that was graded severe. In the VLA1553 arm, there were severe early onset AESI of pyrexia (3/502 [0.6%]), and a combination of severe pyrexia with severe headache (2/502 [0.4%]), or severe arthralgia (1/502 [0.2%]).

Most late onset AESIs were graded as mild (2.8% in VLA1553 vs. 4.0% in placebo) or moderate (2.4% in VLA1553 vs. 1.6% in placebo). Six of 754 (0.8%) participants (3/502 [0.6%] in the VLA1553 arm and 3/252 [1.2%] in the placebo arm) experienced a late onset AESI (pyrexia) that was graded severe.

The majority of early onset AESIs were considered related to trial treatment. Related early onset adverse events were reported for 15/754 (2.0%) participants (14/502 [2.8%] in the VLA1553 arm and 1/252 [0.4%] in the placebo arm).The related early onset AESI symptoms were pyrexia (14/502 [2.8%] in VLA1553 vs. 1/252 [0.4%] in placebo), and a combination of pyrexia with headache (13/502 [2.6%] in VLA1553 vs. 1/252 [0.4%] in placebo), myalgia (9/502 [1.8%] in VLA1553 vs. 1/252 [0.4%] in placebo), arthralgia (6/502 [1.2%] in VLA1553 vs. 1/252 [0.4%] in placebo), or rash (2/502 [0.4%] in VLA1553 vs. none in placebo).

Most related early onset AESIs were graded as mild (1% in VLA1553 vs. none in placebo) or moderate (1% in VLA1553 vs. 0.4% in placebo). For all 4 participants (0.8%) who experienced severe early onset AESIs in VLA1553 arm, there were considered related (vs. none in placebo): severe pyrexia (3/252 [0.6%]), and a combination of severe pyrexia with severe headache (2/252 [0.4%]), or severe arthralgia (1/252 [0.2%]).

No related late onset AESI were reported.

Up to Day 180, all AESIs were resolved.

Among the 61 participants who experienced AESIs with early or late onset (across both treatment arms), 4 participants (VLA1553 group) exhibited both early and late onset AESIs as defined by the protocol.

Early onset AESI were observed between Day 2 and Day 22 (i.e., onset date of the first symptom). Along with fever, the other symptoms included headache (3 subjects), myalgia (2 subjects), arthralgia (1 subject), and rash (1 subject). All symptoms resolved within 1 to 3 days, except for rash, which persisted for 6 days. These events were mostly mild in severity, with moderate fever noted in one subject. All early onset AESI were assessed as related (with a plausible temporal association: first symptom on Day 2 or Day 8), except for one case , where fever and headache on Day 22 were assessed as unrelated.

Late onset AESI occurred between Day 85 and Day 145. Along with fever, the other symptoms included headache (1 subject), myalgia (3 subjects), and arthralgia (1 subject). These symptoms resolved within 1 to 5 days, except for pyrexia and myalgia, which persisted for 10 days . These events were mild or moderate in severity. All late onset AESI were assessed as unrelated to the VLA1553 vaccination by the investigator because of absence of temporal association (i.e., the symptom onset was 3 months or more after the vaccination), and because the data indicated the presence of a respiratory or other common infection, or an acute Dengue infection. On the acute visit, all these 4 subjects were tested negative for CHIKV (by RT-PCR, no viremia detected by qRT-PCR) and Zika virus (by RT-PCR).

None of the AESI, whether early or late onset, were classified as serious and no AESI led to study discontinuation.

Viremia test results on Day 8 and Day 29 was available for all 42 out of the 502 participants in VLA1553 with AESI (sponsor definition) up to Day 180, irrespective of AESI symptom onset. 16 out of the 42 participants with viraemia results reported an early onset AESI and 29 out of the 42 reported a late onset AESI. It is noted that 3 subjects apparently complied with both early and late-AESI-sponsor definitions.

At Day 8, no viraemia was detected in 16/42, 5/42 had an inconclusive result (defined as a mix of positive and negative results in triplicate testing), 9/42 had a result <LOD, 2/42 had a result <LLOQ and 9/42 had a quantifiable result that ranged from 3,226.3 to 190,038.3 GCE/mL. No viraemia data are available for one participant which is included in the list of participants with early onset AESI (sponsor definition) but that did not return to site for the visits.

Two subjects had elevated levels of viraemia in samples mapped at Day 8.

- One subject experienced several adverse events, some of grade 3 severity. Sequencing confirmed match to reference VLA1553 delta5nsP3 strain. Experienced symptoms comply with the early onset AESI (sponsor definition).
- One subject experienced several adverse events early after administration of VLA1553 (all of grade 1) and experienced symptoms complying with late onset AESI (sponsor definition), which were also of grade 1. Sequencing results are pending for this subject.

No late vaccine viremia was detected at Day 29 in any of the tested subjects.

Concerning the 9/42 subjects with Day 8 viraemia >LLOQ, it is noted that 5/16 were subjects with early onset AESI (sponsor definition) and 4/29 were subjects with late onset AESI (sponsor definition). This could be indicative of higher vaccine viraemia associated with increased likelihood to develop early onset AESI. On the other hand, the majority of subjects experiencing early onset AESI sponsor

definition, did not have a quantifiable viraemia at Day 8. Hence, no conclusions can be drawn on a potential correlation between vaccine viraemia levels and safety of VLA1553.

2.5.2.4.2. Chikungunya-like Adverse Reactions (Post-hoc Analysis) (abbreviated: CHIK-like ARs)

The occurrence of certain adverse event combinations, referred to as CHIK-like ARs, was retrospectively evaluated.

CHIK-like ARs were broadly defined, i.e., 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: within 30 days after vaccination, regardless of time of onset, severity or duration of the individual symptoms. These CHIK-like AR occurring within 30 days after vaccination will be referred to as early onset CHIK-like AR. CHIK-like AEAR occurring from 30 days until 6 months post-vaccination will be referred to as late onset CHIK-like AE. CHIK- like AR/AE are presented in this report for all adolescents, by serostatus, by age and by sex, race, ethnicity and BMI.

2.5.2.4.2.1. For all adolescents

The overall incidence of early onset CHIK-like ARs and late onset CHIK-like AEs was 29.5% in the VLA1553 group and 17.5% in the placebo group.

Early onset CHIK-like ARs

Table 27. Subjects with CHIK-like adverse reactions (Safety Population)

	VLA1553 (N=502)	Placebo (N=252)	Total (N=754)
	n (%)	n (%)	n (%)
Any cases with CHIK-like adverse reactions	116 (23.1)	12 (4.8)	128 (17.0)
Any cases with related CHIK-like adverse reactions	113 (22.5)	10 (4.0)	123 (16.3)
Any cases with severe CHIK-like adverse reactions	18 (3.6)	1 (0.4)	19 (2.5)
Any cases with serious CHIK-like adverse reactions	1 (0.2)	0 (0.0)	1 (0.1)
CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like adverse reactions; percentages are based on N. Source: Post Hoc analysis, v3.0, 23-May-2024, Table 1.1.			

Table 28. Symptoms of CHIK-like adverse reactions (subjects with any CHIK-like adverse reaction)

	VLA1553 Participants With CHIK-like Adverse Reaction (N=116)	Placebo Participants With CHIK-like Adverse Reaction (N=12)	Total Participants With CHIK-like Adverse Reaction (N=128)
	n (%) Obs	n (%) Obs	n (%) Obs
Any CHIK-like symptom	116 (100) 409	12 (100) 36	128 (100) 445
Fever	116 (100) 124	12 (100) 12	128 (100) 136
Headache	101 (87.1) 114	11 (91.7) 12	112 (87.5) 126
Myalgia	67 (57.8) 69	3 (25.0) 3	70 (54.7) 72
Fatigue	51 (44.0) 51	6 (50.0) 6	57 (44.5) 57
Arthralgia	33 (28.4) 33	2 (16.7) 2	35 (27.3) 35
Rash	11 (9.5) 11	0 (0.0) 0	11 (8.6) 11
Chills	4 (3.4) 4	0 (0.0) 0	4 (3.1) 4
Hand dermatitis	1 (0.9) 1	0 (0.0) 0	1 (0.8) 1
Paraesthesia	1 (0.9) 1	0 (0.0) 0	1 (0.8) 1
Pruritus	0 (0.0) 0	1 (8.3) 1	1 (0.8) 1
Rash maculo-papular	1 (0.9) 1	0 (0.0) 0	1 (0.8) 1
CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like symptom; percentages are based on N; Obs=number of CHIK-like symptoms. Source: Post Hoc analysis, v3.0, 23-May-2024, Table 2.1.			

Table 29. Related symptoms of CHIK-like adverse reactions (subjects with any CHIK-like adverse reaction)

	VLA1553 Participants With CHIK-like Adverse Reaction (N=116)	Placebo Participants With CHIK-like Adverse Reaction (N=12)	Total Participants With CHIK-like Adverse Reaction (N=128)
	n (%) Obs	n (%) Obs	n (%) Obs
Any related CHIK-like symptoms	113 (97.4) 375	10 (83.3) 24	123 (96.1) 399
Fever	109 (94.0) 111	6 (50.0) 6	115 (89.8) 117
Headache	99 (85.3) 105	9 (75.0) 9	108 (84.4) 114
Myalgia	64 (55.2) 65	2 (16.7) 2	66 (51.6) 67
Fatigue	50 (43.1) 50	5 (41.7) 5	55 (43.0) 55
Arthralgia	30 (25.9) 30	2 (16.7) 2	32 (25.0) 32
Rash	11 (9.5) 11	0 (0.0) 0	11 (8.6) 11
Chills	2 (1.7) 2	0 (0.0) 0	2 (1.6) 2
Paraesthesia	1 (0.9) 1	0 (0.0) 0	1 (0.8) 1
CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with related CHIK-like symptom; percentages are based on N; Obs=number of related CHIK-like symptoms. Source: Post Hoc analysis, v3.0, 23-May-2024, Table 4.			

Table 30. Severe symptoms of CHIK-like adverse reactions (subjects with any CHIK-like adverse reaction)

	VLA1553 Participants With CHIK-like Adverse Reaction (N=116)	Placebo Participants With CHIK-like Adverse Reaction (N=12)	Total Participants With CHIK-like Adverse Reaction (N=128)
	n (%) Obs	n (%) Obs	n (%) Obs
Any severe CHIK-like symptom	18 (15.5) 22	1 (8.3) 1	19 (14.8) 23
Fever	15 (12.9) 15	1 (8.3) 1	16 (12.5) 16
Headache	4 (3.4) 5	0 (0.0) 0	4 (3.1) 5
Arthralgia	1 (0.9) 1	0 (0.0) 0	1 (0.8) 1
Myalgia	1 (0.9) 1	0 (0.0) 0	1 (0.8) 1
CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with severe CHIK-like symptom; percentages are based on N; Obs=number of severe CHIK-like symptoms. Source: Post Hoc analysis, v3.0, 23-May-2024, Table 3.1.			

The proportion of subjects with early onset CHIK-like ARs (within 30 days after vaccination) was 23.1% in the VLA1553 group and 4.8% in the placebo group (Table 23).

The majority of symptoms of early onset CHIK-like ARs in the VLA1553 group were common post-vaccination symptoms of fever in combination with headache (87.1% in VLA1553 vs. 91.7% in placebo), myalgia (57.8% in VLA1553 vs. 25.0% in placebo), fatigue (44.0% in VLA1553 vs. 50.0% in placebo), arthralgia (28.4% in VLA1553 vs. 16.7% in placebo), rash (9.5% in VLA1553 vs. none in placebo), and chills (3.4% in VLA1533 vs. none in placebo). Additionally, one event (0.9%) each of hand dermatitis, maculo-papular rash, and paraesthesia were identified in VLA1553, and 1 event of pruritus in placebo(Table 28).

Serious early onset CHIK-like ARs:

One (0.2%) early onset CHIK-like AR was classified as serious in the VLA1553 group and is discussed in the SAE section.

There were no serious early onset CHIK-like AR reported in the placebo group.

Severe early onset CHIK-like ARs:

The proportion of subjects with early onset severe CHIK-like ARs (i.e., at least one symptom that was assessed as severe by the investigator) was 3.6% (18 subjects / 502) in VLA1553 (15 fever, 4 fever in combination with headache, 1 in combination arthralgia and 1 in combination with myalgia) and 0.4% (1 subjects / 252) in placebo (fever) (Table 30).

Onset and duration of early onset CHIK-like ARs:

The mean onset Day for early onset CHIK-like ARs (i.e. onset date of earliest symptom) was Day 3.4 (median: Day 3) in VLA1553 and Day 2.7 (Median: Day 1.5) in placebo. The mean duration was 7.0 days (median 4 days) in the VLA1553 and 10 days (median 8.5 days) in placebo.

There were no prolonged early onset CHIK-like AR (i.e. at least one symptom with a duration of at least 30 days).

Causality of early onset CHIK-like ARs:

Most early onset CHIK-like ARs were classified as related (i.e., at least one symptom was assessed as related to vaccination by the investigator): 22.5% in VLA1553 and 4.0% in placebo.

The most common related symptoms in the VLA1553 group were fever (94.0% in VLA1533 vs. 50% in placebo), headache (85.3% in VLA1553 vs. 75.0% in placebo), myalgia (55.2% in VLA1553 vs. 16.7% in placebo), fatigue (43.1% in VLA1553 vs. 41.7% in placebo), and arthralgia (25.9% in VLA1553 vs. 16.7% in placebo).

Late-onset CHIK-like AEs

Table 31. Participants with late-onset CHIK-like AEs (Safety Population)

	VLA1553 (N=502)	Placebo (N=252)
	n (%)	n (%)
Any cases with late-onset CHIK-like AE	46 (9.2)	35 (13.9)
Any cases with late-onset related CHIK-like AE	0	1 (0.4)
Any cases with late-onset severe CHIK-like AE	8 (1.6)	5 (2.0)
Any cases with late-onset serious CHIK-like AE	0	1 (0.4)
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like AE; percentages are based on N. Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 1.1.		

Table 32. Symptoms of late-onset CHIK-like AEs (participants with any late-onset CHIK-like AE)

	VLA1553 Participants With Late-Onset CHIK-like AE (N=46)	Placebo Participants With Late-Onset CHIK-like AE (N=35)
	n (%) Obs	n (%) Obs
Any late-onset CHIK-like symptom	46 (100) 162	35 (100) 116
Pyrexia	46 (100) 62	35 (100) 41
Headache	36 (78.3) 50	27 (77.1) 37
Myalgia	26 (56.5) 32	16 (45.7) 19
Fatigue	6 (13.0) 7	5 (14.3) 6
Arthralgia	5 (10.9) 5	4 (11.4) 4
Malaise	3 (6.5) 3	1 (2.9) 1
Chills	1 (2.2) 1	5 (14.3) 5
Pruritus	1 (2.2) 1	0
Rash pruritic	1 (2.2) 1	0
Rash maculo-papular	0	2 (5.7) 2
Erythema	0	1 (2.9) 1
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like symptom; percentages are based on N; Obs=number of CHIK-like symptoms. Note: anosmia, back pain, palpitations were excluded from the list of symptoms of late-onset CHIK-like AE, since these symptoms were not part of the AESI definition according to the sponsor's VLA1553-321 protocol. Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 2.1.		

Table 33. Severe symptoms of late-onset CHIK-like AEs (participants with any late-onset CHIK-like AE)

	VLA1553 Participants With Late-Onset CHIK-like AE (N=46)	Placebo Participants With Late-Onset CHIK-like AE (N=35)
	n (%) Obs	n (%) Obs
Any severe late-onset CHIK-like symptom	8 (17.4) 8	5 (14.3) 5
Pyrexia	7 (15.2) 7	4 (11.4) 4
Rash pruritic	1 (2.2) 1	0
Fatigue	0	1 (2.9) 1
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with severe CHIK-like symptom; percentages are based on N; Obs=number of severe CHIK-like symptoms.		

	VLA1553 Participants With Late-Onset CHIK-like AE (N=46)	Placebo Participants With Late-Onset CHIK-like AE (N=35)
	n (%) Obs	n (%) Obs
Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 3.1.		

The proportion of participants experiencing late-onset CHIK-like AEs (occurring from 30 days to 6 months post-vaccination) was comparable between the VLA1553 arm and the placebo arm (9.2% and 13.9%, respectively). Severe late-onset CHIK-like AEs (at least one symptom assessed as severe) occurred at similar frequencies in the VLA1553 arm and the placebo arm (1.6% and 2.0%, respectively) (Table 31).

In the VLA1553 arm, none of the late-onset CHIK-like AEs were assessed as related, and none were serious.

The median onset (i.e. onset date of earliest symptom) and the median duration (calculated from onset date of earliest symptom to latest end date of a symptom) of late-onset CHIK-like AEs were similar in both groups: median onset Day 79.0 in the VLA1553 arm and Day 75.0 in the placebo arm; median duration 17.0 days and 8.0 days, respectively. In the VLA1553 arm, there were no prolonged late-onset CHIK-like AEs identified (i.e., at least one symptom of a late-onset CHIK-like AE with duration ≥30 days).

In the VLA1553 arm, the most common symptoms, aside from fever, included headache (78.3%), myalgia (56.5%), fatigue (13.0%), and arthralgia (10.9%). Other symptoms were reported with a frequency of less than 10%, including malaise, chills, pruritus, and pruritic rash. All symptoms were assessed as unrelated to VLA1553 vaccination by the investigator. The symptoms and their frequencies in the placebo arm were comparable to those in the VLA1553 arm (Table 32).

Among participants experiencing late-onset CHIK-like AEs, the incidence of severe late-onset CHIK-like symptoms was comparable between the VLA1553 and placebo arms (17.4% and 14.3%, respectively). The frequency of severe fever (i.e., ≥39.0°C) was similar in both arms (15.2% vs. 11.4%, respectively). Severe pruritic rash was observed in one participant (2.2%) in the VLA1553 arm (versus none in the placebo arm) (Table 33).

Viraemia Assessment

Of the 116/502 (23.1%) participants with CHIK-like adverse reactions in the VLA1553 group, 56/116 had available viraemia results and some also had sequencing results available. At Day 8, for 37/56, viraemia was either not detectable (n=34) or <LOD (n=3); 1/56 had a viraemia level <LLOQ and 9/56 had an inconclusive result. In the 9/56 with quantifiable viraemia at Day 8, levels ranged from 7,077.9 GCE/mL to 138,491.9 GCE/mL These subjects were also tested at Day 29, no viraemia was detected.

The viraemic subjects experienced symptoms of fever, arthralgia, fatigue, myalgia, headache, rash, and rash maculo-papular. It is noted that of the 9 subjects with Day 8 viraemia >LLOQ, 5/9 experienced symptoms complying with both early onset AESI (sponsor definition) and CHIK-like AR definition, while 4/9 only experienced symptoms complying with CHIK-like AR definition.

Five subjects had severe symptoms (arthralgia, fever, and headache); the viraemia levels in those subjects ranged from 12,517.9 GCE/ml to 138,491.9 GCE/ml. Three out of the five subjects complied with early onset AESI (sponsor definition). Only in one of the five cases sequencing confirmed match to reference VLA1553 delta5nsP3 strain.

2.5.2.4.2.2. Sub-group analysis by age

Early onset CHIK-like ARs

Table 34. Subjects with CHIK-like adverse reactions by age (Safety Population)

	VLA1553 (N=244) 12 to <15 years	VLA1553 (N=258) 15 to <18 years	Placebo (N=129) 12 to <15 years	Placebo (N=123) 15 to <18 years
	n (%)	n (%)	n (%)	n (%)
Any cases with CHIK-like adverse reactions	64 (26.2)	52 (20.2)	4 (3.1)	8 (6.5)
Any cases with related CHIK-like adverse reactions	62 (25.4)	51 (19.8)	3 (2.3)	7 (5.7)
Any cases with severe CHIK-like adverse reactions	11 (4.5)	7 (2.7)	0 (0.0)	1 (0.8)
Any cases with serious CHIK-like adverse reactions	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)
CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like adverse reaction, percentages are based on N. Source: Post Hoc analysis, v3.0, 23-May-2024, Table 1.1.				

Table 35. Symptoms of CHIK-like adverse reactions by age (subjects with any CHIK-like adverse reaction)

	VLA1553 Participants With CHIK-like Adverse Reaction 12 to <15 years (N=64)	VLA1553 Participants With CHIK-like Adverse Reaction 15 to <18 years (N=52)	Placebo Participants With CHIK-like Adverse Reaction 12 to <15 years (N=4)	Placebo Participants With CHIK-like Adverse Reaction 15 to <18 years (N=8)
	n (%) Obs	n (%) Obs	n (%) Obs	n (%) Obs
Any CHIK-like Symptom	64 (100) 203	52 (100) 206	4 (100) 14	8 (100) 22
Fever	64 (100) 66	52 (100) 58	4 (100) 4	8 (100) 8
Headache	54 (84.4) 60	47 (90.4) 54	4 (100) 4	7 (87.5) 8
Myalgia	31 (48.4) 32	36 (69.2) 37	2 (50.0) 2	1 (12.5) 1
Fatigue	23 (35.9) 23	28 (53.8) 28	2 (50.0) 2	4 (50.0) 4
Arthralgia	11 (17.2) 11	22 (42.3) 22	2 (50.0) 2	0 (0.0) 0
Rash	7 (10.9) 7	4 (7.7) 4	0 (0.0) 0	0 (0.0) 0
Chills	2 (3.1) 2	2 (3.8) 2	0 (0.0) 0	0 (0.0) 0
Hand dermatitis	0 (0.0) 0	1 (1.9) 1	0 (0.0) 0	0 (0.0) 0
Paraesthesia	1 (1.6) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Pruritus	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	1 (12.5) 1
Rash maculo-papular	1 (1.6) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like symptom, percentages are based on N, Obs=number of CHIK-like symptoms. Source: Post Hoc analysis, v3.0, 23-May-2024, Table 2.1.				

Table 36. Severe symptoms of CHIK-like adverse reactions by age (subjects with any CHIK-like adverse reaction)

	VLA1553 Participants With CHIK-like Adverse Reaction 12 to <15 years (N=64)	VLA1553 Participants With CHIK-like Adverse Reaction 15 to <18 years (N=52)	Placebo Participants With CHIK-like Adverse Reaction 12 to <15 years (N=4)	Placebo Participants With CHIK-like Adverse Reaction 15 to <18 years (N=8)
	n (%) Obs	n (%) Obs	n (%) Obs	n (%) Obs
Any severe CHIK-like Symptom	11 (17.2) 11	7 (13.5) 11	0 (0.0) 0	1 (12.5) 1
Fever	8 (12.5) 8	7 (13.5) 7	0 (0.0) 0	1 (12.5) 1
Headache	3 (4.7) 3	1 (1.9) 2	0 (0.0) 0	0 (0.0) 0
Arthralgia	0 (0.0) 0	1 (1.9) 1	0 (0.0) 0	0 (0.0) 0
Myalgia	0 (0.0) 0	1 (1.9) 1	0 (0.0) 0	0 (0.0) 0

CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like symptom, percentages are based on N, Obs=number of CHIK-like symptoms.
Source: Post Hoc analysis, v3.0, 23-May-2024, Table 3.1.

In the VLA1553 group, subjects aged 12 to <15 years had slightly more early onset CHIK-like ARs compared to subjects aged 15 to <18 years (26.2% and 20.2%, respectively). This difference was not observed in the placebo arm. Most early onset CHIK-like ARs were classified as related in the age subgroups of both treatment groups (Table 34).

Among the subjects with early onset CHIK-like ARs in the VLA1553 group, the frequency of headache (22.1% in 12 to <15 yoa vs. 18.2% in 15 to <18 yoa) and rash (2.9% vs. 1.6%, respectively) was slightly higher in subjects aged 12 to <15 years compared to subjects aged 15 to <18 years. Myalgia (12.7% vs. 14%, respectively), fatigue (9.4% vs. 10.9%, respectively), arthralgia (4.5% vs. 8.5%, respectively), and chills (0.8% in both arms) was similar in both age categories. However, the interpretation is limited due to the small subject number in the subgroups.

The proportion of subjects aged 12 to <15 years with severe early onset CHIK-like ARs was slightly higher compared to subjects aged 15 to <18 years (4.5% and 2.7%, respectively): severe fever (3.3% in 12 to <15 yoa vs. 2.7% in 15 to <18 yoa), severe headache (1.2% vs. 0.4%, respectively). This difference was not observed in the placebo arm. One event each of severe myalgia and severe arthralgia was identified in subjects aged 15 to <18 years (versus none in the younger).

The subject who had a serious early onset CHIK-like AR, was in the age subgroup 12 to <15 years.

Late onset CHIK-Like AE

Table 37. Participants with late-onset CHIK-like AEs by age (Safety Population)

	VLA1553 (N=244)	VLA1553 (N=258)	Placebo (N=129)	Placebo (N=123)
	12-<15 years	15-<18 years	12-<15 years	15-<18 years
	n (%)	n (%)	n (%)	n (%)
Any cases with late-onset CHIK-like AE	32 (13.1)	14 (5.4)	21 (16.3)	14 (11.4)
Any cases with late-onset related CHIK-like AE	0	0	0	1 (0.8)
Any cases with late-onset severe CHIK-like AE	7 (2.9)	1 (0.4)	4 (3.1)	1 (0.8)
Any cases with late-onset serious CHIK-like AE	0	0	1 (0.8)	0
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like AE; percentages are based on N. Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 1.1.				

Table 38. Participants with symptoms of late-onset CHIK-like AEs by age (participants with any late-onset CHIK-like AE)

	VLA1553 Participants With Late-Onset CHIK-like AE 12 to <15 years (N=32)	VLA1553 Participants With Late-Onset CHIK-like AE 15 to <18 years (N=14)	Placebo Participants With Late-Onset CHIK-like AE 12 to <15 years (N=21)	Placebo Participants With Late-Onset CHIK-like AE 15 to <18 years (N=14)
	n (%) Obs	n (%) Obs	n (%) Obs	n (%) Obs
Any CHIK-like Symptom	32 (100) 113	14 (100) 49	21 (100) 68	14 (100) 48
Pyrexia	32 (100) 42	14 (100) 20	21 (100) 23	14 (100) 18
Headache	26 (81.3) 37	10 (71.4) 13	17 (81.0) 23	10 (71.4) 14
Myalgia	20 (62.5) 25	6 (42.9) 7	12 (57.1) 14	4 (28.6) 5
Fatigue	2 (6.3) 2	4 (28.6) 5	2 (9.5) 2	3 (21.4) 4
Arthralgia	2 (6.3) 2	3 (21.4) 3	2 (9.5) 2	2 (14.3) 2
Malaise	2 (6.3) 2	1 (7.1) 1	0 (0.0) 0	1 (7.1) 1
Chills	1 (3.1) 1	0 (0.0) 0	2 (9.5) 2	3 (21.4) 3
Pruritus	1 (3.1) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Rash pruritic	1 (3.1) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Rash maculo-papular	0 (0.0) 0	0 (0.0) 0	2 (9.5) 2	0 (0.0) 0
Erythema	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	1 (7.1) 1
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like symptom, percentages are based on N, Obs=number of CHIK-like symptoms. Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 2.1.				

Table 39. Participants with severe symptoms of late-onset CHIK-like AEs by age (participants with any late-onset CHIK-like AE)

	VLA1553 Participants With Late-Onset CHIK-like AE 12 to <15 years (N=32)	VLA1553 Participants With Late-Onset CHIK-like AE 15 to <18 years (N=14)	Placebo Participants With Late-Onset CHIK-like AE 12 to <15 years (N=21)	Placebo Participants With Late-Onset CHIK-like AE 15 to <18 years (N=14)
	n (%) Obs	n (%) Obs	n (%) Obs	n (%) Obs
Any severe CHIK-like symptom	7 (21.9) 7	1 (7.1) 1	4 (19.0) 4	1 (7.1) 1
Pyrexia	6 (18.8) 6	1 (7.1) 1	3 (14.3) 3	1 (7.1) 1
Fatigue	0 (0.0) 0	0 (0.0) 0	1 (4.8) 1	0 (0.0) 0
Rash pruritic	1 (3.1) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like symptom, percentages are based on N, Obs=number of CHIK-like symptoms. Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024				

In the VLA1553 arm, the frequencies of late-onset CHIK-like AEs and severe late-onset CHIK-like AEs (at least one symptom assessed as severe) were higher in participants aged 12 to <15 years (13.1% and 2.9%) compared to participants aged 15 to <18 years (5.4% and 0.4%). However, a similar trend was observed in the placebo arm: the frequencies of late-onset CHIK-like AEs and severe late-onset CHIK-like AEs were higher in participants aged 12 to <15 years (16.3% and 3.1%) compared to participants aged 15 to <18 years (11.4% and 0.8%) (Table 37).

The difference observed for the frequencies of late-onset CHIK-like AEs is mainly due to the frequency of myalgia which was higher in participants aged 12 to <15 years (62.5%) compared to participants aged 15 to <18 years (42.9%) in VLA1553 arm (difference also observed in placebo arm: 57.1% and 28.6%, respectively). A small increase of frequency of headache is also observed in participants aged 12 to <15 years (81.3%) compared to participants aged 15 to <18 years (71.4%) in VLA1553 arm (difference also observed in placebo arm: 81.0% and 71.4%, respectively). Other data interpretation is limited due to the small number of subjects in the age subgroups with each other symptoms (Table 38).

Among VLA1553 participants experiencing late-onset CHIK-like AEs, the incidence of severe late-onset CHIK-like symptoms was higher in the 12 to <15 years age subgroup compared to the 15 to <18 years age subgroup (21.9% vs. 7.1%), primarily due to a higher frequency of severe fever (18.8% and 7.1%, respectively). These percentages were comparable to the placebo arm, where the frequency of severe late-onset CHIK-like symptoms was 19.0% and 7.1% in participants aged 12 to <15 years and 15 to <18 years, respectively, and the frequency of severe fever was 14.3% and 7.1%, respectively. One event each of severe pruritic rash was observed in the age subgroup 12 to <15 years in the VLA1553 arm (Table 39).

2.5.2.4.2.3. Sub-group analyses by sex, race, ethnicity and BMI

Early onset CHIK-like AR

In the VLA1553 group, female subjects had slightly lower rates of CHIK-like ARs compared to males (20.4% and 26.2%, respectively). A similar trend was observed in the placebo group (2.9% vs. 7.0%, respectively).

The highest frequencies of CHIK-like ARs were observed for the following subjects: black or African American (27.3%), white (25.7%), other (22.1%), and American Indian or Alaska Native (17.5%).

The frequency of CHIK-like ARs was slightly higher for the Hispanic or Latino (25.4%) than for the non-Hispanic or Latino (17.1%), and slightly higher in the BMI subgroup ≥25 kg/m^2 to <30 kg/m^2 (32.3%) compared to the other groups (<25 kg/m^2, ≥30 kg/m^2 to <35 kg/m^2, and ≥35 kg/m^2: between 20.0% vs. 25.0%).

Table 40. Subjects with CHIK-like adverse reactions stratified by sex, race, ethnicity, and BMI (Safety Population)

	VLA1553 (N=502)	Placebo (N=252)
CHIK-like adverse reactions	**n/N* (%)**	**n/N* (%)**
By Sex		
Male	61/233 (26.2)	8/115 (7.0)
Female	55/269 (20.4)	4/137 (2.9)
By Race		
White	43/167 (25.7)	5/78 (6.4)
Black or African American	18/66 (27.3)	2/31 (6.5)
Asian	0/2 (0.0)	0/0 (0.0)
American Indian or Alaska Native	2/2 (100)	0/2 (0.0)
Multiracial	21/120 (17.5)	1/72 (1.4)
Other	32/145 (22.1)	4/69 (5.8)
By Ethnicity		
Hispanic or Latino	91/358 (25.4)	8/172 (4.7)
Non-Hispanic or Latino	24/140 (17.1)	4/79 (5.1)
By BMI		
BMI <25 kg/m^2	88/406 (21.7)	8/198 (4.0)
BMI ≥ 25 kg/m^2 and BMI<30 kg/m^2	20/62 (32.3)	1/40 (2.5)
BMI ≥ 30 kg/m^2 and BMI<35 kg/m^2	6/24 (25.0)	3/11 (27.3)
BMI ≥35 kg/m^2	2/10 (20.0)	0/3 (0.0)

BMI=body mass index; CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like adverse reaction, percentages are based on subgroup N (*); N=total number of participants
Source: Post Hoc analysis, v3.0, 23-May-2024, Tables 13, 14, 16, 17, 19, 20, 22 and 23.

Late-onset CHIK-like AEs

In the VLA1553 arm, the rates of late-onset CHIK-like AEs were comparable between female and male participants (7.8% vs. 10.7%, respectively), a trend also observed in the placebo arm (14.6% vs. 13.0%, respectively).

Regarding race, meaningful comparisons were possible for Black/African American, White, multiracial, and other participants. In the VLA1553 arm, most late-onset CHIK-like AEs were identified in White and Black participants (10.8% and 15.2%, respectively), with a similar trend in the placebo arm (10.3% and 22.6%, respectively). The proportion of VLA1553 participants with late-onset CHIK-like AEs in the multiracial category was within a similar range as for other participants (5.8% and 7.6%, respectively); this trend was also observed in the placebo arm (12.5% and 14.5%, respectively).

Late-onset CHIK-like AEs were observed in both ethnicity subgroups, with comparable frequencies in participants of Hispanic/Latino ethnicity and non-Hispanic/Latino ethnicity (10.1% vs. 7.1%, respectively), a trend also observed in the placebo arm (15.1% vs. 11.4%, respectively).

Late-onset CHIK-like AEs were observed with lower frequency in participants with a BMI of <25 kg/m²in the VLA1553 arm (7.9%) vs. the placebo arm (14.6%), and with higher frequencies in those with a BMI of ≥25 kg/m² to <30 kg/m² in the VLA1553 arm (16.1%) vs. the placebo arm (7.5%). However, the

interpretation is limited due to the small number of participants with a BMI of ≥25 kg/m² to <30 kg/m² (62 in VLA1553 vs. 40 in placebo) (and in higher BMI categories not discussed here).

Table 41. Participants with late-onset CHIK-like AEs stratified by sex, race, ethnicity, and BMI (Safety Population)

	VLA1553 (N=502)	Placebo (N=252)
Late-onset CHIK-like AEs	**n/N* (%)**	**n/N* (%)**
By Sex		
Male	25/233 (10.7)	15/115 (13.0)
Female	21/269 (7.8)	20/137 (14.6)
By Race		
White	18/167 (10.8)	8/78 (10.3)
Black or African American	10/66 (15.2)	7/31 (22.6)
Asian	0/2 (0.0)	0/0 (0.0)
American Indian or Alaska Native	0/2 (0.0)	1/2 (50.0)
Multiracial	7/120 (5.8)	9/72 (12.5)
Other	11/145 (7.6)	10/69 (14.5)
By Ethnicity		
Hispanic or Latino	36/358 (10.1)	26/172 (15.1)
Non-Hispanic or Latino	10/140 (7.1)	9/79 (11.4)
By BMI		
BMI <25 kg/m^2	32/406 (7.9)	29/198 (14.6)
BMI ≥ 25 kg/m^2 and BMI<30 kg/m^2	10/62 (16.1)	3/40 (7.5)
BMI ≥ 30 kg/m^2 and BMI<35 kg/m^2	2/24 (8.3)	1/11 (9.1)
BMI ≥35 kg/m^2	2/10 (20.0)	2/3 (66.7)
BMI=body mass index; CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like AE, percentages are based on subgroup N (*); N=total number of participants Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 13, Table 14, Table 16, Table 17, Table 19, Table 20, Table 22 and Table 23.		

2.5.2.4.3. Medically attended events (MAAEs)

Up to 6 months, the frequencies of any medically attended AEs were similar between VLA1553 arm (22.1%) and placebo arm (22.2%).

The most common medically attended AEs (≥2.0% of subjects) in the VLA1553 arm were pyrexia (40/502 [8.0%]), headache (34/502 [6.8%]), and myalgia (24/502 [4.8%]).

Most medically attended AEs were graded as mild (10% in VLA1553 vs. 10.3% in placebo) or moderate (9.4% VLA1553 vs. 9.1% placebo). In total, 21/754 (2.8%) subjects (14/502 [2.8%] in the VLA1553 arm and 7/252 [2.8%] in the placebo arm) experienced at least one medically attended AE that was graded severe.

The frequency of related MAAEs was slightly higher in the VLA1553 arm (8%) compared to the placebo arm (6%).

Related medically attended solicited AEs were reported in 38/502 (7.6%) and 14/252 (5.6%) subjects 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%) subjects in the VLA1553 and placebo arms, respectively.

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 (initial MAA). 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).

2.5.2.4.4. Discontinuation due to adverse events

There were no AE leading to study withdrawal in both groups.

2.5.2.4.5. Laboratory findings

2.5.2.4.5.1. Clinical laboratory evaluation

The most common abnormal laboratory parameters were (based on an immunogenicity subset of 328 Ixchiq recipients, and 56 placebo recipients):

- neutropenia/neutrophils decrease: VL1553 40.2% (64 grade 1 + 45 grade 2 + 22 grade 3 + 1 grade 4 = 132) vs. placebo 28.57% (9 grade 1 + 5 grade 2 + 2 grade 3 =16)
- anaemia/red blood cell haemoglobin decrease: VL1553 40.2% (99 grade 1 + 33 grade 2 = 132) vs. placebo 48.2% (19 grade 1 + 8 grade 2 = 27)
- alkaline phosphatase increased: VL1553 17.7% (46 grade 1 + 7 grade 2 + 5 grade 3 = 58) vs. placebo 14.3% (6 grade 1 + 2 grade 2 = 8)
- leukopenia/leukocytes decrease: VL1553 16.8% (49 grade 1 + 6 grade 2 = 55) vs. placebo 5.4% (3 grade 1)
- hypernatremia: VL1553 14.9% (36 grade 1 + 8 grade 2 + 2 grade 3 + 3 grade 4 = 49) vs. placebo 16.1% (7 grade 1 + 1 grade 2 + 1 grade 4 = 9)
- leucocytosis/leukocytes increase: VL1553 14.6% (44 grade 1 + 4 grade 2 = 48) vs. placebo 10.7% (4 grade 1 + 2 grade 2 = 6)
- AST increase: VLA1553 12.8% (31 grade 1 + 10 grade 2 + 1 grade 3 = 42) vs. placebo 17.9% (9 grade 1 + 1 grade 4 = 10)
- lymphopenia/lymphocyte decrease: VL1553 11.6% (25 grade 1 + 11 grade 2 + 2 grade 3 = 38) vs. placebo 8.9% (3 grade 1 + 2 grade 2 = 5)
- ALT increase: VLA1553 11.3% (33 grade 1 + 4 grade 2 = 37) vs. placebo 12.5% (6 grade 1 + 1 grade 3 = 7)
- Hypokalaemia: VL1553 10.4% (27 grade 1 + 5 grade 2 + 2 grade 3 = 34) vs. Placebo 5.4% (2 grade 1 + 1 grade 2 = 3)

2.5.2.4.5.2. Vital signs, physical findings, and other observations

No relevant changes from baseline were observed for vital sign and physical findings in the VLA1553 or placebo arms. One case of grade 4 was reported in combination with arthralgia, myalgia and headache and is considered in the AESI section.

2.5.2.4.5.3. Viraemia

In study VLA1553-321, plasma samples were collected from all subjects for clinically indicated retrospective investigation of viraemia by RT-qPCR (collected on vaccination Day 1 and on Days 8, 29, 85, 180 and 365, and if applicable, at the Early Termination Visit). Viraemia samples were also collected and analysed for acute and convalescent visits (AV/CV) throughout the trial for all participants with suspected CHIK cases for analysis of the exploratory endpoints.

For all subjects included in the viraemia subset (a randomly selected subgroup of approximately 75 subjects from the immunogenicity subset), Day 1 and Day 8 samples were analysed for viraemia by RT-qPCR. Day 29 sample were analysed only if Day 8 sample was positive, or had an inconclusive result, or was out of the visit window. Eventually 78 subjects were included in the viraemia subset. A total of 76/78 were vaccinated, 52 with VLA1553 (respectively 43 and 9 were either seronegative or seropositive at baseline by µPRNT) and 24 with placebo (respectively 20 and 4 were either seronegative or seropositive at baseline by µPRNT).

Viraemia results for the viraemia subset were already assessed at time of MAA. In summary, viraemia was not detected in any of the participant on Day 1 (only one subject in the VLA1553 arm had an invalid result at Day 1); it was not detected at Day 8 in any of the subject of the placebo arm (irrespective of baseline serostatus); and it was not detected at Day 8 in any of the 9 baseline seropositive subjects of the VLA1553 arm. In baseline seronegative subjects administered VLA1553, vaccine viraemia was detected in 9/42 tested participants (4 had a quantifiable result and 5 had a result below LLOQ); 30/42 subjects had a result indicating absence of viraemia and 3/42 had an inconclusive result. The mean plasma viral RNA on Day 8 was 56,876.68 GCE/mL (range 4,882.10 to 190,038.30).

Within submission of Part C, the MAH committed to submit separate Tables summarizing viraemia results for the different categories of subjects. More specifically, a table summarizing data for the randomly selected subjects of the viraemia subset; and different tables summarizing data for the subjects that had retrospective investigation of viraemia or that had viraemia investigation during acute visits are expected to be included in VLA1553-321 part C CSR. When relevant, safety aspects triggering retrospective viraemia assessment or acute visits should also be specified. Corresponding separate viraemia listing should also be submitted.

2.5.3. Adverse events per serostatus

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.

In the VLA1553 arm, in the seronegative sub-group, there were 210 subjects aged 12 to <15 years, and 198 subjects aged 15 to <18 years (total of 408 subjects). In the seropositive sub-group, there were 34 subjects aged 12 to <15 years, and 60 subjects aged 15 to <18 years (total of 94 subjects). Because of the low number of seropositive adolescents in each age category, the comparison is not done for them hereafter.

2.5.3.1. Solicited adverse events

Solicited systemic and injection sites AE were collected until 10 days after single vaccination.

Table 42. Summary of Solicited Adverse Events up to Day 180 – Stratified by µPRNT Baseline Serostatus

Category [n (%) m]		Stratum: Seronegative by µPRNT			Stratum: Seropositive by µPRNT		
		VLA1553 (N=408)	Placebo (N=206)	Total (N=614)	VLA1553 (N=94)	Placebo (N=45)	Total (N=139)
Any Solicited Systemic Adverse Events		277 (67.9) 715	91 (44.2) 177	368 (59.9) 892	42 (44.7) 85	15 (33.3) 27	57 (41.0) 112
95% CI		63.1, 72.4	37.3, 51.2	55.9, 63.8	34.4, 55.3	20.0, 49.0	32.7, 49.7
P-value[a]				*<0.0001*			0.2689
Any Solicited Systemic Adverse Events by Maximum Severity	Mild	192 (47.1)	67 (32.5)	259 (42.2)	36 (38.3)	14 (31.1)	50 (36.0)
	Moderate	69 (16.9)	23 (11.2)	92 (15.0)	5 (5.3)	1 (2.2)	6 (4.3)
	Severe	16 (3.9)	1 (0.5)	17 (2.8)	1 (1.1)	0	1 (0.7)
Any Related Solicited Systemic Adverse Events		273 (66.9) 702	87 (42.2) 164	360 (58.6) 866	40 (42.6) 77	14 (31.1) 26	54 (38.8) 103
95% CI		62.1, 71.5	35.4, 49.3	54.6, 62.6	32.4, 53.2	18.2, 46.6	30.7, 47.5
P-value[a]				*<0.0001*			0.2644
Any Related Solicited Systemic Adverse Events by Maximum Severity	Mild	189 (46.3)	64 (31.1)	253 (41.2)	35 (37.2)	13 (28.9)	48 (34.5)
	Moderate	68 (16.7)	22 (10.7)	90 (14.7)	4 (4.3)	1 (2.2)	5 (3.6)
	Severe	16 (3.9)	1 (0.5)	17 (2.8)	1 (1.1)	0	1 (0.7)
Any Solicited Injection Site Adverse Events		132 (32.4) 198	54 (26.2) 81	186 (30.3) 279	28 (29.8) 38	7 (15.6) 9	35 (25.2) 47
95% CI		27.8, 37.1	20.3, 32.8	26.7, 34.1	20.8, 40.1	6.5, 29.5	18.2, 33.2
P-value[a]				0.1367			0.0945
Any Solicited Injection Site Adverse Events by Maximum Severity	Mild	127 (31.1)	51 (24.8)	178 (29.0)	28 (29.8)	7 (15.6)	35 (25.2)
	Moderate	3 (0.7)	2 (1.0)	5 (0.8)	0	0	0
	Severe	2 (0.5)	1 (0.5)	3 (0.5)	0	0	0
Any Related Solicited Injection Site Adverse Events		132 (32.4) 198	54 (26.2) 81	186 (30.3) 279	28 (29.8) 38	7 (15.6) 9	35 (25.2) 47
95% CI		27.8, 37.1	20.3, 32.8	26.7, 34.1	20.8, 40.1	6.5, 29.5	18.2, 33.2
P-value[a]				0.1367			0.0945
Any Related Solicited Injection Site Adverse Events by Maximum Severity	Mild	127 (31.1)	51 (24.8)	178 (29.0)	28 (29.8)	7 (15.6)	35 (25.2)
	Moderate	3 (0.7)	2 (1.0)	5 (0.8)	0	0	0
	Severe	2 (0.5)	1 (0.5)	3 (0.5)	0	0	0

AE=adverse event; CI=confidence interval; m=number of events; µPRNT=Micro Plaque Reduction Neutralization Test; MedDRA=Medical Dictionary for Regulatory Activities; n=number of participants

[a] P-value from Fisher's exact test for difference between the trial arms.

Note: Adverse events were coded using MedDRA version 24.1. Adverse events with causality reported as Possible or Probable were considered as Related to IMP. AEs with missing causality were classed as related. Two-sided exact Clopper-Pearson 95% CIs are presented.

One randomized participant (REC01-008) had no µPRNT sample collected. This impacts all stratified tables with µPRNT baseline serostatus.

Solicited local AEs (injection site pain, tenderness, erythema/redness, induration, and swelling)

Solicited injection site AEs were reported with similar frequency in subjects with both serostatus in the VLA1553 arm: 32.4% in seronegative vs. 29.8% in seropositive. In both strata, this frequency was higher in the VLA1553 vs. placebo (seronegative: 32.4% vs. 26.2%, respectively; seropositive: 29.8% vs. 15.6%, respectively).

In the VLA1553 arm, solicited injection site AEs were reported with similar frequency in seronegative subjects aged 12 to <15 years and seronegative subjects aged 15 to <18 years.

The proportion of subjects who experienced tenderness was numerically higher in the VLA1553 arm of the seronegative stratum compared to the seropositive stratum (21.6% and 12.8% subjects, respectively). This difference was also observed in the placebo arm (16% vs. 6.7%, respectively). The other solicited injection site AEs occurred at a similar frequency in each stratum.

Overall, most solicited injection site AEs were graded as mild in both strata and in both arms.

Moderate and severe solicited injection site AEs were reported only in the seronegative stratum. Five of 754 (0.7%) subjects experienced a moderate solicited injection site AE (3/502 [0.6%] in the VLA1553

arm and 2/252 [0.8%] in the placebo arm) (all in the seronegative strata). Only 3/754 (0.4%) subjects experienced a severe solicited injection site AE: 2/502 [0.4%] in the VLA1553 arm [1 event of induration and 1 of erythema/redness] and 1/252 [0.4%] in the placebo arm (all in the seronegative strata). In the VLA1553 arm, the 2 severe solicited injection site AEs were reported with in seronegative subjects aged 12 to <15 years (none reported in seronegative subjects aged 15 to <18 years).

All solicited injection site AEs were considered related to trial treatment.

Solicited systemic AEs (fever, fatigue, headache, nausea, vomiting, muscle pain/myalgia, joint pain/arthralgia, and rash)

Solicited systemic AEs were reported more frequently in seronegative subjects in the VLA1553 arm: 67.9% in seronegative vs. 44.7% in seropositive. Solicited systemic AEs were slightly more reported in seronegative subjects in the placebo arm: 44.2% in seronegative vs. 33.3% in seropositive.

There was a significant difference between trial arms in the seronegative stratum for solicited systemic AEs (VLA1553: 277/408 [67.9%]; placebo: 91/206 [44.2%], p<0.0001); no significant difference between treatment arms in the seropositive stratum was seen (VLA1553: 42/94 [44.7%]; placebo: 15/45 [33.3%], p=0.2689).

In the VLA1553 arm, solicited systemic AEs were reported with similar frequency in seronegative subjects aged 12 to <15 years and seronegative subjects aged 15 to <18 years.

For each solicited systemic AE, in the VLA1553 arm, the proportion of subjects with solicited systemic AE was higher in the seronegative stratum compared to the seropositive stratum. In the placebo arm, a similar difference was only observed for headache, myalgia and nausea.

In the VLA1553 and placebo arms, most cases were mild (seronegative: 46.3% vs. 31.1%, respectively; seropositive: 37.2% vs. 28.9%, respectively) or moderate (seronegative: 16.7% vs. 10.7%, respectively; seropositive: 4.3% vs. 2.2%, respectively).

The majority of severe solicited systemic AE, occurred in subjects in the seronegative stratum (VLA1553: 16/408 [3.9%] subjects; placebo: 1/206 [0.5%] subject, p<0.0162). One subject in the VLA1553 arm of the seropositive stratum also experienced a severe solicited systemic AE. All severe solicited systemic AEs were considered related to trial treatment.

In the seronegative stratum, severe solicited systemic AEs reported in the VLA1553 arm were fever (13 subjects), headache (four subjects), arthralgia (one subject), and myalgia (one subject) compared with headache (one subject) in the placebo arm. In the seropositive stratum, only fever was reported in the VLA1553 arm (1 subject). Some subjects in the VLA1553 experienced several severe solicited systemic AEs.

In the VLA1553 arm, severe solicited systemic AEs were reported with higher frequency in seronegative subjects aged 12 to <15 years (5.2%) compared to subjects aged 15 to <18 years (2.5%).

In the VLA1553 and placebo arms, most of the solicited systemic AEs were assessed as related to the vaccine. In the VLA1553 arm, the proportion of subjects with related solicited systemic AEs was higher in the seronegative stratum (66.9%) than the seropositive stratum (42.6%). Within the seronegative stratum, related solicited systemic events were seen more frequently in the VLA1553 arm than the placebo arm (66.9% and 42.2%, respectively, p<0.0001). No significant difference was seen between the treatment arms in the seropositive stratum (42.6% and 31.1%, respectively, p=0.2644). Most related solicited systemic AEs were graded as mild or moderate. All severe solicited systemic AEs were considered as related to trial treatment.

2.5.3.2. Unsolicited adverse events

Table 43. Summary of Unsolicited Adverse Events up to Day 29 and Day 180 – Stratified by µPRNT Baseline Serostatus

		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 Unsolicited Adverse Events up to Day 29		161 (39.5) 301	64 (31.1) 128	225 (36.6) 429	36 (38.3) 60	17 (37.8) 41	53 (38.1) 101
95% CI		34.7, 44.4	24.8, 37.9	32.8, 40.6	28.5, 48.9	23.8, 53.5	30.0, 46.7
P-value				*0.0420*			>0.9999
Any Unsolicited Adverse Events up to Day 29 by Maximum Severity	Mild	127 (31.1)	45 (21.8)	172 (28.0)	20 (21.3)	12 (26.7)	32 (23.0)
	Moderate	29 (7.1)	19 (9.2)	48 (7.8)	11 (11.7)	2 (4.4)	13 (9.4)
	Severe	5 (1.2)	0	5 (0.8)	5 (5.3)	3 (6.7)	8 (5.8)
Any Related Unsolicited Adverse Events up to Day 29		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				*<0.0001*			0.2449
Any Related Unsolicited Adverse Events up to Day 29 by Maximum Severity	Mild	33 (8.1)	1 (0.5)	34 (5.5)	1 (1.1)	2 (4.4)	3 (2.2)
	Moderate	4 (1.0)	0	4 (0.7)	0	0	0
	Severe	2 (0.5)	0	2 (0.3)	0	0	0
Any Unsolicited Adverse Events up to Month 6		256 (62.7) 895	124 (60.2) 500	380 (61.9) 1395	53 (56.4) 179	24 (53.3) 83	77 (55.4) 262
95% CI		57.9, 67.5	53.2, 66.9	57.9, 65.7	45.8, 66.6	37.9, 68.3	46.7, 63.8
P-value[a]				0.5394			0.8555
Any Unsolicited Adverse Events up to Month 6 by Maximum Severity	Mild	151 (37.0)	75 (36.4)	226 (36.8)	26 (27.7)	11 (24.4)	37 (26.6)
	Moderate	84 (20.6)	40 (19.4)	124 (20.2)	15 (16.0)	8 (17.8)	23 (16.5)
	Severe	21 (5.1)	9 (4.4)	30 (4.9)	12 (12.8)	5 (11.1)	17 (12.2)
Any Related Unsolicited Adverse Events up to Month 6		39 (9.6) 61	1 (0.5) 1	40 (6.5) 62	2 (2.1) 2	3 (6.7) 5	5 (3.6) 7
95% CI		6.9, 12.8	0.0, 2.7	4.7, 8.8	0.3, 7.5	1.4, 18.3	1.2, 8.2
P-value[a]				*<0.0001*			0.3287
Any Related Unsolicited Adverse Events up to Month 6 by Maximum Severity	Mild	33 (8.1)	1 (0.5)	34 (5.5)	2 (2.1)	2 (4.4)	4 (2.9)
	Moderate	4 (1.0)	0	4 (0.7)	0	1 (2.2)	1 (0.7)
	Severe	2 (0.5)	0	2 (0.3)	0	0	0
Any Related Severe Unsolicited Adverse Events (entire reporting period i.e., up to Month 6)		2 (0.5) 5	0	2 (0.3) 5	0	0	0
95% CI		0.1, 1.8	0.0, 1.8	0.0, 1.2	0.0, 3.8	0.0, 7.9	0.0, 2.6

CI=confidence interval; IMP=investigational medicinal product; µPRNT=micro plaque reduction neutralization test; m=number of events; MedDRA=Medical Dictionary for Regulatory Activities; n=number of participants

[a] P-value from Fisher's Exact test for difference between the trial arms.

Note: Adverse events were coded using MedDRA version 24.1. Adverse events with causality reported as Possible or Probable were considered as Related to IMP. Adverse events with missing causality were classed as related. Two-sided exact Clopper-Pearson 95% CIs are presented.

One randomized participant (REC01-008) had no µPRNT sample collected. This impacts all stratified tables with µPRNT baseline serostatus.

Unsolicited AE up to 28 days post vaccination

The proportion of subjects who experienced unsolicited AEs up to 28 days post-vaccination was similar in the VLA1553 groups of each stratum (baseline seronegative: 39.5%; baseline seropositive: 38.3%) and was comparable to the proportion of subjects with unsolicited AEs in the placebo group (baseline seronegative: 31.1%; baseline seropositive: 37.8%). No relevant difference between serostatus strata was observed.

Overall, amongst the most frequently reported unsolicited AEs in VLA1553:

- Headache, odynophagia, cough and neutropenia were reported with similar rate in both strata.
- Pyrexia was less reported in the seronegative stratum (3.2%) vs. the seropositive stratum (7.4%).
- Eye pain (3.7%) and abdominal pain (3.2%) were only reported in the seronegative stratum.

Overall, most unsolicited AEs were graded as mild or moderate, regardless of the serostatus stratum

Severe unsolicited AEs were mostly reported in the seropositive stratum (5/94 [5.3%] subjects in VLA1553 and 3/45 [6.7%] in placebo) vs. the seronegative stratum (5/408 [1.2%] subjects in VLA1553 and none in placebo).

A significantly higher frequency of related unsolicited AEs was reported in the VLA1553 arm compared with the placebo arm (9.6% versus 0.5%; p<0.0001) in the seronegative stratum (difference not observed in the seropositive stratum).

Related unsolicited AEs were mostly reported in the seronegative stratum (39/408 [9.6%] subjects in VLA1553 and 1/206 [0.5%] in placebo) vs. the seropositive stratum (1/94 [1.1%] subjects in VLA1553 and 2/45 [4.4%] in placebo). Most related unsolicited AEs were graded as mild or moderate.

Two of the 502 subjects (0.4%) in the VLA1553 arm experienced at least one related unsolicited AE that was graded severe (headache), both in the seronegative stratum (versus none in the placebo arm).

Unsolicited AE up to 6 months post vaccination

The proportion of subjects who experienced unsolicited AEs up to 6 months post-vaccination was similar in the VLA1553 groups of each stratum (baseline seronegative: 62.7%; baseline seropositive: 56.4%) and was comparable to the proportion of subjects with unsolicited AEs in the placebo group (baseline seronegative: 60.2%; baseline seropositive: 53.3%). No relevant difference between serostatus strata was observed.

There was no difference in the unsolicited AE frequency in subjects aged 12 to <15 years and subjects aged 15 to <18 years in the VLA1553 group when stratified by baseline serostatus.

Overall, the most frequently reported unsolicited AEs in VLA1553 (pyrexia, headache, and cough) were reported with similar rate in both strata.

Most unsolicited AEs after vaccination were graded as mild.

Severe unsolicited AEs were mostly reported in the seropositive stratum (seronegative: 21/408 [5.1%] subjects in the VLA1553 arm and 9/206 [4.4%] in the placebo arm; seropositive: 12/94 [12.8%] subjects in the VLA1553 arm and 5/45 [11.1%] in the placebo arm).

No difference was observed between treatment groups within the seronegative stratum (VLA1553: 5.1%; placebo: 4.4%) and within the seropositive stratum (VLA1553 12.8% and placebo 11.1%).

The severe unsolicited AE frequency was similar between subjects aged 12 to <15 years and subjects aged 15 to <18 years in the seronegative stratum of the VLA1553 group (4.8% and 5.6%).

Related unsolicited AEs were reported more frequently in subjects in the VLA1553 arm of the seronegative stratum vs. the VLA1553 arm of the seropositive stratum (39/408 [9.6%] and 2/94 [2.1%] subjects, respectively). Within the seronegative stratum, a significant difference was observed between treatment arms (VLA1553: 39/408 [9.6%] subjects; placebo: 1/206 [0.5%] subject; p<0.0001). No significant difference between treatment arms in the seropositive stratum was observed (VLA1553 2/94 [2.1%] and placebo 3/45 [6.7%] subjects, p=0.3287).

The related unsolicited AE frequency was similar between subjects aged 12 to <15 years and subjects aged 15 to <18 years in the seronegative stratum of the VLA1553 group (9.0% and 10.1%).

Overall, most related unsolicited AEs were graded as mild or moderate.

Related severe unsolicited AEs were seen in 2 subjects in the VLA1553 arm of the seronegative stratum only (headache).

Two (0.5%) subjects in the seronegative stratum of the VLA1553 group had related severe unsolicited AEs (one subject each in the 12 to <15 years of age subgroup and 15 to <18 years of age subgroup); no related severe unsolicited were reported in the seropositive strata. One subject (age group 12 to <15 years) had related severe headache of short duration ; one subject (age group 15 to < 18 years) had related severe headache of intermittent occurrence, each occurrence resolved on the same day of onset.

2.5.3.3. Serious adverse events/deaths/other significant events

There were no deaths in study VLA3221-321.

The proportion of subjects who experienced SAEs up to Month 6 was similar in the VLA1553 groups of each stratum (seronegative: 1.2% [7 events in 5 subjects]; seropositive: 2.1% [2 events in 2 subjects]) and was comparable to the proportion of subjects with SAEs in the placebo group (seronegative: 1.0% [6 events in 2 subjects]; seropositive: 4.4% [2 events in 2 subjects]).

The SOC for which most SAEs were documented was infections and infestations (2/502 [0.4%] participants in the VLA1553 arm and 2/252 [0.8%] participants in the placebo arm; all in the seronegative stratum). The most frequently reported SAE was pyrexia (3/754 [0.4%] participants), followed by pneumonia (2/754 [0.3%] participants). All other SAEs were reported once only.

In the seronegative stratum, the following SAEs were reported:

- VLA1553: 1 pneumonia, 1 appendicitis, 2 pyrexia, 1 juvenile myoclonic epilepsy, 1 abdominal pain, 1 hyperkalaemia
- Placebo: 1 pneumonia, 1 pharyngitis, 1 pyrexia, 1 fatigue ; 1 headache, 1 suicidal ideation

In the seropositive stratum, the following SAEs were reported:

- VLA1553: 1 neutropenia, 1 lower limb fracture
- Placebo: 1 prothrombin time prolonged, 1 transaminases increased

There was no difference in the SAE frequency between subjects aged 12 to <15 years and subjects aged 15 to <18 years in the seronegative stratum of the VLA1553 group (1.0% and 1.5%) and in the seropositive stratum of the VLA1553 group (2.9% and 1.7%).

Only 1 related SAE (grade 4 fever) was reported, which was a solicited AE in the VLA1553 arm of the seronegative stratum.

2.5.3.4. Adverse events of special interest (AESI)

2.5.3.4.1. AESI (protocol definition)

Table 44. Summary of Adverse Events of Special Interest up to Day 180 – Stratified by µPRNT Baseline Serostatus

		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 AESI		38 (9.3) 44	16 (7.8) 17	54 (8.8) 61	4 (4.3) 4	3 (6.7) 3	7 (5.0) 7
Any AESI by Maximum Severity	Mild	15 (3.7)	10 (4.9)	25 (4.1)	2 (2.1)	0	2 (1.4)
	Moderate	18 (4.4)	5 (2.4)	23 (3.7)	0	1 (2.2)	1 (0.7)
	Severe	5 (1.2)	1 (0.5)	6 (1.0)	2 (2.1)	2 (4.4)	4 (2.9)
Any Early Onset Adverse Events of Special Interest		17 (4.2) 17	1 (0.5) 1	18 (2.9) 18	0	1 (2.2) 1	1 (0.7) 1
Any Early Onset Adverse Events of Special Interest by Maximum Severity	Mild	7 (1.7)	0	7 (1.1)	0	0	0
	Moderate	6 (1.5)	1 (0.5)	7 (1.1)	0	1 (2.2)	1 (0.7)
	Severe	4 (1.0)	0	4 (0.7)	0	0	0
Any Related Early Onset Adverse Events of Special Interest		14 (3.4) 14	0	14 (2.3) 14	0	1 (2.2) 1	1 (0.7) 1
Any Related Early Onset Adverse Events of Special Interest by Maximum Severity	Mild	5 (1.2)	0	5 (0.8)	0	0	0
	Moderate	5 (1.2)	0	5 (0.8)	0	1 (2.2)	1 (0.7)
	Severe	4 (1.0)	0	4 (0.7)	0	0	0
Any Late Onset Adverse Events of Special Interest		25 (6.1) 27	15 (7.3) 16	40 (6.5) 43	4 (4.3) 4	2 (4.4) 2	6 (4.3) 6
Any Late Onset Adverse Events of Special Interest by Maximum Severity	Mild	12 (2.9)		10 (4.9)		22 (3.6)	2 (2.1)
	Moderate	12 (2.9)		4 (1.9)		16 (2.6)	0
	Severe	1 (0.2)		1 (0.5)		2 (0.3)	2 (2.1)

AESI=adverse event of special interest; m=number of events; MedDRA=Medical Dictionary for Regulatory Activities; µPRNT=Micro Plaque Reduction Neutralization Test; n=number of participants
Note: Adverse events were coded using MedDRA version 24.1.
One randomized participant (REC01-008) had no µPRNT sample collected. This impacts all stratified tables with µPRNT baseline serostatus.

AESIs (early and late onset) were reported with slightly higher frequency in the VLA1553 arm for subjects in the seronegative stratum (38/408 [9.3%]) compared to the seropositive stratum (4/94 [4.3%]). No difference was observed for the placebo arm between the seronegative stratum (16/206 [7.8%]) and the seropositive stratum (3/45 [6.7%]).

The rate of AESIs was slightly higher in subjects aged 12 to <15 years compared to subjects aged 15 to <18 years in the seronegative stratum of the VLA1553 group (11.4% and 7.1%); within the seropositive stratum, the rate of AESIs was higher in subjects aged 12 to <15 years compared to subjects aged 15 to <18 years (11.8% and 0%); the rate of AESIs in baseline seropositive subjects aged 12 to <15 years (11.8%) was comparable to the rate of AESIs in subjects aged 12 to <15 years in the seronegative stratum (11.4%).

The most frequent AESI was a combination of headache and pyrexia:

- seronegative: 32/408 (7.8%) in VLA1553 and 15/206 (7.3%) in placebo

- seropositive: 4/94 (4.3%) in VLA1553 and 3/45 (6.7%) in placebo.

The second most frequent AESI was a combination of pyrexia and myalgia seen in:

- seronegative: 22/408 (5.4%) in VLA1553 and 11/206 (5.3%) in placebo
- seropositive: 4/94 (4.3%) in VLA1553 and 2/45 (4.4%) in placebo.

By Day 180, the majority of AESIs were late onset AESIs. In total, 19/754 (2.5%) subjects experienced an early onset AESI: 17/408 [4.2%] in the VLA1553 arm and 1/206 [0.5%] in the placebo arm of the seronegative stratum, and none in the VLA1553 arm and only 1/45 [2.2%] in the placebo arm of the seropositive stratum.

Late onset AESIs were reported for 46/754 (6.1%) subjects overall: 25/408 (6.1%) in the VLA1553 arm and 15/206 (7.3%) in the placebo arm in the seronegative stratum, and 4/94 (4.3%) in the VLA1553 arm and 2/45 (4.4%) in the placebo arm in the seropositive stratum. Up to Day 180, all AESIs were resolved.

The most common early onset AESI symptoms, in the seronegative stratum, were a combination of pyrexia and headache (16/408 [3.9%] in the VLA1553 arm and 1/206 [0.5%] in the placebo arm), myalgia (10/408 [2.5%] in the VLA1553 arm and 1/206 [0.5%] in the placebo arm), or arthralgia (7/408 [1.7%] in VLA1553 vs. none in placebo).

There was only 1 early onset AESI in the seropositive stratum: combination of fever with headache, myalgia, and arthralgia (placebo arm).

The most common late onset AESI symptoms, in the seronegative stratum, were a combination of pyrexia and headache (17/408 [4.2%] in the VLA1553 arm and 14/206 [6.8%] in the placebo arm), or myalgia (14/408 [3.4%] in the VLA1553 arm and 10/206 [4.9%] in the placebo arm).

The most common late onset AESI symptoms, in the seropositive stratum, were a combination of pyrexia and headache (4/94 [4.3%] in the VLA1553 arm and 2/45 [4.4%] in the placebo arm), or myalgia (4/94 [4.3%] in the VLA1553 arm and 1/45 [2.2%] in the placebo arm).

In the seronegative stratum, most AESIs were graded as mild (4.1%) or moderate (3.7%) (including both arms). In the seropositive stratum, most AESIs were graded as severe (2.9%) (including both arms).

Ten of 754 (1.3%) subjects experienced an AESI that was graded severe, comprising 5/408 (1.2%) subjects in the VLA1553 arm and 1/206 (0.5%) subject in the placebo arm of the seronegative stratum, and 2/94 (2.1%) subjects in the VLA1553 arm and 2/45 (4.4%) subjects in the placebo arm of the seropositive stratum.

Severe AESI were reported in both age groups of the baseline seronegative stratum of the VLA1553 group (12 to <15 years: 1.4%, 3/210 subjects; 15 to <18 years: 1.0%, 2/198 subjects).

Severe AESIs were only reported in subjects 12 to <15 years of age in the seropositive stratum of the VLA1553 group (12 to <15 years: 5.9%, 2/34 subjects; 15 to <18 years: 0/60 subjects) (VLA1553-321 post hoc analysis AEs by serostatus and age group, v2.0, 12-Feb-2025, Table 2).

Severe AESIs of pyrexia, headache, and arthralgia were reported. Severe pyrexia occurred in 4/408 (1.0%) participants in the VLA1553 arm and 1/206 (0.5%) participant in the placebo arm of the seronegative stratum, and in 2/94 (2.1%) participants in the VLA1553 arm and 2/45 (4.4%) participant in the placebo arm of the seropositive stratum. Severe headache occurred in 2/408 (0.5%) participants in the VLA1553 arm of the seronegative stratum, and severe arthralgia occurred in 1/408 (0.2%) participant in the VLA1553 arm of the seronegative stratum.

Of the 10 participants who experienced a severe AESI, 4 participants experienced severe early onset AESIs (all in the VLA1553 arm of the seronegative stratum: 3 severe pyrexia, 2 severe headache, and 1 severe arthralgia), and 6 experienced a severe late onset AESI (2 in the seronegative stratum [1 in each arm] and 4 in the seropositive stratum [2 in each arm]).

The majority of early onset AESIs were considered related to trial treatment; related early onset AESIs were reported for 15/754 (2.0%) subjects: 14/408 (3.4%) subjects in the VLA1553 arm of the seronegative stratum (none in the placebo arm), and 1/45 (2.2%) subject in the placebo arm of the seropositive stratum (none in the VLA1553 arm).

In the seronegative stratum, VLA1553 arm, the most common related early onset AESI symptoms were a combination of pyrexia and headache (13/408 [3.2%]), myalgia (9/408 [2.2%]), or arthralgia (6/408 [1.5%]).

Most related early onset AESIs (mainly in the seronegative stratum VLA1553 arm) were graded as mild or moderate. Four of 754 (0.5%) subjects experienced a related early onset AESI that was graded severe, all in the VLA1553 arm of the seronegative stratum: symptoms of pyrexia (3/408 [0.7%]), headache (2/408 [0.5%]), and arthralgia (1/408 [0.2%]).

No related late onset AESIs were reported. Up to Day 180, all AESIs were resolved.

2.5.3.4.2. Chikungunya-like Adverse Reactions (Post-hoc Analysis) (abbreviated: CHIK-like ARs)

Early onset CHIK-like ARs

Table 45. Subjects with CHIK-like adverse reactions by serostatus at baseline (Safety Population)

	VLA1553 (N=94) Baseline Seropositive	VLA1553 (N=408) Baseline Seronegative	Placebo (N=45) Baseline Seropositive	Placebo (N=206) Baseline Seronegative
	n (%)	n (%)	n (%)	n (%)
Any CHIK-like adverse reaction	6 (6.4)	110 (27.0)	4 (8.9)	8 (3.9)
Any related CHIK-like adverse reaction	5 (5.3)	108 (26.5)	4 (8.9)	6 (2.9)
Any severe CHIK-like adverse reaction	1 (1.1)	17 (4.2)	1 (2.2)	0 (0.0)
Any serious CHIK-like adverse reaction	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)
CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like adverse reaction, percentages are based on N. Source: Post Hoc analysis, v3.0, 23-May-2024, Table 10, Table 11.				

Table 46. Symptoms of CHIK-like adverse reactions by CHIKV serostatus at baseline (subjects with* any CHIK-like adverse reac*tion)

	VLA1553 (N=6) Participants With CHIK-like Adverse Reaction Baseline Seropositive	VLA1553 (N=110) Participants With CHIK-like Adverse Reaction Baseline Seronegative	Placebo (N=4) Participants With CHIK-like Adverse Reaction Baseline Seropositive	Placebo (N=8) Participants With CHIK-like Adverse Reaction Baseline Seronegative
	n (%) Obs	n (%) Obs	n (%) Obs	n (%) Obs
Any CHIK-like symptom	6 (100) 17	110 (100) 392	4 (100) 13	8 (100) 23
Fever	6 (100) 6	110 (100) 118	4 (100) 4	8 (100) 8
Headache	5 (83.3) 6	96 (87.3) 108	3 (75.0) 4	8 (100) 8
Myalgia	5 (83.3) 5	62 (56.4) 64	2 (50.0) 2	1 (12.5) 1
Fatigue	0 (0.0) 0	51 (46.4) 51	1 (25.0) 1	5 (62.5) 5
Arthralgia	0 (0.0) 0	33 (30.0) 33	1 (25.0) 1	1 (12.5) 1
Rash	0 (0.0) 0	11 (10.0) 11	0 (0.0) 0	0 (0.0) 0
Chills	0 (0.0) 0	4 (3.6) 4	0 (0.0) 0	0 (0.0) 0
Hand dermatitis	0 (0.0) 0	1 (0.9) 1	0 (0.0) 0	0 (0.0) 0
Paraesthesia	0 (0.0) 0	1 (0.9) 1	0 (0.0) 0	0 (0.0) 0
Pruritus	0 (0.0) 0	0 (0.0) 0	1 (25.0) 1	0 (0.0) 0
Rash maculo-papular	0 (0.0) 0	1 (0.9) 1	0 (0.0) 0	0 (0.0) 0

CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with CHIK-like symptom, percentages are based on N, Obs=number of CHIK-like symptoms.
Source: Post Hoc analysis, v3.0, 23-May-2024, Table 2.1.

Table 47. Severe symptoms of CHIK-like adverse reactions by CHIKV serostatus at baseline (subjects with any CHIK-like adverse reaction)

	VLA1553 (N=6) Participants With CHIK-like Adverse Reaction Baseline Seropositive	VLA1553 (N=110) Participants With CHIK-like Adverse Reaction Baseline Seronegative	Placebo (N=4) Participants With CHIK-like Adverse Reaction Baseline Seropositive	Placebo (N=8) Participants With CHIK-like Adverse Reaction Baseline Seronegative
	n (%) Obs	n (%) Obs	n (%) Obs	n (%) Obs
Any severe CHIK-like symptom	1 (16.7) 1	17 (15.5) 21	1 (25.0) 1	0 (0.0) 0
Fever	1 (16.7) 1	14 (12.7) 14	1 (25.0) 1	0 (0.0) 0
Headache	0 (0.0) 0	4 (3.6) 5	0 (0.0) 0	0 (0.0) 0
Arthralgia	0 (0.0) 0	1 (0.9) 1	0 (0.0) 0	0 (0.0) 0
Myalgia	0 (0.0) 0	1 (0.9) 1	0 (0.0) 0	0 (0.0) 0

CHIK-like adverse reaction=Chikungunya-like adverse reaction; n=number of participants with severe CHIK-like symptom, percentages are based on N, Obs=number of severe CHIK-like symptoms.
Source: Post Hoc analysis, v3.0, 23-May-2024, Table 3.1.

Early onset CHIK-like ARs were reported with higher frequency in the VLA1553 arm for subjects of the seronegative stratum (110/408 [27%]) compared to the seropositive stratum (6/94 [6.4%]). For the placebo arm, they were slightly less reported in the seronegative stratum (8/206 [3.9%]) compared to the seropositive stratum (4/45 [8.9%]) (Table 45).

Among the baseline seronegative subjects with early onset CHIK-like ARs in the VLA1553 group, the most common symptoms were fever in combination with headache (87.3%), myalgia (56.4%), fatigue (46.4%), and arthralgia (30.0%).

The baseline seropositive subjects with early onset CHIK-like ARs in the VLA1553 group experienced, beside fever, the symptoms of headache and myalgia (Table 46).

In CHIKV baseline seronegative VLA1553 participants, the percentage of early onset CHIK-like ARs (all, related, severe) was slightly higher in participants aged 12 to <15 years compared to 15 to <18 years (all: 29.0% and 24.7%, related: 28.6% and 24.2%, severe: 5.2% and 3.0%, respectively; p>0.05). The number of CHIKV baseline seropositive VLA1553 participants within the age subgroups was small (12 to <15 years: n=34, 15 to <18 years: n=60) which limits the interpretation of data.

Serious early onset CHIK-like ARs:

The only subject who had a serious CHIK-like AR was baseline seronegative in the VLA1553 arm (Grade 4 fever, refer to SAE section)

There were no serious early onset CHIK-like AR reported in the placebo group.

Severe early onset CHIK-like ARs:

Severe early onset CHIK-like ARs were reported with higher frequency in the VLA1553 arm for subjects of the seronegative stratum (17/408 [4.2%]) compared to the seropositive stratum (1/94 [1.1%]). No difference was observed for the placebo arm between the seronegative stratum (placebo: 0/206 [0%]) and the seropositive stratum (placebo: 1/45 [2.2%]).

Among the baseline seronegative subjects with CHIK-like ARs in the VLA1553 group, the most common severe symptoms were fever (12.7%); other severe symptoms were headache (3.6%), arthralgia and myalgia (0.9% each). There was one severe event of fever each in the baseline seropositive VLA1553 and placebo group, respectively (Table 47).

Causality of early onset CHIK-like ARs:

Most early onset CHIK-like ARs were classified as related (i.e., at least one symptom was assessed as related to vaccination by the investigator): 26.5% in the VLA1553 arm and 2.9% in the placebo arm of the seronegative stratum, and 5.3% and 8.9%, respectively, in the seropositive stratum.

Late-onset CHIK-like AEs

Table 48. Participants with late-onset CHIK-like AEs by CHIKV baseline serostatus (Safety Population)

	VLA1553 (N=408)	VLA1553 (N=94)	Placebo (N=206)	Placebo (N=45)
	Baseline seronegative	Baseline seropositive	Baseline seronegative	Baseline seropositive
	n (%)	n (%)	n (%)	n (%)
Any cases with late-onset CHIK-like AE	38 (9.3)	8 (8.5)	29 (14.1)	6 (13.3)
Any cases with late-onset related CHIK-like AE	0	0	0	1 (2.2)
Any cases with late-onset severe CHIK-like AE	4 (1.0)	4 (4.3)	3 (1.5)	2 (4.4)
Any cases with late-onset serious CHIK-like AE	0	0	1 (0.5)	0

	VLA1553 (N=408)	VLA1553 (N=94)	Placebo (N=206)	Placebo (N=45)
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like AE; percentages are based on N. Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 1.2, Table 1.3.				

Table 49. Participants with symptoms of late-onset CHIK-like AEs by CHIKV baseline serostatus (participants with any late-onset CHIK-like AE)

	VLA1553 Participants With Late-Onset CHIK-like AE (N=38)	VLA1553 Participants With Late-Onset CHIK-like AE (N=8)	Placebo Participants With Late-Onset CHIK-like AE (N=29)	Placebo Participants With Late-Onset CHIK-like AE (N=6)
	Baseline seronegative	Baseline seropositive	Baseline seronegative	Baseline seropositive
	n (%)	n (%)	n (%)	n (%)
Any CHIK-like symptom	38 (100) 135	8 (100) 27	29 (100) 100	6 (100) 16
Pyrexia	38 (100) 53	8 (100) 9	29 (100) 35	6 (100) 6
Headache	28 (73.7) 40	8 (100) 10	22 (75.9) 32	5 (83.3) 5
Myalgia	20 (52.6) 25	6 (75.0) 7	15 (51.7) 18	1 (16.7) 1
Fatigue	6 (15.8) 7	0 (0.0) 0	4 (13.8) 5	1 (16.7) 1
Arthralgia	4 (10.5) 4	1 (12.5) 1	2 (6.9) 2	2 (33.3) 2
Malaise	3 (7.9) 3	0 (0.0) 0	1 (3.4) 1	0 (0.0) 0
Chills	1 (2.6) 1	0 (0.0) 0	4 (13.8) 4	1 (16.7) 1
Pruritus	1 (2.6) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Rash pruritic	1 (2.6) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
Rash maculo-papular	0 (0.0) 0	0 (0.0) 0	2 (6.9) 2	0 (0.0) 0
Erythema	0 (0.0) 0	0 (0.0) 0	1 (3.4) 1	0 (0.0) 0

Table 50. Participants with severe symptoms of late-onset CHIK-like AEs by CHIKV baseline serostatus (participants with any late-onset CHIK-like AE)

	VLA1553 Participants With Late-Onset CHIK-like AE (N=38)	VLA1553 Participants With Late-Onset CHIK-like AE N=8)	Placebo Participants With Late-Onset CHIK-like AE (N=29)	Placebo Participants With Late-Onset CHIK-like AE (N=6)
	Baseline seronegative	Baseline seropositive	Baseline seronegative	Baseline seropositive
	n (%)	n (%)	n (%)	n (%)
Any severe CHIK-like symptom	4 (10.5) 4	4 (50.0) 4	3 (10.3) 3	2 (33.3) 2
Pyrexia	3 (7.9) 3	4 (50.0) 4	2 (6.9) 2	2 (33.3) 2
Fatigue	0 (0.0) 0	0 (0.0) 0	1 (3.4) 1	0 (0.0) 0
Rash pruritic	1 (2.6) 1	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0
CHIK-like AE=Chikungunya-like adverse event; n=number of participants with CHIK-like symptom, percentages are based on N, Obs=number of CHIK-like symptoms. Source: Post hoc analysis late onset CHIK-like AE, v1.0, 12-Dec-2024, Table 3.1.				

In the VLA1553 arm, the frequency of late-onset CHIK-like AEs was comparable between baseline seronegative and baseline seropositive participants (9.3% and 8.5%, respectively); same was observed in the placebo arm (14.1% and 13.3%, respectively). The proportion of baseline seronegative VLA1553 participants with severe late-onset CHIK-like AEs was slightly lower compared to baseline seropositive participants (1.0% and 4.3%, respectively); a similar trend was observed in the placebo arm (1.5% and 4.4%, respectively) (Table 48).

Among the 38 baseline seronegative participants with late-onset CHIK-like AEs in the VLA1553 arm, the most common symptoms were fever with headache (73.7%), myalgia (52.6%), fatigue (15.8%), and arthralgia (10.5%). Similar frequencies were reported in the 29 baseline seronegative participants of the placebo arm. The 8 baseline seropositive participants with late-onset CHIK-like AEs in the VLA1553 arm experienced symptoms of headache (100%), myalgia (75%), and arthralgia (12.5%), in addition to fever. The comparison between seronegative and seropositive subgroups is not possible due to the small number of participants in the seropositive subgroup (Table 49).

In CHIKV baseline seronegative VLA1553 participants, the percentage of late onset CHIK-like AEs (all, severe) was slightly higher in participants aged 12 to <15 years compared to 15 to <18 years (all: 11.9% and 6.6%, severe: 1.4% and 0.5%, respectively; p>0.05). No baseline seronegative participants with related late onset CHIK-like AEs were identified in either age group. The number of CHIKV baseline seropositive VLA1553 participants within the age subgroups was small (12 to <15 years: n=34, 15 to <18 years: n=60) which limits the interpretation of data.

Among baseline seronegative participants experiencing late-onset CHIK-like AEs, the incidence of severe late-onset CHIK-like symptoms was comparable between the VLA1553 arm and placebo arm (10.5% and 10.3%, respectively), with a comparable frequency of severe fever (7.9% and 6.9%, respectively). One event each of severe pruritic rash was observed in the baseline seronegative subgroup in the VLA1553 arm (Table 50).

2.5.3.5. Medically attended events

Up to 6 months, within the VLA1553 arm, a numerically higher proportion of subjects experienced a MAAEs (100/408 [24.5%] subjects) in the seronegative stratum compared to the seropositive stratum (11/94 [11.7%] subjects). Within the placebo arm, a numerically higher proportion of subjects also experienced a MAAEs (53/206 [25.7%] subjects) in the seronegative stratum compared to the seropositive stratum (3/45 [6.7%] subjects). No difference was observed between treatment groups within the seronegative stratum (VLA1553: 24.5%; placebo: 25.7%) and within the seropositive stratum (VLA1553: 11.7%; placebo: 6.7%).

By CHIKV Baseline Serostatus and Age: There was no difference in the MAAE frequency between subjects aged 12 to <15 years and subjects aged 15 to <18 years in the seronegative stratum of the VLA1553 group (24.3% and 24.7%); within the seropositive stratum, the rate of MAAEs was higher in subjects aged 12 to <15 years compared to subjects aged 15 to <18 years (20.6% and 6.7%); the rate of MAAEs in baseline seropositive subjects aged 12 to <15 years (20.6%) was comparable to the rate of MAAEs in subjects aged 12 to <15 years in the seronegative stratum (24.3%).

In the seronegative stratum, the most common MAAEs (≥5.0% of subjects) in the VLA1553 arm were pyrexia (39/408 [9.6%]), headache (32/408 [7.8%]), and myalgia (23/408 [5.6%]). In the seropositive stratum, the only MAAEs reported at least twice was headache.

Most medically attended AEs were graded as mild or moderate in both strata. In total, 21/754 (2.8%) subjects (14/502 [2.8%] in the VLA1553 arm and 7/252 [2.8%] in the placebo arm) experienced at least one medically attended AE that was graded severe.

In the seronegative stratum, 12/408 (2.9%) subjects in the VLA1553 arm and 6/206 (2.9%) in the placebo arm experienced a severe MAAEs. In the seropositive arm, 2/94 (2.1%) and 1/45 (2.2%) subjects in the VLA1553 and placebo arms, respectively, experienced a severe MAAEs.

Within the VLA1553 arm, a numerically higher proportion of subjects experienced a related MAAEs (38/408 [9.3%] subjects) in the seronegative stratum compared to the seropositive stratum (2/94 [2.1%] subjects). Within the placebo arm, a numerically higher proportion of subjects also experienced

a related MAAEs (15/206 [7.3%] subjects) in the seronegative stratum compared to the seropositive stratum (none). No difference was observed between treatment groups within the seronegative stratum (VLA1553: 9.3%; placebo: 7.3%) and within the seropositive stratum (VLA1553: 2.1%; placebo: 0%).

By CHIKV Baseline Serostatus and Age: The related MAAE frequencies were similar between subjects aged 12 to <15 years and subjects aged 15 to <18 years in the seronegative stratum of the VLA1553 group (7.6% and 11.1%); and also in the seropositive stratum of the VLA1553 group (2.9% and 1.7%).

2.5.3.6. Analysis performed across trials

Although the limitations of comparing different clinical studies are acknowledged, the MAH was required to present and discuss the safety in adolescents vaccinated with VL1553 (study VLA1553 321) compared to the adults (Pooled Safety Population studies VLA1553 301, VLA1553 302, and VLA1553 101: mainly seronegative).

Table 51. Overall safety summary up to Day 180 by Baseline µPRNT Serostatus (Safety Population)

	Adolescents (VLA1553-321)				**Adults (pooled safety population: VLA1553-301, VLA1553-302, and VLA1553-101)**	
	Seronegative [a]		**Seropositive [b]**		**Mainly seronegative [c]**	
Arm	VLA1553	PBO	VLA1553	PBO	VLA1553	PBO
N	408	206	94	45	3610	1033
Solicited local AEs	32.4%	26.2%	29.8%	15.6%	15.2%	11.1%
Tenderness	21.6%	16.0%	12.8%	6.7%	10.8%	8.1%
Pain	18.6%	14.1%	22.3%	8.9%	6.1%	3.7%
Induration	4.2%	4.4%	4.3%	4.4%	1.4%	0.8%
Erythema/ Redness	2.5%	1.5%	1.1%	0%	1.6%	1.5%
Swelling	1.7%	3.4%	0%	0%	0.7%	0.8%
Solicited Systemic AEs	67.9%	44.2%	44.7%	33.3%	51.1%	26.9%
Headache	54.7%	36.9%	35.1%	22.2%	32.0%	14.6%
Myalgia	28.7%	13.1%	19.1%	6.7%	23.7%	7.4%
Fatigue	24.8%	9.2%	11.7%	11.1%	29.4%	12.6%
Fever	28.2%	2.4%	6.4%	8.9%	13.8%	0.8%
Nausea	17.4%	14.1%	9.6%	4.4%	11.4%	5.6%
Arthralgia	14.5%	5.3%	6.4%	4.4%	16.6%	4.8%
Vomiting	2.9%	4.4%	1.1%	0%	2.0%	1.0%
Rash	4.2%	0.5%	1.1%	2.2%	2.4%	0.5%
Unsolicited AEs	62.7%	60.2%	56.4%	53.3%	31.6%	23.9%
Related unsolicited AEs	9.6%	0.5%	2.1%	6.7%	11.6%	4.6%
SAEs	1.2%	1.0%	2.1%	4.4%	1.4%	0.8%
Related SAEs	0.2%	0%	0%	0%	0.1%	0%
MAAEs	24.5%	25.7%	11.7%	6.7%	12.3%	11.3%
Related MAAEs	9.3%	7.3%	2.1%	0%	1.9%	0.7%

	Adolescents (VLA1553-321)				**Adults (pooled safety population: VLA1553-301, VLA1553-302, and VLA1553-101)**	
	Seronegative [a]		**Seropositive [b]**		**Mainly seronegative [c]**	
Arm	VLA1553	PBO	VLA1553	PBO	VLA1553	PBO
N	408	206	94	45	3610	1033
AESI (≤6 months after vaccination)	9.3%	7.8%	4.3%	6.7%	0.3%	0.1%
Early onset AESI (≤21 days after vaccination)	4.2%	0.5%	0%	2.2%	0.3%	0.1%
Late onset AESI (>21d, ≤6 months after vaccination)	6.1%	7.3%	4.3%	4.4%	n/a[d]	n/a[d]
Related AESI (≤6 months after vaccination)	3.4%	0%	0%	2.2%	0.3%	0.1%
Related early onset AESI (≤21 days after vaccination)	3.4%	0%	0%	2.2%	0.3%	0.1%
Related late onset AESI (>21d, ≤6 months after vaccination	0%	0%	0%	0%	n/a[d]	n/a[d]
CHIK-like ARs (≤6 months after vaccination)	32.8%	17.0%	14.9%	20.0%	12.1%	0.6%
Early onset CHIK-like ARs (≤30 days after vaccination)	27.0%	3.9%	6.4%	8.9%	12.1%	0.6%
Late onset CHIK-like ARs (>30d, ≤6 months after vaccination)	9.3%	14.1%	8.5%	13.3%	n/a[e]	n/a[e]
Related CHIK-like ARs (≤6 months after vaccination)	26.5%	2.9%	5.3%	11.1%	11.6%	0.6%
Related early onset CHIK-like ARs (≤30 days after vaccination)	26.5%	2.9%	5.3%	8.9%	11.6%	0.6%
Related late onset CHIK-like ARs (>30d, ≤6 months after vaccination)	0%	0%	0%	2.2%	n/a[e]	n/a[e]

n/a=not applicable

[a] $\mu PRNT_{50}$ ≤40 at baseline
[b] $\mu PRNT_{50}$ >40 at baseline
[c] In the VLA1553-301 trial, 99.9% of participants in the VLA1553 arm and 99.7% in the placebo arm were baseline CHIKV seronegative ($\mu PRNT_{50}$ ≤40). In the VLA1553-302 trial, 98.0% of participants were baseline CHIKV seronegative ($\mu PRNT_{50}$ ≤40), and in the VLA1553-101 trial, 99.2% of participants were baseline CHIKV seronegative ($\mu PRNT_{50}$ ≤40).
[d] In trials VLA1553-101, -301 and -302, only early onset AESI were documented.
[e] In trials VLA1553-101, -301 and -302, only early onset AESI were documented, therefore, no post hoc analysis for late onset CHIK-like AEs was conducted.

Table 52. Summary of AEs up to Day 180 for the pooled dataset including relative risks (Adults (pooled safety population: VLA1553-301, VLA1553-302, and VLA1553-101))

	Statistic	VLA1553 (N=3610)	Placebo (N=1033)	Relative Risk VLA1553/Placebo	Relative Risk CI VLA1553/Placebo
Any solicited AE	n (%) [95% CI]	1940 (53.7) [52.1, 55.4]	331 (32.0) [29.3, 35.0]	1.677	[1.529, 1.845]
Any related solicited AE	n (%) [95% CI]	1806 (50.0) [48.4, 51.7]	301 (29.1) [26.4, 32.0]	1.717	[1.555, 1.901]
Any unsolicited AE	n (%) [95% CI]	1140 (31.6) [30.1, 33.1]	247 (23.9) [21.4, 26.6]	1.321	[1.174, 1.489]
Any related unsolicited AE	n (%) [95% CI]	420 (11.6) [10.6, 12.7]	48 (4.6) [3.5, 6.1]	2.504	[1.878, 3.349]
Any serious AE	n (%) [95% CI]	52 (1.4) [1.1, 1.9]	8 (0.8) [0.4, 1.5]	1.860	[0.902, 3.845]
Any related serious AE	n (%) [95% CI]	2 (0.1) [0.0, 0.2]	0 (0.0) [0.0, 0.4]	NC	[0.069, 29.788]
Any medically attended AE	n (%) [95% CI]	445 (12.3) [11.3, 13.4]	117 (11.3) [9.5, 13.4]	1.088	[0.900, 1.319]
Any related medically attended AE	n (%) [95% CI]	70 (1.9) [1.5, 2.4]	7 (0.7) [0.3, 1.4]	2.861	[1.347, 6.100]
Any early onset AESI (protocol definition)	n (%) [95% CI]	11 (0.3) [0.2, 0.5]	1 (0.1) [0.0, 0.5]	3.148	[0.524, 18.961]
Any early onset related AESI (protocol definition)	n (%) [95% CI]	10 (0.3) [0.2, 0.5]	1 (0.1) [0.0, 0.5]	2.861	[0.473, 17.340]
Any early onset CHIK-like AR	n (%) [95% CI]	436 (12.1) [11.1, 13.2]	6 (0.6) [0.3, 1.3]	20.794	[9.530, 45.532]
Any early onset related CHIK-like AR	n (%) [95% CI]	418 (11.6) [10.6, 12.7]	6 (0.6) [0.3, 1.3]	19.935	[9.135, 43.662]

CI...confidence interval
Two-sided 95% confidence intervals are calculated according to Altman (Wilson score interval) for adverse event frequency.
Two-sided 95% score-based confidence intervals are calculated. When there is a 0% response or a 100% response in both groups, the Wald-Modified confidence intervals will instead be calculated. When division by zero occurs, the relative risk will be set to NC, but the Wald-Modified CI will be shown.
Source: Table 1, post hoc analysis AE categories and RR (pooled dataset), v1.0, 12-Dec-2024.

Table 53. Summary of AEs for study VLA1553-321 (Part B) including relative risks (Safety Population, seronegative by µPRNT at baseline)

	Statistic	VLA1553 (N=408)	Placebo (N=206)	Relative Risk VLA1553/Placebo	Relative Risk CI VLA1553/Placebo
Any solicited AE	n (%) [95% CI]	304 (74.5) [70.1, 78.5]	105 (51.0) [44.2, 57.7]	1.462	[1.273, 1.703]
Any related solicited AE	n (%) [95% CI]	299 (73.3) [68.8, 77.3]	102 (49.5) [42.8, 56.3]	1.480	[1.283, 1.732]
Any unsolicited AE	n (%) [95% CI]	256 (62.7) [58.0, 67.3]	124 (60.2) [53.4, 66.6]	1.042	[0.916, 1.198]
Any related unsolicited AE	n (%) [95% CI]	39 (9.6) [7.1, 12.8]	1 (0.5) [0.1, 2.7]	19.691	[3.475, 113.42]
Any serious AE	n (%) [95% CI]	5 (1.2) [0.5, 2.8]	2 (1.0) [0.3, 3.5]	1.262	[0.286, 5.619]
Any related serious AE	n (%) [95% CI]	1 (0.2) [0.0, 1.4]	0 (0.0) [0.0, 1.8]	NC	[0.062, 37.064]
Any medically attended AE	n (%) [95% CI]	100 (24.5) [20.6, 28.9]	53 (25.7) [20.2, 32.1]	0.953	[0.718, 1.275]
Any related medically attended AE	n (%) [95% CI]	38 (9.3) [6.9, 12.5]	15 (7.3) [4.5, 11.7]	1.279	[0.730, 2.264]
Any AESI (protocol definition)	n (%) [95% CI]	38 (9.3) [6.9, 12.5]	16 (7.8) [4.8, 12.2]	1.199	[0.693, 2.094]
Any related AESI (protocol definition)	n (%) [95% CI]	14 (3.4) [2.1, 5.7]	0 (0.0) [0.0, 1.8]	NC	[0.879, 244.53]
Any early onset AESI (protocol definition)	n (%) [95% CI]	17 (4.2) [2.6, 6.6]	1 (0.5) [0.1, 2.7]	8.583	[1.478, 50.566]
Any related early onset AESI (protocol definition)	n (%) [95% CI]	14 (3.4) [2.1, 5.7]	0 (0.0) [0.0, 1.8]	NC	[0.879, 244.53]
Any late onset AESI (protocol definition)	n (%) [95% CI]	25 (6.1) [4.2, 8.9]	15 (7.3) [4.5, 11.7]	0.842	[0.459, 1.552]
Any related late onset AESI (protocol definition)	n (%) [95% CI]	0 (0.0) [0.0, 0.9]	0 (0.0) [0.0, 1.8]	NC	[0.010, 25.385]
Any CHIK-like AR	n (%) [95% CI]	134 (32.8) [28.5, 37.5]	35 (17.0) [12.5, 22.7]	1.933	[1.398, 2.708]
Any related CHIK-like AR	n (%) [95% CI]	108 (26.5) [22.4, 31.0]	6 (2.9) [1.3, 6.2]	9.088	[4.192, 20.041]

2.5.4. Safety in special populations

Within this procedure, data have been submitted specifically for healthy adolescents, and no other special population.

Use in pregnancy

Up to Part B of study VLA1553-321, positive urine pregnancy tests were reported for one subject at Visit 4 (Day 85) and for another subject at Visit 5 (Day 180). No follow-up information regarding the pregnancies was available at the time of this reporting. Follow-up information reported after Month 6 (Day 180) will be included in the Part C CSR.

2.5.5. Post marketing experience

Ixchiq was approved for adults by the US FDA On 9 June 2023 and received a marketing authorisation valid throughout the EU on 28 June 2024.

To date, one single report on off-label use of Ixchiq in a <12-year-old child was received. Allergic dermatitis, pain, swelling, feeling hot and erythema were reported one day after vaccination with Ixchiq. Events suggesting a local reaction at the vaccination site on the administered arm were considered as possibly related.

2.5.6. Discussion on clinical safety

Safety in adolescent participants 12 to <18 years was assessed in 502 participants in Brazil who received one dose of VLA1553 with a follow-up of 6 months (study VLA1553-321 Part B, versus 252 adolescents in placebo arm). In the VLA1553 arm, the median age is 15 year and there are 53.6% females (i.e.

slightly more than males - 46.4%) (comparable with the placebo arm: median age of 14 year, 54.4% females, 45.6% males).

Part C is still ongoing on the immunogenicity subset (12 months after vaccination): 328 participants in VLA1553 arm and 56 in placebo. The MAH intends to submit VLA1553-321 study part C CSR in March 2025.

The most common vaccination site reactions, reported within 10 days post-vaccination, were tenderness (19.9%) and pain (19.3%). The most common systemic adverse reactions were headache (51%), myalgia (26.9%), fever (24.1%), fatigue (22.3%), nausea (15.9%) and arthralgia (12.9%). Most cases were mild or moderate.

Within 6 months post-vaccination, the overall incidence of unsolicited AEs was not significantly different between the VLA1553 arm and the placebo arm (61.6% versus 58.7%). Overall, pyrexia was the most frequently reported unsolicited AEs in VLA1553 (19.3% vs. 23.8%), followed by headache (15.1% vs. 19.8%), and cough (10% vs. 10.3%). Most cases were mild or moderate.

A significantly higher frequency of related unsolicited AEs was reported in the VLA1553 arm compared with the placebo arm (8.4% versus 1.6%). Related unsolicited AEs reported in ≥0.5% for participants were eye pain (1.2% vs. 0%, respectively), pyrexia (1.0% vs. 0%, respectively), headache (0.8% vs. 0.4%, respectively), and neutropenia (1% vs. 0%, respectively).

There were no death and no AE leading to study withdrawal in study VLA3221-321. SAEs were reported with similar rate in both arms: 1.4% (7/502) in VLA1553 arm (9 SAEs) versus 1.6% (4/252) in placebo arm (8 SAEs). In the VLA1553 arm, the following SAEs were reported: 1 pneumonia, 1 appendicitis, 2 pyrexia (including 1 considered as related), 1 juvenile myoclonic epilepsy, 1 neutropenia, 1 abdominal pain, 1 lower limb fracture and 1 hyperkalaemia. From the 7 of subjects vaccinated with VLA1553 who had 9 SAEs, 1 was considered as related to the vaccine (fever) and was part of an AESI in combination with mild arthralgia in arms and hands, mild myalgia, and mild headache.

AESIs (protocol definition) for VLA1553 include fever in combination with signs and symptoms potentially indicative of an acute stage CHIKV-associated event (with a duration ≥ 3 days). They have been captured 21 days post-vaccination. The cluster of symptoms that constitute an AESI but starting after 21 days post vaccination until study end was defined as late onset AESI.

By Day 180, AESIs (early and late onset) were reported in 42/502 (8.4%) subjects in the VLA1553 arm and 19/252 (7.5%) subjects in the placebo arm (i.e. total of 61 subjects). The majority of AESIs were late onset AESIs: 17/502 (3.4%) in VLA1533 and 2/252 (0.8%) in placebo had an early onset AESI, and 29/502 (5.8%) in VLA1533 and 17/252 (6.7% i.e. higher than in the active arm) in placebo had a late onset AESI (i.e. a total of 65 cases).

In total, including both arms, there were 61 subjects with AESIs (early and late onset), but 19 subjects with early onset AESIs and 46 subjects with late onset AESIs, i.e. a total of 65 cases, including 4 participants (VLA1553 group) who exhibited both early and late onset AESIs.

The early onset AESI symptoms were a combination of pyrexia with headache, myalgia, arthralgia, rash, or maculo-papular rash, and they were in majority graded as mild or moderate. Most of the early onset AESIs were considered as related, as at least one symptom of the combination was a solicited AE (such as fever), occurring in close temporal proximity to the vaccination, and without alternative cause reported for the majority of subjects. The late onset AESI symptoms were the same as described for the early ones and graded as mild or moderate. However, none of the late onset AESI was considered as related due to the absence of a temporal association with vaccination (3 late onset AESIs began 33 days to 36 days post-vaccination; all other began even later: ≥2 months post-vaccination), or due to the presence of other underlying events.

The occurrence of CHIK-like ARs in adolescents was retrospectively evaluated. CHIK-like ARs were broadly defined, i.e., occurrence of fever (≥38°C) (vs. ≥37.8°C for the definition in adults) 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; within 30 days after vaccination, regardless of time of onset, severity or duration of the individual symptoms.

The proportion of subjects with CHIK-like ARs (within 30 days after vaccination) was 23.1% in the VLA1553 group and 4.8% in the placebo group. Most of them were classified as related (i.e., at least one symptom was assessed as related to vaccination by the investigator). These will be named early onset CHIK-like ARs hereafter.

Among those in the VLA1553 group, combinations of fever with headache (87.1%), myalgia (57.8%), fatigue (44%), or arthralgia (28.4%) were the most common, all other symptoms were reported in fewer than 10% participants. Most of them were classified as related. 3.6% of participants reported at least one severe symptom, most commonly fever or headache. Only 1 serious early onset CHIK-like AR was reported (fever in the VLA1553 arm). Median onset was 2 days after vaccination, and median time to resolution was 4 days. There were no longer lasting CHIK-like AR reported in adolescents (i.e., at least one symptom with duration ≥30 days).

Overall, the proportions of participants experiencing late-onset CHIK-like AEs (occurring from 30 days to 6 months post-vaccination), severe late-onset CHIK-like AEs (at least one symptom assessed as severe) and severe late-onset CHIK-like symptoms were comparable between the VLA1553 arm and the placebo arm, with similar median onset and median duration of late-onset CHIK-like AEs. In the VLA1553 arm, none of the late-onset CHIK-like AEs were assessed as related, and none were serious. In both arms, the most common symptoms, aside from fever, included headache, myalgia, fatigue, and arthralgia.

The overall incidence of early onset CHIK-like ARs and late onset CHIK-like AEs was 29.5% in the VLA1553 group and 17.5% in the placebo group. As expected, this is higher than the total frequency of AESIs (including early and late onset): 8.4% in the VLA1553 arm and 7.5% in the placebo arm.

Overall, the time-to-onset was based on the first symptom of the combination. Most second symptoms of AESIs/CHIK-like ARs/AEs in the VLA1553 arm occurred within the first month following vaccination (AESIs: 3.4%, CHIK-like ARs: 23.1%) as all participants with early onset AESIs or CHIK-like ARs fell within this period. As anticipated, during Month 1, the frequency of AESIs/CHIK-like ARs/AEs was higher in the VLA1553 arm compared to the placebo arm (AESIs: 3.4% and 0.8%, respectively; CHIK-like ARs: 23.1% and 4.8%, respectively). Beyond Month 1, the frequency of AESIs and CHIK-like AEs based on the onset of the second symptom was similar between the VLA1553 and placebo arms. For AESIs, frequencies ranged from 0.4% to 2.0% in the VLA1553 arm and 0.4% to 2.4% in the placebo arm. For CHIK-like AEs, frequencies ranged from 0.4% to 3.2% in the VLA1553 arm and 1.2% to 4.8% in the placebo arm. Notably, all second symptoms of related CHIK-like ARs/AEs occurred within Month 1, except for one participant in the placebo arm. In conclusion, in VLA1553 arm, and in opposite to placebo arm, the onset of most of the CHIK-like symptoms is covered by protocol defined AESI (early onset) focussing on the first 21 days after vaccination and by CHIK-like ARs comprising up to 30 days after vaccination.

There were no cases of prolonged AESIs/CHIK-like ARs/AEs (i.e., with at least one symptom lasting ≥30 days), no chronic CHIK-like symptoms (i.e. without fever), long-term effects, and possible complications, such as long-lasting musculoskeletal stiffness or pain (myalgia), joint stiffness or pain (arthralgia), joint swelling/effusion, synovitis, arthritis, osteoarthritis, or neurological symptoms.

There was only one related event of prolonged solicited arthralgia (duration: 32 days) reported in a seropositive subject at baseline (without fever); and the longest duration of related myalgia was 20 days

(without fever). One event of seizure and one event of juvenile myoclonic epilepsy were assessed as unrelated to VLA1553 vaccination (due to the absence of temporal association: 53 and 55 days after vaccination, respectively) and resolved after medical intervention.

Retinitis or uveitis were not reported in either treatment arm, and eye disorders related to VLA1553 vaccination were all mild or moderate in severity and resolved within 13 days (eye pain, conjunctival hyperaemia, photophobia and eyelid oedema). Conjunctival hyperaemia has been included in section 4.8 of the SmPC. The events of conjunctivitis were all short-lived and unrelated to VLA1553 vaccination.

In the VLA1553 group, subjects aged 12 to <15 years (26.2%: 64 out of 244) had slightly earlier onset CHIK-like ARs compared to subjects aged 15 to <18 years (20.2%: 52 out of 258) (difference not observed in the placebo arm). The proportion of subjects aged 12 to <15 years with severe early onset CHIK-like ARs (4.5%: 11 out of 244) was also slightly higher compared to subjects aged 15 to <18 years (2.7%: 7 out of 258) (difference not observed in the placebo arm). In the VLA1553 arm, the frequencies of late-onset CHIK-like AEs and severe late-onset CHIK-like AEs were higher in participants aged 12 to <15 years (13.1% and 2.9%) compared to participants aged 15 to <18 years (5.4% and 0.4%). However, a similar trend was observed in the placebo arm.

The most common abnormal laboratory parameters (that could be selected as investigation ADRs) were: neutropenia (40.2%), alkaline phosphatase increased (17.7%), leukopenia (16.8%), leukocytosis (14.6%), lymphopenia (11.6%) and hypokalaemia (10.4%) (based on an immunogenicity subset of 328 VLA1553 recipients) (neutropenia, leukopenia and lymphopenia were listed as ADRs in the initial MA). The changes in haematology and clinical chemistry parameters were considered expected and consistent with a normal physiologic response to a live-attenuated viral vaccine.

As already discussed at the time of initial MA, based on the limited viraemia data collectively generated in all phase 3 studies (including VLA1553-321) in terms of number of subjects included and tested time-points, no conclusions can be drawn on a potential correlation between vaccine viraemia levels and safety of VLA1553. Within Part C CSR submission, the MAH has committed to submit viraemia data for all the 116/502 (23.1%) participants with CHIK-like adverse reactions in the VLA1553 group.

By serostatus at baseline:

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. Because of the small number of seropositive subjects in both arms, the interpretation for these sub-groups is limited.

The proportion of participants who experienced solicited systemic AEs was higher in baseline seronegative participants vaccinated with VLA1553 than in baseline seropositive participants vaccinated with VLA1553 (67.9% and 44.7% respectively). The proportion of participants who experienced solicited systemic AEs was also higher in baseline seronegative participants vaccinated with placebo than in baseline seropositive participants vaccinated with placebo (44.2% and 33.3% respectively). In the VLA1553 arm, solicited systemic AEs were reported with similar frequency in seronegative subjects aged 12 to <15 years (64.3% of 210 subjects) and seronegative subjects aged 15 to <18 years (71.7% of 198 subjects).

The proportion of participants who experienced solicited local AEs, and unsolicited AEs & SAEs (up to 6 months after vaccination) was similar in the VLA1553 arms of each stratum.

AESIs (early and late onset until 6 months after vaccination) (as assessed by the investigator, sponsor protocol definition) were reported with slightly higher frequency in the VLA1553 arm for subjects in the seronegative stratum (9.3%) compared to the seropositive stratum (4.3%). No difference was observed

for the placebo arm between the seronegative stratum (7.8%) and the seropositive stratum (6.7%). The frequencies of early onset AESIs were higher in the VLA1553 arm compared to the placebo arm in seronegative adolescents. However, in seropositive adolescents, the frequencies of the early onset AESIs was similar in both arms. The frequencies of the late onset AESIs was similar in both arms (for both serostatus sub-groups). The rate of AESIs was slightly higher in subjects aged 12 to <15 years (11.4%) compared to subjects aged 15 to <18 years in the seronegative stratum of the VLA1553 group (7.1%).

The proportion of participants who experienced early onset CHIK-like ARs (retrospective analyse of combination events within 30 days after vaccination) was higher in baseline seronegative participants (27.0%) than in baseline seropositive participants vaccinated with VLA1553 (6.4%). For the placebo arm, they were slightly less early onset CHIK-like ARs reported in the seronegative stratum (3.9%) compared to the seropositive stratum (8.9%). Early onset CHIK-like ARs were much more reported in the VL1553 arm vs. the placebo arm in seronegative adolescents. However, in seropositive adolescents, the frequencies of the early onset CHIK-like ARs were similar in both arms.

The frequency of late-onset CHIK-like AEs was comparable between baseline seronegative and baseline seropositive participants, and the frequency of severe late-onset CHIK-like AEs was slightly lower in baseline seronegative participants compared to baseline seropositive participants.

By age categories:

When comparing the seronegative adolescents (VLA1553-321) to the adults (pooled safety population: VLA1553-301, VLA1553-302, and VLA1553-101: mainly seronegative, initial MAA):

- Solicited local and systemic AEs, unsolicited AEs and MAAEs were more frequently reported in both arms (VLA1553 and placebo). Nevertheless, the increases of frequencies observed in VLA1553 versus placebo seem comparable in the adolescents and in the adults. For instance, in seronegative adolescents, headache has been reported by 54.7% in VLA1553 arm vs. 36.9% in placebo arm; fever by 28.2% vs. 2.4%; tenderness by 19.9% vs. 14.7%; pain at injection site: 19.3% vs. 13.5%. In adults, headache has been reported by 32.0% in VLA1553 arm vs. 14.6% in placebo arm; fever by 13.8% vs. 0.8%; tenderness by 10.8% vs. 8.1%; pain at injection site: 6.1% vs. 37%.
- The AESIs (early and late onset) were much more frequent in both arms for the adolescents (VLA1553: 9.3%, placebo: 7.8%) versus the adults (VLA1553: 0.3%, placebo: 0.1%).
- The early onset CHIK-like ARs were more frequent in both arms for the adolescents (VLA1553: 27%, placebo: 3.9%) versus the adults (VLA1553: 12.1%, placebo: 0.6%).

In the adult pooled safety population, the highest relative risk (RR) with VLA1553 versus placebo arm (> 3) was observed for the early onset AESIs (RR 3.15; 95% CI, 0.52 to 18.96), early onset CHIK-like ARs (RR 20.79, 95% CI, 9.53 to 45.53), and related early onset CHIK-like ARs (RR 19.94, 95% CI, 9.14 to 43.66). The RR for the related early onset AESIs was 2.86 (95% CI, 0.47, 17.34).

In the CHIKV baseline seronegative adolescents, the highest risk (>3) was for the related unsolicited AEs (RR 19.70; 95% CI, 3.48 to 113.42), early onset AESIs (RR 8.58; 95% CI, 1.48 to 50.57), early onset CHIK-like ARs (RR 6.94; 95% CI, 3.54 to 13.85), and related early onset CHIK-like ARs (RR 9.09; 95% CI, 4.19 to 20.04). The RR for the related early onset AESIs could not be calculated as there was no events in the placebo arm. For the late onset AESIs, which were part of the VLA1553-321 protocol only (and not monitored in the adult studies VLA1553-101, VLA1553-301, and VLA1553-302), the risk was lower in the VLA1553 group compared to the placebo group (RR 0.84; 95% CI, 0.46 to 1.55); similar findings were shown for late-onset CHIK-like AEs (RR, 0.66; 95% CI, 0.42 to 1.04).

Therefore, when comparing the RR of:

- Solicited AEs, it was similar in the adolescents (RR 1.46; 95% CI, 1.27 to 1.70) and the adults (RR 1.68; 95% CI, 1.53 to 1.85).
- Unsolicited AEs, it was similar in the adolescents (RR 1.04; 95% CI, 0.92 to 1.20) and the adults (RR 1.32; 95% CI, 1.17 to 1.49).
- MAAEs, it was similar in the adolescents (RR 0.95; 95% CI, 0.72 to 1.28) and the adults (RR 1.09; 95% CI, 0.9 to 1.32).
- Early onset AESIs, it was slightly higher in the adolescents (RR 8.58; 95% CI, 1.48 to 50.57) compared to the adults (RR 3.15; 95% CI, 0.52 to 18.96).
- Related early onset CHIK-like ARs, it was slightly lower in the adolescents (RR 9.09; 95% CI, 4.19 to 20.04) compared to the adults (RR 19.94, 95% CI, 9.14 to 43.66).

Nevertheless, it is acknowledged that data interpretation is limited due to the small participant numbers in some AE categories, and the overall smaller adolescent number in the VLA1553-321 trial compared to the pooled adult number.

In conclusion, overall, and because of all limitations, it is considered that the trends regarding the relative risk in VLA1553 recipients vs. placebo for the AE categories were similar between adults and adolescents.

2.5.7. Conclusions on clinical safety

The most common vaccination site reactions in adolescents 12 to <18 years of age were tenderness and pain . The most common systemic adverse reactions were headache myalgia , fever, fatigue, nausea and arthralgia. Related unsolicited AEs reported in ≥0.5% for participants were eye pain , pyrexia , headache), and neutropenia. The most common abnormal laboratory parameters were neutropenia, alkaline phosphatase increased, leukopenia, leukocytosis, lymphopenia and hypokalaemia (neutropenia, leukopenia and lymphopenia being selected as ADRs in the SmPC).

The proportion of participants who experienced solicited systemic AEs was higher in baseline seronegative participants vaccinated with VLA1553 than in baseline seropositive participants vaccinated with VLA1553. However, this was also true when comparing the participants vaccinated with placebo. The proportion of participants who experienced solicited local AEs and unsolicited AEs was similar in the VLA1553 arms of each stratum. However, because of the small number of seropositive subjects in both arms, the interpretation for these sub-groups is limited.

Overall, the reactogenicity was higher in both arms (VLA1553 and placebo) when comparing seronegative adolescents (VLA1553-321) to adults (pooled safety population: VLA1553-301, VLA1553-302, and VLA1553-101, mainly seronegative – initial MAA) (in particular for headache, fever, tenderness and pain at the injection site). Nevertheless, the increases of frequencies observed in VLA1553 versus placebo seem comparable in the adolescents and in the adults, and differences could be considered acceptable given the overall frequency and intensity. Moreover, increased rates of AESIs (early and late onset) and of early onset CHIK-like ARs have been reported with adolescents (compared to the adults) that could reflect the observed increased frequency of fever (symptom in combination in both definitions). However, overall, and because of all limitations, it is considered that the trends regarding the relative risk in VLA1553 recipients vs. placebo for the AE categories were similar between adults and adolescents.

For the adolescents, most reported AESIs were with a late onset but only the early ones were assessed as related. The frequencies of early onset AESIs were higher in the VLA1553 arm compared to the placebo arm in seronegative adolescents. However, in seropositive adolescents, the frequencies of the

early onset AESIs was similar in both arms. The frequencies of the late onset AESIs was similar in both arms (for both serostatus sub-groups).

Early onset CHIK-like ARs were much more reported in the VL1553 arm vs. the placebo arm in seronegative adolescents. In seropositive adolescents, the frequencies of the early onset CHIK-like ARs were similar in both arms. Most of the early onset CHIK-like ARs were classified as related. Overall, the proportions of participants experiencing late-onset CHIK-like AEs (occurring from 30 days to 6 months post-vaccination) were comparable between the VLA1553 arm and the placebo arm, with similar median onset and median duration. In the VLA1553 arm, none of the late-onset CHIK-like AEs were assessed as related, and none were serious. The frequency of late-onset CHIK-like AEs was comparable between baseline seronegative and baseline seropositive participants.

Overall, there were no acute symptom/sign that were of concern. In particular, in the AESIs / CHIK-like ARs, only 1 symptom was serious (fever assessed as related to the vaccine); none were associated to meningoencephalitis or other serious acute disease/sign; and none were associated to arthritis.

Finally, there were no cases of prolonged AESIs/CHIK-like ARs/AEs (i.e., with at least one symptom lasting ≥30 days), no chronic CHIK-like symptoms (i.e. without fever), long-term effects, and possible complications, such as long-lasting musculoskeletal stiffness or pain (myalgia), joint stiffness or pain (arthralgia), joint swelling/effusion, synovitis, arthritis, osteoarthritis, or neurological symptoms. There was only one related event of prolonged solicited arthralgia (duration: 32 days) reported in a seropositive subject at baseline (without fever); and the longest duration of related myalgia was 20 days (without fever). Retinitis or uveitis were not reported in either treatment arm, and eye disorders related to VLA1553 vaccination were all mild or moderate in severity and resolved within 13 days. Conjunctival hyperaemia has been included in section 4.8 of the SmPC.

Overall, the safety profile of VLA1553 for healthy adolescents is considered similar to the safety profile in the healthy adults and therefore acceptable.

2.5.8. PSUR cycle

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.

2.6. Risk management plan

The MAH submitted an updated RMP version 2.1 with this application.

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

The PRAC considered that the risk management plan version 2.1 is acceptable.

Safety concerns

Table 54. 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

Pharmacovigilance plan

Table 55. Summary table of 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			
VLA1553-303 Ongoing	Primary objective: To evaluate persistence of antibodies annually from 1 to 10 years after single immunization with VLA1553. Secondary objective: To evaluate long-term safety (i.e. SAEs) 6 months to 2 years after single immunization with VLA1553.	Long-term safety	First Participant In: 02 April 2021 The overall trial duration (First Participant In – Last Participant Out) is estimated to be approximately 122 months. Completion: CSR Part A (Visit 1, Year 1): 01 March 2023 CSR Part B (Visit 2, Year 2): 17 January 2024 CSR Part C (Visit 3, Year 3): planned Q4 / 2024 CSR Part D (Visit 4, Year 4): planned Q4 / 2025 CSR Part E (Visit 5, Year 5): planned Q4 / 2026 and accordingly up to CSR Part J (Visit 10, Year 10): planned Q1 2031

Study / Status	Summary of objectives	Safety concerns addressed	Milestones / Due dates
VLA1553-321 Ongoing	Primary objective: to evaluate the immunogenicity and safety of the full dose of the live-attenuated CHIKV vaccine candidate (VLA1553) 28 days following vaccination in adolescents aged 12 years to <18 years after a single immunisation. Secondary objectives: to assess the immunogenicity and safety of the full dose of VLA1553 following vaccination in adolescents aged 12 years to <18 years up to Month 12 after a single immunization. In addition, the immunogenicity and safety of VLA1553 in participants previously exposed to CHIKV are assessed.	Chikungunya-like adverse reactions (broad definition) Vaccine-associated arthritis Cardiac events Long-term safety	First participant in: 14 Feb 2022 Last participant out: 16 Feb 2024 CSR Part A (Visit 3, Day 29): 21 Dec 2023 CSR Part B (Visit 5, Month 6): 24 May 2024 CSR Part C (Visit 6, Month 12): planned Q1 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: 05 Mar 2024 Start of data collection: 01 Oct 2025. Last participant in: 30 Jun 2026. Study completion: 30 Sep 2027. Final report submission: 31 Dec 2027.
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 update submission: 31 Jan 2025 Start of data collection: 31 Mar 2025 Interim Study Report: 31 May 2027 Last participant:31 May 2028 End of data collection: 30 Nov 2028 Final study report: 31 May 2029
Prospective Safety Cohort Study VLA1553-406 Planned	To estimate the incidence rates of a predefined set of adverse events (AEs) which constitute safety concerns according to the VLA1553 Risk Management Plan (RMP) following the administration of the live-attenuated VLA1553 vaccine in individuals that are target for the pilot vaccination program, within a defined risk window following vaccination.	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	Final protocol with SAP: 31 Mar 2025 Start of data collection: 01 Oct 2025 Study completion: 31 Dec 2026 Final study report: 31 Dec 2027

The MAH communicated that the interventional category 3 study VLA1553-304 (moderately immunocompromised adult participants infected with human immunodeficiency virus), included in the initial RMP, was cancelled due to recruitment issues and the study has been removed from the RMP. Two other post-authorisation studies are planned to address safety in patients with autoimmune or inflammatory disorders (i.e. VLA1553- 401 and VLA1553-406).
The MAH committed to include adolescents in the post-authorisation safety study VLA1553-401 after label extension to adolescents is approved by the US. The RMP should then be updated accordingly to

indicate that adolescents will be included in all relevant studies.

Risk minimisation measures

Table 56. 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. Clinical Trial VLA1553-321 in adolescents.
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. Clinical Trial VLA1553-321 in adolescents.
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. Clinical trial VLA1553-321 in adolescents.

Safety concern	Risk minimisation measures	Pharmacovigilance activities
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 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.
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. Clinical Trial VLA1553-321 in adolescents.
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. Update of the Product information

As a consequence of this new indication, sections 4.1, 4.2, 4.8 and 5.1 of the SmPC are updated. Annex II and the Package Leaflet are updated in accordance, seeAttachment 1.

2.7.1. User consultation

A justification for not performing a full user consultation with target patient groups on the package leaflet has been submitted by the MAH and has been found acceptable for the following reasons:

No full user consultation with target patient groups on the package leaflet has been performed on the basis of a bridging report making reference to the original product, as authorised for the adult population. The posology, safety profile, container, strength and pharmaceutical form, and all other information in the package leaflet remains identical, except for the age range of the patient population. The bridging report submitted by the MAH has been found acceptable. An additional user testing is thus not considered necessary.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The MAH seeks an extension of the approved indication to children and adolescents from 12 to 17 years of age. The proposed indication for Ixchiq (also referred to as VLA1553) is for active immunisation to prevent disease caused by Chikungunya virus (CHIKV) in individuals 12 years and older.

Chikungunya (CHIK) disease (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 Ae. albopictus*). Human-to-human transmission (vertical and blood-borne transmission) of CHIKV has been described. Both adults and children can become infected and be symptomatic with chikungunya.

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 report 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 for 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 >100 countries and >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. Autochthonous cases have been reported in France between July and October 2024 (1 in mainland France, 11 in La Réunion). 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.

Ixchiq is approved in EU since June 2024 for active immunisation for the prevention of disease caused by CHIKV in individuals 18 years and older.

On 30th January 2025, the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion on another CHIK vaccine (recombinant, adsorbed) (Vimkunya, manufactured by Bavarian Nordic), intended for the prevention of chikungunya disease in individuals 12 years and older.

Given the absence of authorised products for prevention or treatment of CHIK in the EU for adolescents, 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 in adolescents is acknowledged.

3.1.3. Main clinical studies

The current application is supported by data from study VLA1553-321.

VLA1553-321 is a randomised, placebo-controlled, double-blind, multicentre, Phase 3 clinical trial evaluating the safety and immunogenicity of a single dose of Ixchiq in adolescents aged 12-17 years for 6 months after vaccination (12 months within the Immunogenicity subset).

The study is still ongoing. The MAH submitted Part A (Data up to Day 29) and Part B analysis (Data up to Month 6). Part C analysis (up to Month 12) will be reported in March 2025.

The study was conducted at 10 study sites in Brazil which is an endemic country for chikungunya. Ixchiq was administered at the final dose selected for adults (1x10E4 TCID50 per 0.5 mL). Participants aged 12-17 years (n=765) were randomly allocated in a 2:1 ratio to receive a single dose of Ixchiq (n=510) or Placebo (n=255) intramuscularly with a target of 20% seropositive participants, based on CHIKV ELISA performed at screening.

The Safety population included 754 participants. Of those, 81.5% (n=614) participants were CHIKV seronegative (µPRNT50 ≤40) at baseline (408 and 206 participants respectively in the Ixchiq and Placebo arms) and 18.5% (n=139) participants were CHIKV seropositive (µPRNT50 >40) (94 and 45 participants respectively in the Ixchiq and placebo arms). Baseline µPRNT data were not available for 1 participant.

Immunogenicity was assessed in a subset of 351 participants (PP population, 303 in the Ixchiq arm and 48 in the placebo arm), including 293 baseline CHIKV seronegative (µPRNT50 ≤40) (251 and 42 in the Ixchiq and in the placebo arm, respectively) and 58 were baseline CHIKV seropositive (µPRNT50 >40) (52 and 6 in the Ixchiq and in the placebo arm, respectively).

3.2. Favourable effects

The primary endpoint of trial VLA1553-321 was met. At 28 days post-vaccination, 98.8% (248/251, [95% CI: 96.5-98.8]) of the baseline CHIKV seronegative participants vaccinated with a single dose of Ixchiq had an antibody titre of at least the threshold accepted as reasonably likely to predict protection (≥150 µPRNT50) (PP population). The lower bound of the 95% CI around the proportion exceeds the non-acceptance threshold of 70%. Only 1/42 of the placebo participants reached the threshold at Day 29.

The point estimate and lower bound 95% CI were the same as those observed in study VLA1553-301, which is the pivotal trial conducted in adults in the US. Results are also in line with study VLA1553-302. Both studies VLA1553-301 and VLA1553-302 were conducted in adults in the US and supported the approval of the initial MA.

The proportion of baseline CHIKV seronegative participants achieving the threshold was still very high up to 6 months post-vaccination (99.1% [95% CI: 96.9-99.9]), similarly to the proportion observed in adults up to 2 years post-vaccination (97.1% [95% CI: 94.4-98.7], study VLA1553-303 which is a follow up study of VLA1553-301).

3.3. Uncertainties and limitations about favourable effects

There are no efficacy data from clinical trials. Efficacy trials were considered not feasible pre-authorisation due to unpredictable and short-lived outbreaks. There is no established immune correlate of protection (ICP) for Chikungunya. Hence, an alternative approach was followed to establish the

effect of Ixchiq based on neutralizing antibodies which are known to have a major role in protecting against CHIKV infection and/or disease.

The primary endpoint of the VLA1553-321 trial (as for the pivotal adult trial VLA1553-301) 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 Réunion 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 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 MAH 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 therefore around how this threshold actually translates into protection against Chikungunya and/or CHIKV infection.

Uncertainty thus 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 Ixchiq (study VLA1553-404) planned to be conducted in different countries/regions. Feasibility evaluations are currently ongoing.

The protocol of the pragmatic randomised clinical trial VLA1553-404 (in endemic country) is being revised to reflect that adolescents (12-17 yrs at the time of vaccination) will be added to the trial population.

Since Ixchiq is a live-attenuated vaccine, induced immune responses might resemble those resulting from natural infection. Data are lacking however to support this assumption. Day 29 GMTs induced by Ixchiq in the baseline CHIKV seronegative participants are similar to the titres observed at baseline in CHIKV seropositive participants who have had a natural infection in the past. Of note, the baseline GMTs of the CHIKV seropositive participants does not reflect the peak antibody level reached shortly after natural infection. The time elapsed between the past infection and the study baseline sampling is unknown. The peak of antibody level post-acute infection is likely to be much higher than the peak antibody level induced by Ixchiq. At 6 months post-vaccination, GMTs in baseline CHIKV seronegative vaccinees are lower compared to the baseline GMTs of the CHIKV seropositive participants in the Ixchiq arm, suggesting that antibody titres induced by Ixchiq are lower than those induced by a natural infection over the longer term. The clinical relevance of these observations is not known.

It remains uncertain if protection would differ according to the infecting CHIKV strain (homologous and/or heterologous strains/genotypes). Investigations are ongoing with respect to cross-neutralization of wild-type CHIKV strains of different circulating genotypes.

No neutralizing antibody responses is observed 7 days after vaccination. Early protection via other mechanisms is possible.

Persistence of CHIKV-specific neutralizing antibodies at levels above the threshold of 150 µPRNT50 in a very high proportion of the vaccinated individuals has been shown up to 6 months post-vaccination in adolescents VLA1553-321 and 2 years post-vaccination in adults in study VLA1553-303. Data up to 12

months post-vaccination (immunogenicity subset only) in adolescents will be reported with part C of VLA1553-321 and data up to 5 years post-vaccination in adults will be obtained in study VLA1553-303.

Concomitant administration with other vaccines has not been studied but is planned in the risk management plan.

Immune responses induced by Ixchiq does not appear to be impacted by concomitant use of anti-inflammatory and anti-rheumatic products or analgesics (VLA1553-301, VLA1553-302 and VLA1553-321).

Data in CHIKV seropositive adult participants involved in the trials supporting MA were scarce, as these trials were conducted in the US. Data from study VLA1553-321 in endemic area, in which the vast majority of participants with pre-existing immunity to CHIKV present antibody titres above the predefined threshold of 150 µPRNT50 at baseline (50/52), show that Ixchiq does not induce a boost of natural immunity (GMFI at 1.3 [95% CI: 1.0-1.6] at Day 29 post-vaccination). This suggests that the vaccine virus is neutralized in the presence of CHIKV pre-existing antibodies. Whether a booster effect of natural immunity might be observed in individuals with low neutralising antibody titres is not known. However, since natural infection is believed to induce long-term (even maybe life-long) protection, and hence the risk of re-infection is likely absent/very low, the added value of any booster effect of natural immunity in terms of clinical protection would probably be lacking/very limited anyway. Therefore, individuals who have been previously infected by CHIKV might not benefit from the vaccine.

Only generally healthy adolescents were included in VLA1553-321, similarly to the initial MA studies that included only generally adults. No immunogenicity data have been obtained in participants immunocompromised due to medical condition or due to immunosuppressive treatments.

3.4. Unfavourable effects

Safety in adolescent participants 12 to <18 years was assessed in 502 participants in Brazil who received one dose of Ixchiq with a follow-up of 6 months. 18.7% of these participants had pre-existing antibodies against chikungunya virus.

The most common vaccination site reactions in adolescents 12 to <18 years of age (both serostatus included) were tenderness (19.9%) and pain (19.3%). The most common systemic adverse reactions were headache (51%), myalgia (26.9%), fever (24.1%) fatigue (22.3%), nausea (15.9%) and arthralgia (12.9%). Most cases were mild or moderate.

Within 6 months post-vaccination, the overall incidence of unsolicited AEs was not significantly different between the Ixchiq arm and the placebo arm. Overall, pyrexia was the most frequently reported unsolicited AEs in Ixchiq, followed by headache, and cough. Most cases were mild or moderate. Related unsolicited AEs reported in ≥0.5% for participants were eye pain (1.2% Ixchiq vs. 0% placebo), pyrexia (1.0% vs. 0%, respectively), headache (0.8% vs. 0.4%, respectively), and neutropenia (1% vs. 0%, respectively).

The most common abnormal laboratory parameters were neutropenia (40.2%), alkaline phosphatase increased (17.7%), leukopenia (16.8%), leucocytosis (14.6%), lymphopenia (11.6%) and hypokalaemia (10.4%) (neutropenia, leukopenia and lymphopenia being selected as ADRs in the SmPC).

There were no deaths and no AEs leading to study withdrawal in study VLA3221-321. SAEs were reported with similar rates in both arms. In the Ixchiq arm, the following SAEs were reported: 1 pneumonia, 1 appendicitis, 2 pyrexia (including 1 considered as related), 1 juvenile myoclonic epilepsy, 1 neutropenia, 1 abdominal pain, 1 lower limb fracture and 1 hyperkalaemia.

AESIs (protocol definition) for Ixchiq include fever in combination with signs and symptoms potentially indicative of an acute stage CHIKV-associated event (with a duration ≥ 3 days). By Day 180, AESIs (early onset: 1st symptom starting within 21 days after vaccination; and late onset: 1st symptom starting from 22 days after vaccination) were reported in 42/502 (8.4%) subjects in the Ixchiq arm and 19/252 (7.5%) subjects in the placebo arm. The early and late onset AESI symptoms were a combination of pyrexia with headache, myalgia, arthralgia, rash, or maculo-papular rash, and they were in majority graded as mild or moderate. Most reported AESIs were with a late onset and only the early ones were assessed as related. The frequencies of early onset AESIs were higher in the Ixchiq arm compared to the placebo arm. The frequencies of the late onset AESIs was similar in both arms.

The occurrence of CHIK-like ARs was retrospectively evaluated. CHIK-like ARs were broadly defined, i.e., occurrence of fever (≥38°C) (vs. ≥37.8°C for the definition in adults) and at least one other symptom also reported for acute-stage chikungunya illness, within 30 days after vaccination, regardless of time of onset, severity or duration of the individual symptoms. These "early onset" CHIK-like ARs were reported by 23.1% of adolescents in the Ixchiq group and 4.8% in the placebo group. Among those in the Ixchiq group, combinations of fever with headache (87.1%), myalgia (57.8%), fatigue (44%), or arthralgia (28.4%) were the most common, all other symptoms were reported in fewer than 10% participants. Most of them were classified as related. 3.6% of participants reported at least one severe symptom, most commonly fever or headache. Only 1 serious early onset CHIK-like AR was reported (fever in the Ixchiq arm). Median onset was 2 days after vaccination, and median time to resolution was 4 days. Overall, the proportions of participants experiencing late-onset CHIK-like AEs (occurring from 30 days to 6 months post-vaccination) were comparable between the Ixchiq arm and the placebo arm, with similar median onset and median duration. In the Ixchiq arm, none of the late-onset CHIK-like AEs were assessed as related, and none were serious.

Finally, there were no cases of prolonged AESIs/CHIK-like ARs/AEs (i.e., with at least one symptom lasting ≥30 days), no chronic CHIK-like symptoms (i.e. without fever), long-term effects, and possible complications, such as long-lasting musculoskeletal stiffness or pain (myalgia), joint stiffness or pain (arthralgia), joint swelling/effusion, synovitis, arthritis, osteoarthritis, or neurological symptoms. There was only one related event of prolonged solicited arthralgia (duration: 32 days) reported in a seropositive subject at baseline (without fever); and the longest duration of related myalgia was 20 days (without fever). Retinitis or uveitis were not reported in either treatment arm, and eye disorders related to Ixchiq vaccination were all mild or moderate in severity and resolved within 13 days. Conjunctival hyperaemia has been included in section 4.8 of the SmPC.

The important identified risks (CHIK-like ARs) and the important potential risks (vaccine-associated arthritis, cardiac events, and safety in pregnant or breastfeeding women) identified for the adults are kept for the adolescents.

3.5. Uncertainties and limitations about unfavourable effects

Because of the small number of seropositive subjects in both arms (94 vaccinated with Ixchiq and 45 with placebo), the interpretation of differences and similarities of reactogenicity and safety between the seropositive and seronegative subjects is limited. A prospective safety cohort study VLA1553-406 is planned in endemic areas (Brazil) and will further characterise the safety concerns of Ixchiq. Nevertheless, based on VLA1553-321, the following have been observed:

- The proportion of participants who experienced solicited systemic AEs was higher in baseline seronegative participants vaccinated with Ixchiq than in baseline seropositive participants vaccinated with Ixchiq. However, this was also true when comparing the participants vaccinated with placebo.

- The proportion of participants who experienced solicited local AEs and unsolicited AEs was similar in the Ixchiq arms of each stratum.
- AESIs (early and late onset) (protocol definition) were reported with slightly higher frequency in the Ixchiq arm for subjects in the seronegative stratum compared to the seropositive stratum (difference not observed for the placebo arm between the seronegative and seropositive stratum). The frequencies of early onset AESIs were higher in the Ixchiq arm compared to the placebo arm in seronegative adolescents. In seropositive adolescents, the frequencies of the early onset AESIs was similar in both arms. The frequencies of the late onset AESIs was similar in both arms (for both serostatus sub-groups).
- The proportion of participants who experienced early onset CHIK-like ARs (i.e. within 30 days after vaccination) was higher in baseline seronegative participants than in baseline seropositive participants vaccinated with Ixchiq. For the placebo arm, they were slightly less reported in the seronegative stratum compared to the seropositive stratum. Early onset CHIK-like ARs were much more reported in the VL1553 arm vs. the placebo arm in seronegative adolescents. In seropositive adolescents, the frequencies of the early onset CHIK-like ARs were similar in both arms. The frequency of late-onset CHIK-like AEs was comparable between baseline seronegative and baseline seropositive participants.

No cases of prolonged AESI/CHIK-like ARs/AEs have been reported. Overall, the reactogenicity was higher in both arms (Ixchiq and placebo) when comparing seronegative adolescents (VLA1553-321) to adults (pooled safety population: VLA1553-301, VLA1553-302, and VLA1553-101, mainly seronegative – initial MAA) (in particular for headache, fever, tenderness and pain at the injection site). Nevertheless, the increases of frequencies observed in Ixchiq versus placebo seem comparable in the adolescents and in the adults, and differences could be considered acceptable given the overall frequency and intensity. Moreover, increased rates of AESIs (early and late onset) and of early onset CHIK-like ARs have been reported with adolescents (compared to the adults) could reflect the observed increased frequency of fever (symptom in combination in both definitions). However, overall, and because of all limitations (i.e. small participant numbers in some AE categories, and the overall smaller adolescent number in the VLA1553-321 trial compared to the pooled adult number), it is considered that the trends regarding the relative risk in Ixchiq recipients vs. placebo for the AE categories were similar between adults and adolescents.

The list of safety concerns identified for the adults are kept for the adolescents. No new safety concern has been identified in the adolescent population.

The following protocols are currently revised:

- US Post-Authorization Safety study VLA1553-401 to assess the safety of Ixchiq in approximately 5,000 travellers (age approved per label) vaccinated with Ixchiq, planning to visit endemic areas. Therefore, although enrolment should be open to adolescent (upon approval of Ixchiq for use in this population by US FDA), the protocol will not prespecify a target proportion of adolescents in the trial. Participants should be followed up for 6 months. This study should cover the important identified risk of Chikungunya-like adverse reactions and the important potential risks of vaccine-associated arthritis and cardiac events, as well as missing information.

  Pragmatic RCT VLA1553-404 (in endemic country) to reflect that adolescents (12-17 yrs at the time of vaccination) will be added to the trial population (randomized 1:1 to receive Ixchiq or control). The overall sample size of at least 10,000 individuals in the Ixchiq arm will remain unchanged. For the safety, the following should be followed: follow-up of any ongoing CHIK-like ARs (with or without fever) beyond the 24 weeks standard safety follow-up period in support of assessment of chronicity; collection of SAEs incl. chikungunya leading to hospitalization and

CHIK-like AEs (suspected CHIK cases presenting with negative PCR) throughout the trial, until the event resolves, stabilizes, or the participant becomes unreachable or until global end of trial (i.e. once sufficient cases have been accrued to evaluate effectiveness).

Currently, it is unclear if adolescents will be enrolled in the prospective safety cohort study VLA1553-406 in Brazil.

The RMP will be updated in due time to show that adolescents will be included in the relevant studies.

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 Ixchiq, 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 Ixchiq. Recent literature indicates an association between clinical symptoms and viral loads during natural CHIKV infection (e.g. Raghavendhar et al.; Sagar et al.). The clinical studies conducted so far with Ixchiq were not adequately designed and powered to establish if such an association also applies to Ixchiq and the adverse reactions.

3.6. Effects Table

Table 57. Effects Table for Ixchiq for the active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in adolescents (study VLA1553-321 parts A and B – 6 months follow-up)

Effect	Short Description	Unit	Ixchiq	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the Ixchiq arm achieving a Day 29 CHIKV neutralizing antibody titre µPRNT50 ≥150	% [95% CI] (n/N)	98.8% [96.5-99.8] (248/251)	2.4% [0.1-12.6] (1/42)	**SoE:** At Day 29 post-vaccination, proportion of baseline Ixchiq 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	VLA1553-321 – Part B Clinical Study Report v1.0 24 May 2024

Effect	Short Description	Unit	Ixchiq	Placebo	Uncertainties/ Strength of evidence	References
Immunogenicity	Proportion of baseline CHIKV seronegative participants in the Ixchiq arm achieving a Day 180 CHIKV neutralizing antibody titre µPRNT50 ≥150	% [95% CI] (n/N)	99.1% [96.9-99.9] (232/234)	0.0% [0.0, 9.0] (0/39)	**SoE:** At Day 180 post-vaccination, proportion of baseline Ixchiq 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	VLA1553-321 – Part B Clinical Study Report v1.0 24 May 2024
Unfavourable Effects						
Solicited systemic AEs	Headache	%	51.0	34.5	502 vaccinated adolescents with Ixchiq	table 2.7.4-25 clinical safety summary
	Fatigue	%	22.3	9.5	Idem	Idem
	Myalgia	%	26.9	12.3	Idem	Idem
	Arthralgia	%	12.9	5.6	Idem	Idem
	Fever	%	24.1	3.6	Idem	Idem
	Nausea	%	15.9	12.3	Idem	Idem
	Rash	%	3.6	0.8	Idem	Idem
	Vomiting	%	2.6	3.6	Idem	Idem
Solicited local AEs	Tenderness	%	19.9	14.7	502 vaccinated adolescents with Ixchiq	table 2.7.4-25 clinical safety summary
	Vaccination site pain	%	19.3	13.5	Idem	Idem
	Erythema	%	2.2	1.2	Idem	Idem
	Induration	%	4.2	4.4	Idem	Idem
	Swelling	%	1.4	2.8	Idem	Idem

Effect	Short Description	Unit	Ixchiq	Placebo	Uncertainties/ Strength of evidence	References
Unsolicited AEs (≥1% in Ixchiq and > than placebo) (not solicited)	Abdominal pain	%	4.6	3.6	502 vaccinated adolescents with Ixchiq	Table 60 CSR
	Upper respiratory tract infection	%	3.4	0.8	Idem	Idem
	Eye pain	%	3.4	0.8	Idem	Idem
	Dizziness	%	2	1.2	Idem	Idem
	Ear pain	%	1.6	0	Idem	Idem
	Sinusitis	%	1.2	0.4	Idem	Idem
White blood cell count decreased	Neutropenia (neutrophile decreased)	%	40.2	28.57	328 vaccinated adolescents with Ixchiq	Table 14.3.3.1.3 CSR
	Leukopenia (leukocyte decreased)	%	16.8	5.4	Idem	Idem
	Lymphopenia (lymphocyte decreased)	%	11.6	8.9	Idem	Idem
Other abnormal laboratory parameters (% in Ixchiq > than placebo)	Alkaline phosphatase increased	%	17.7	14.3	Idem	Table 14.3.3.2.3 CSR
	Leucocytosis (leukocytes increase)	%	14.6	10.7	Idem	Table 14.3.3.1.3 CSR
	Hypokalaemia	%	10.4	5.4	Idem	Table 14.3.3.2.3 CSR
AESIs (early and late onset)	Up to 6 months after vaccination	%	8.4	7.5	502 vaccinated adolescents with Ixchiq	Table 2.7.4-32 clinical safety summary
CHIK-like ARs (early onset)	Up to 30 days after vaccination	%	23.1	4.8	Idem	Table 1 CSR Addendum

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Chikungunya constitutes an important disease burden worldwide. 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). 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. In 2013, a second major outbreak occurred when a strain from the Asian lineage emerged in Saint Martin Island rapidly spreading to neighbouring islands and Central, South, and North America. 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 colonised by *Aedes albopictus*. One autochthonous case was recently detected in continental EU (France) and 11 in La Réunion. 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.

Ixchiq is approved in EU since June 2024 for active immunisation for the prevention of disease caused by CHIKV in individuals 18 years and older. On 30th January 2025, the EMA CHMP adopted a positive opinion on another CHIK vaccine (recombinant, adsorbed) (Vimkunya, manufactured by Bavarian Nordic) intended for the prevention of chikungunya disease in individuals 12 years and older.

To date there is no other prophylactic vaccine licensed in EU and no specific treatment, other than supportive, exists.

There are no efficacy data for Ixchiq. The conduct of efficacy trials was considered not feasible pre-authorisation due to unpredictable and short-lived outbreaks. Although immunogenicity results of the VLAA553-321 in adolescents are compelling, as they were in adults' studies, they are based on a neutralising antibody titre threshold reasonably likely to predict protection (supported by animal and sero-epidemiological data) and not on an established ICP. Uncertainties remain around how this threshold actually translates into protection against disease and/or infection. Therefore, the actual protection of Ixchiq remains to be confirmed.

Two post-approval effectiveness studies are currently planned and were discussed in the initial MA. 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 characterise the clinical protection offered by the vaccine. Feasibility assessments are ongoing. Adolescents (12-17 yrs at the time of vaccination) will be added to the trial population of the pragmatic RCT, and the TNCC study will include participants within the age limits approved per label in Brazil.

Overall, the reactogenicity was higher in both arms (Ixchiq and placebo) when comparing seronegative adolescents (VLA1553-321) to adults (pooled safety population: VLA1553-301, VLA1553-302, and VLA1553-101, mainly seronegative) (in particular for headache, fever, tenderness and pain at the injection site). Nevertheless, the increases of frequencies observed in Ixchiq versus placebo seem comparable in the adolescents and in the adults, and differences could be considered acceptable given the overall frequency and intensity. Moreover, increased rates of AESIs (early and late onset) and of early onset CHIK-like ARs have been reported with adolescents (compared to the adults) that could reflect the observed increased frequency of fever (symptom in combination in both definitions). However, overall, and because of all limitations, it is considered that the trends regarding the relative risk in Ixchiq recipients vs. placebo for the AE categories were similar between adults and adolescents.

Safety issues associated with Ixchiq in adolescents include very common white blood cell count decreased (leukopenia, neutropenia and lymphopenia), and chikungunya-like adverse reactions (such as fever associated with headache, myalgia, fatigue or arthralgia). These reactions are reflected in the SmPC.

However, there were no acute symptom/sign that were of concern. In particular, in the AESIs / CHIK-like ARs, only 1 symptom was serious (fever assessed as related to the vaccine); none were associated to meningoencephalitis or other serious acute disease/sign; and none were associated to arthritis. Finally, there were no cases of prolonged AESIs/CHIK-like ARs/AEs (i.e., with at least one symptom lasting ≥30 days), no chronic CHIK-like symptoms (i.e. without fever), long-term effects, and possible complications, such as long-lasting musculoskeletal stiffness or pain (myalgia), joint stiffness or pain (arthralgia), joint swelling/effusion, synovitis, arthritis, osteoarthritis, or neurological symptoms. CHIK-like AR is an important identified risk in the RMP, and it will be further characterised with post-authorisation safety studies.

3.7.2. Balance of benefits and risks

Ixchiq has been shown to induce robust CHIKV-specific neutralising antibody responses in adolescents, with titres above the threshold reasonably likely to predict protection in most participants, up to 6 months post-vaccination. Uncertainties remain around how this threshold actually translates into protection against disease and/or infection.

The actual protection of Ixchiq remains to be confirmed. Two post-approval efficacy/effectiveness studies are currently planned.

Overall, the safety profile of Ixchiq for healthy adolescents is considered similar to the safety profile in the healthy adults.

3.8. Conclusions

The overall benefit/risk balance of Ixchiq in adolescents 12 years of age and older is positive.

4. Recommendations

Outcome

Based on the review of the submitted data, the CHMP considers the following variation acceptable and therefore recommends the variation to the terms of the Marketing Authorisation, concerning the following change:

Variation accepted		**Type**	**Annexes affected**
C.I.6.a	C.I.6.a - Change(s) to therapeutic indication(s) - Addition of a new therapeutic indication or modification of an approved one	Type II	I, II and IIIB

Extension of indication to include active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in adolescents 12 years and older for Ixchiq, based on interim 6 months results from study VLA1553-321; this is a randomised, double-blinded, multicentre study to evaluate the immunogenicity and safety of the adult dose of VLA1553 6 months following vaccination in adolescents from 12 years to less than 18 years of age after a single immunisation. As a

consequence, sections 4.1, 4.2, 4.8 and 5.1 of the SmPC are updated. The Annex II and the Package Leaflet are updated in accordance. In addition, the MAH took the opportunity to introduce minor editorial changes to the PI. The RMP version 2.1 has been agreed.

The variation leads to amendments to the Summary of Product Characteristics, Annex II and Package Leaflet and to the Risk Management Plan (RMP).

Amendments to the marketing authorisation

In view of the data submitted with the variation, amendments to Annexes I, II and IIIB and to the Risk Management Plan are recommended.

This recommendation is subject to the following amended condition:

Description	Due date
Post-authorisation efficacy study (PAES): In order to confirm the efficacy of IXCHIQ in individuals 12 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

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 12 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

Paediatric data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0501/2023 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.