30 January 2025
EMA/59925/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Vimkunya

Common name: chikungunya vaccine (recombinant, adsorbed)

Procedure No. EMEA/H/C/005470/0000

Note

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

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List of abbreviations

µBCA	Micro bicinchoninic acid
µg	Microgram
AAS	Atomic absorption spectroscopy
ADME	Absorption, distribution, metabolism, and excretion
ADP	Assembled and labelled Drug Product
AE	Adverse event
AESI	Adverse event of special interest
AET	Analytical evaluation threshold
AEX	Anion exchange chromatography
ANOVA	Analysis of variance
AS	Active substance
AUC	Area under curve
BALB/c	Laboratory-bred mouse strain
BDP	Bulk drug product
BL	Break loose
BMI	Body mass index
BN	Bavarian Nordic A/S
BN-K	BN-Kvistgaard
C	Capsid protein of chikungunya virus Senegal strain 37997
CCI	Container closure integrity
CCS	Container closure system
CHIKV	Chikungunya virus
CHIKV VLP	Chikungunya virus virus-like particle
CHIKV VLP vaccine	Vaccine consisting of CHIKV VLP composed of E1, E2 and capsid proteins of CHIKV strain 37997
CHIKV-*luc*	CHIKV 181/25 containing firefly luciferase enzyme transgene
CHKVLP059	VRC-CHKVLP059-00-VP vaccine consisting of CHIKV VLP composed of E1, E2 and capsid proteins of CHIKV strain 37997 formulated without adjuvant
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CMR	Commercial
CoA	Certificate of analysis
CPMP	Committee for proprietary medicinal products
CPP	Critical process parameter
CPV	Continued Process Verification
CQA	Critical quality attribute (n/p: non/potential)
CSR	Clinical study report
CTM	Clinical trial material
CV	Coefficient of variation

DART	Developmental and reproductive toxicology
DF	Diafiltration
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
DP	Drug product
DS	Drug substance
DSP	Downstream process
DTT	Dithiothreitol
EBV	Epstein Barr virus
E	Envelope (protein)
E1	Envelope 1 protein
E2	Envelope 2 protein
eAF	Electronic application form
EDC	Electronic data capture
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
Emergent	Emergent Travel Health Inc.
EOP	End of production
EOS	End of study
ER	Engineering run
EU	European Union
FDA	U.S. Food and Drug Administration
FDP	Labelled finished drug product
F-PERT	Fluorescent product enhanced reverse transcriptase
GD	Gestation Day
GF	Gliding force
GFP	Green fluorescent protein
GLP	Good Laboratory Practice
GMFI	Geometric mean fold increase
GMP	Good Manufacturing Practice
GMT	Geometric mean titre
HAAV	Human adeno-associated virus
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCMV	Human cytomegalovirus
HCP	Host cell protein
HCV	Hepatitis C virus
HEK	Human embryonic kidney
HHV	Human herpes virus
HIC	Hydrophobic interaction
HILIC	Hydrophilic interaction chromatography
HIV	Human immunodeficiency virus
HMW	High molecular weight
HPLC	High performance liquid chromatography

HTLV	Human T-cell lymphotropic virus
HZ	Horizontal (storage)
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP-OES	Inductively couple plasma with optical emission spectroscopy
ID	Intradermal
IFN	Interferon
Ifnar$^{-/-}$	Type 1 interferon signalling (interferon-α/β receptor-1) knockout mice
IgG	Immunoglobulin G antibody isotype
IM	Intramuscular
IP	Investigational product
IPC	In-process control
IPT	In-process test
IR	Immunoreactivity
IV	Intravenous
kg	Kilogram
KPA	Key performance attribute
KPP	Key process parameter
LC	Liquid chromatography
LD	Lactation Day
LER	Low endotoxin recovery
LIVCA	Limit of *in vitro* cell age
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LOQ	Limit of quantification
luc	Firefly luciferase transgene
MAAE	medically attended adverse events
mAb	Monoclonal antibody
Max	Maximum
MCB	Master cell bank
mg	Milgram
Min	Minimum
mL	Millilitre
MMV/MVM	Minute virus of mice
MO	Major objection
MS	Mass spectrometry
N/A	Not applicable
NGS	Next generation sequencing
NHP	Non-human primate
NIH VRC	National Institutes of Health Vaccine Research Centre
NLT	Not less than
NMT	Not more than

NOR	Normal operating range
NT_{80}	Neutralising titre 80%
OECD	Organisation for Economic Co-operation and Development
OOS	Out of specification
PAES	post-authorisation efficacy study
PAR	Proven acceptable range
PASS	post-authorisation safety study
PaxVax	PaxVax Inc.
PBT	Polybutylene terephthalate
PCR	Polymerase chain reaction
PCV	Porcine circovirus
pd	protocol deviation
PETG	Polyethylene terephthalate glycol
PFS	Pre-filled syringe
PFU	Plaque forming units
Ph. Eur.	European Pharmacopoeia
PPQ	Process performance qualification
PV	pharmacovigilance
PXVX0317	Original nomenclature for the CHIKV VLP vaccine
QA	Quality assurance
QC	Quality control
QP	Qualified person
qPCR	Quantitative polymerase chain reaction
Q-SEC	Quaternary amine anion exchange – size exclusion chromatography
QTPP	Quality target product profile
RCB	Research cell bank
RH	Relative humidity
RMM	risk minimisation measures
RMP	risk management plan
RNA	Ribonucleic acid
RP	Reversed phase
RSD	Relative standard deviation
RT	Room temperature
RT-qPCR	Reverse-transcription quantitative polymerase chain reaction
S.E.M.	Standard error of the mean
SAE	Serious adverse event
SC	Subcutaneous
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SFV	Semliki Forest virus
SMC	Safety monitoring committee
SmPC	Summary of product characteristics

SNA	Serum neutralizing antibody
SOP	Standard operating procedure
TAMC	Total aerobic microbial count
TEM	Transmission electron microscopy
TI	Tolerance interval
TOR	Time out of refrigeration
TYMC	Total yeasts and molds count
UF/DF	Ultrafiltration/Diafiltration
US	United States (of America)
USP	Upstream process/United States Pharmacopoeia
UV	Ultraviolet
VLP	Virus-like particle
VT	Vertical (storage)
WCB	Working cell bank
WFI	Water for injection
WHO	World Health Organization

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Bavarian Nordic A/S submitted on 26 June 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Vimkunya, through the centralised procedure under Article 28 of Regulation (EC) No 1901/2006. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 19 September 2019.

The applicant applied for the following indication: Active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 12 years and older. The use of this vaccine should be in accordance with official recommendations.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

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

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

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision (P/0159/2021) on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0159/2021 was not yet completed as some measures were deferred.

The PDCO agreed to grant a deferral for children less than 12 years of age.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

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

1.5. Applicant's request(s) for consideration

1.5.1. Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

1.5.2. New active Substance status

The applicant requested the active substance Chikungunya virus (CHIKV) virus-like particle (VLP) proteins (capsid protein (C) and envelope proteins E1 and E2) derived from CHIKV Senegal strain 37997, produced in human embryonic kidney cells by recombinant DNA technology, and which are adsorbed on aluminium hydroxide, hydrated, contained in the above medicinal product, to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. PRIME

Vimkunya was granted eligibility to PRIME on 19 September 2019 in the following indication: Active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 12 years and older. Eligibility to PRIME was granted at the time in view of the following:

- The seriousness of the condition is acknowledged in particular due to persistent polyarthritis and polyarthralgia, as well as the potential for severe chikungunya fever which can manifest as encephalopathy and encephalitis, myocarditis, hepatitis, and multiorgan failure.
- There are no authorised products for prevention or treatment of the target indication, and management remains supportive; Therefore, the unmet need in the sought indication is acknowledged.
- Immunogenicity of the product has been examined in several in vivo models and antibodies are reported to be neutralising.
- In non-human primates challenged with the virus, vaccination was protective against viraemia and development of arthritis.
- An ongoing clinical study in healthy volunteers' seroconversion after one dose was reported in approximately 85% of patients within a week.

Upon granting of eligibility to PRIME, Andrea Laslop was appointed by the CHMP as rapporteur.

A kick-off meeting was held on 28 February 2020. The objective of the meeting was to discuss the development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

Implementation of previous scientific advice, with follow-up advice as appropriate.

Post-authorisation evidence generation and specifically the design of an antibody and persistence study to be discussed in a scientific advice.

1.7. Scientific advice

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

Date	Reference	Co-ordinators
12 December 2019	EMEA/H/SA/4283/1/2019/PR/III	*Hans Ovelgönne, Mair Powell*

30 April 2020	EMEA/H/SA/4283/1/FU/1/2020/PR/II	*Andrea Laslop, Mair Powell*
23 July 2020	EMEA/H/SA/4283/1/FU/2/2020/PR/II	*Mair Powell, Andrea Laslop*
20 May 2021	EMA/SA/0000053546	*Dieter Deforce, Andrea Laslop*
16 December 2021	EMA/SA/0000071591	*Ingrid Schellens, Andrea Laslop*
13 October 2022	EMA/SA/0000100081	*Mair Powell, Andrea Laslop*
24 January 2023	EMA/SA/0000115136	*Florian Klinglmueller*

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

- *Strategy regarding potency assay, stability and shelf-life, DS and DP characterisation and specification.*
- *Feedback regarding the manufacturing facilities and open operations for the cell culture stage.*
- *Changes in the drug substance manufacturing process.*
- *Strategy to demonstrate comparability between Phase III CTM and PPQ/Commercial manufacturing process.*
- *Use stability data from the phase 3 clinical batches as primary stability data for a future MAA and timelines regarding the MAA submission.*
- *Agreement that a human factor validation is not needed for the pre-filled syringe.*
- *Design and endpoints for the passive transfer study in NHP.*
- *Agreement that conducting a field efficacy trial in a chikungunya-endemic area is not logistically feasible.*
- *Agreement that serum neutralizing antibody (SNA) is the appropriate surrogate immune marker to support marketing authorisation.*
- *Agreement that the CHIKV-Luc assay method is appropriate for measurement of SNA.*
- *Agreement with the proposed protective human SNA threshold titre level.*
- *Design of Phase 3 studies EBSI-CV-317-004 and EBSI-CV-317-005 including primary and secondary endpoints, safety monitoring plan, sample size and statistical power.*
- *Agreement on the clinical development and safety database to support MAA.*
- *Strategy to support duration of protection in the Summary of Product Characteristics (SmPC).*
- *The design of EBSI-CV-317-008 study to evaluate antibody persistence, anamnestic response and responses to booster doses.*
- *Suitability of the post-approval efficacy EBSI-CV-317-007 trial design including CHIKV case definitions, choice of endpoints, screening and enrolment strategy, safety management strategy and choice of analysis sets.*

1.8. Steps taken for the assessment of the product

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

Rapporteur: Daniela Philadelphy Co-Rapporteur: Jean-Michel Race

The application was received by the EMA on	26 June 2024
Accelerated Assessment procedure was agreed-upon by CHMP on	22 February 2024
The procedure started on	18 July 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	18 September 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	24 September 2024
In accordance with Article 6(3) of Regulation (EC) No 726/2004, the CHMP Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	03 October 2024
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	15 October 2024
The applicant submitted the responses to the CHMP consolidated List of Questions on	08 November 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	28 November 2024
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	05 December 2024
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	10 December 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues on	20 December 2024 and 24 January 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	16 January 2025
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	23 January 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Vimkunya on	30 January 2025
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	30 January 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Chikungunya virus is an arthropod-borne alphavirus of the family *Togaviridae*. The virion contains a positive-sense single-strand ribonucleic acid (RNA) genome with a long open reading frame coding for C, envelope (E1, E2, E3) and 6K structural proteins, together with four non-structural proteins (nsP1–4). Chikungunya virus is transmitted to humans through the bite of mosquitoes infected with CHIKV, primarily *Aedes aegypti* and *Aedes albopictus*. Once infected, a mosquito is infectious for the rest of its life and can transmit virus to multiple hosts. Although mosquitoes are the primary mode of transmission of CHIKV, blood-borne transmission via needle stick is possible and maternal-foetal transmission has been documented during pregnancy (Contopoulos-Ioannidis et al., PLoS Negl Trop Dis. 2018).

In the human host, the virus incubates for 1 to 12 days before reaching high viremia levels and inducing initial symptoms of fever and flu-like syndrome (Vairo et al., Infect Dis Clin North Am. 2019). Acute febrile chikungunya cases commonly present with fever and joint pain and other symptoms may include joint stiffness or swelling, muscle pain, lack of appetite, fatigue, headache, or rash (Suhrbier, Nat Rev Rheumatol. 2019). The most classic symptom of acute CHIKV disease is a debilitating polyarthralgia that is present in greater than 90% of cases (Schilte et al., PLoS Negl Trop Dis. 2013). Atypical manifestations are possible, such as encephalitis, Guillain-Barré syndrome, or myocarditis (Suhrbier, Nat Rev Rheumatol. 2019). Sepsis and septic shock have been reported in adults, adolescents, and infants (Gupta et al., Indian J Crit Care Med. 2018; Sharma et al., Emerg Infect Dis. 2018). Hospitalization and death are rare but do occur and more commonly in the very young and in the elderly population with comorbidities (Costa et al., Trop Med Infect Dis. 2023). In nearly half of cases, chronic symptoms such as continual or recurrent arthralgias, depression, and mood and sleep disorders (Paixao et al., Trans R Soc Trop Med Hyg. 2018; Suhrbier, Nat Rev Rheumatol. 2019) persist for months or years, with significant impact on quality of life and daily productivity, primarily due to the reduced mobility associated with joint pain. Older age and more severe acute symptoms are associated with worse arthritic sequelae (van Aalst et al., Travel Med Infect Dis. 2017).

2.1.2. Epidemiology and risk factors

First recognized in what is now Tanzania in 1952 (Ross, J Hyg (Lond). 1956), chikungunya has been reported from every continent except Antarctica (Silva et al., Acta Trop. 2018) and has spread over the past two decades from Asia and Africa to Europe and the Americas, and from the tropics and subtropics to temperate regions. A literature review of the epidemiology of chikungunya from 1999 to 2020, encompassing 97 outbreak reports from 45 countries, confirms the disease's status as an emerging global public health concern (Bettis et al., PLoS Negl Trop Dis. 2022). The Coalition for Epidemic Preparedness Innovations (CEPI) lists CHIKV as a priority pathogen with epidemic and pandemic potential (CEPI, Priority diseases. 2024); moreover, chikungunya is on the World Health Organization (WHO) 2024-2026 Vaccines Prequalification Priority List (Medium-Priority Vaccine) (WHO, 2024).

In the past 70 years, CHIKV has emerged several times to cause large, region-wide outbreaks of disease (Weaver et al., Annu Rev Entomol. 2020; Mourad et al., Curr Infect Dis Rep. 2022). In 2004, an outbreak in coastal Kenya spread to Indian Ocean islands and then to India, causing explosive

epidemics (Weaver & Lecuit, N Engl J Med. 2015). Cases imported by travellers found their way into Europe and local transmission followed, with 205 cases reported in northern Italy in 2007 (Rezza et al., Lancet. 2007). This 2007 outbreak was the first reported autochthonous transmission in a temperate region due to the wide distribution of the vector, *Aedes albopictus* (also called tiger mosquito), in many parts of Europe and the United States of America (US) (Chen & Wilson, Curr Opin Infect Dis. 2010). A second outbreak of locally transmitted chikungunya occurred in Italy in 2017, with approximately 400 total cases (Rezza, J Travel Med. 2018). While more than half the cases spread within the immediate area of the outbreak, secondary cases were documented as far as 60 miles (~100 km) away (Guzzetta et al., BMC Med. 2020).

Globally, over two million cases have been reported since 2005. Chikungunya disease has now been identified in over 110 countries in Asia, Africa, Europe, and the Americas (WHO, Chikungunya. 2024). The onward transmission of CHIKV in mainland US and Europe is mostly linked to importation of the virus by viraemic travellers into receptive areas with established and active competent vectors (*Aedes mosquitoes*), which are established in Europe and the US (Leta et al., Int J Infect Dis. 2018; Grabenstein et al., Travel Med Infect Dis. 2023). While most US and European cases of chikungunya remain imported, locally acquired cases were reported sporadically in the European Union (EU) (Italy and France in 2007, 2010, 2014, and 2017 (ECDC, Autochthonous transmission of chikungunya virus in mainland EU/EEA, 2007–present. 2023) and in US or US Territories (CDC, Chikungunya in the US. Last reviewed October 11, 2024) and are susceptible to increase. The likelihood of the occurrence of local transmission events of CHIKV in areas where the vectors are present remains high, as the environmental conditions are favourable for vector activity and virus replication in vectors. The recent surge in cases in Latin America, partially attributed to warmer than average temperatures in the region (Giovanetti et al., Emerg Infect Dis. 2023), underscores the multiple factors contributing to the global persistence of CHIKV as a public threat.

2.1.3. Clinical presentation, diagnosis

Diagnosis of CHIKV infection can be based either on the presence of the CHIKV or on serology. During the acute phase of disease, there are generally high levels of viremia, and reverse transcription quantitative polymerase chain reaction (RT-qPCR) or isothermal amplification can be used to detect viral RNA (Simo et al., Trop Med Infect Dis. 2023). Immunoglobulin M (IgM) antibodies against CHIKV develop within one week after infection and may mediate early control of infection; IgM may persist for several months. Immunoglobulin G (IgG) antibodies typically develop after IgM and persist for months to years and perhaps decades (Kam et al., J Virol. 2012; Kam et al., J Infect Dis. 2012; Nitatpattana et al., Virol J. 2014; Galatas et al., PLoS Negl Trop Dis. 2016). Protection against subsequent infection has been shown to be associated with the presence of anti-CHIKV antibodies that neutralize the virus in vitro (Yoon et al., PLoS Negl Trop Dis. 2015).

2.1.4. Management

The treatment of CHIKV disease is symptomatic, including non-salicylate analgesics and non-steroid anti-inflammatory therapy. There are no specific antiviral drugs against CHIKV. Prevention of CHIKV infection is mostly restricted to protection against mosquito bites.

From a Public Health perspective, a prophylactic vaccine against disease caused by CHIKV is of major interest, as CHIKV infection can result in severe disabling arthralgia, which occurs as recurrent joint pain that can persist for months to years' post-infection in a large proportion of infected individuals.

Recently, an EU market-authorisation was issued on 28/06/2024 for a monovalent chikungunya live-attenuated vaccine (LAV) (Ixchiq, manufactured by Valneva), indicated for active immunisation for the

prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older after receiving a positive opinion from the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP) on 30/05/2024.

2.2. About the product

Vimkunya, or Chikungunya virus (CHIKV) virus-like particle (VLP) vaccine, abbreviated as CHIKV VLP vaccine (previously designated PXVX0317), is a sterile aluminium hydroxide adjuvanted vaccine. The CHIKV VLP component of the vaccine consists of VLPs that are produced in human embryonic kidney 293 cells and contain three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997: Capsid (C, ~35 kDa), Envelope 1 (E1, ~55 kDa), and Envelope 2 (E2, ~50 kDa), which self-assemble to form a spherical, highly ordered VLP (Akahata et al., Nat Med. 2010). Comparison of the CHIKV VLP and the native virus by transmission electron microscopy and cryo-electron microscopy demonstrate that the particles are structurally indistinguishable (Basore et al., Cell. 2019). The drug product is comprised of 40 µg CHIKV VLP adsorbed on aluminium hydroxide (corresponding to approximately 300 µg of aluminium) and stabilized with formulation buffer. It is supplied as a glass pre-filled syringe (PFS) with 0.80 mL deliverable dose volume.

The VLP platform is a well-established technology with decades-long track records of efficacy and safety and can overcome drawbacks from LAVs such as their infectious nature, their potential for reversion to a virulent form, their risk of mutation which contradicted them to special populations (pregnant women, immunocompromised subjects).

For details on the mechanism of action, refer to section 2.6.2.2. The product has not yet been approved in any market.

The indication is active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 12 years and older.

The proposed posology is a single dose of 0.8 ml (40 µg CHIKV VLP vaccine adsorbed on aluminium hydroxide, hydrated) to be administered by intramuscular (IM) injection in the deltoid muscle.

The anatomical therapeutic chemical (ATC) code for CHIKV VLP vaccine has not been assigned yet.

2.3. Type of Application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the therapeutic innovation of the candidate vaccine, in the context of an identified unmet medical need in individuals 12 years and older.

2.4. Quality aspects

2.4.1. Introduction

The filled drug product (FDP) i.e. labelled finished drug product), VIMKUNYA is presented as a sterile 40 µg Chikungunya virus (CHIKV) virus-like particle (VLP) suspension for injection adsorbed on aluminium hydroxide, hydrated (corresponding to approximately 300 µg of aluminium) and stabilised with formulation buffer containing sucrose, dipotassium phosphate, potassium dihydrogen phosphate, sodium citrate and water for injection (WFI).

The CHIKV VLP component of the vaccine consists of VLPs that are produced in human embryonic kidney 293 (HEK293) cells and contain the envelope (E1 and E2) and capsid (C) proteins of CHIKV Senegal West African (WA) strain 37997.

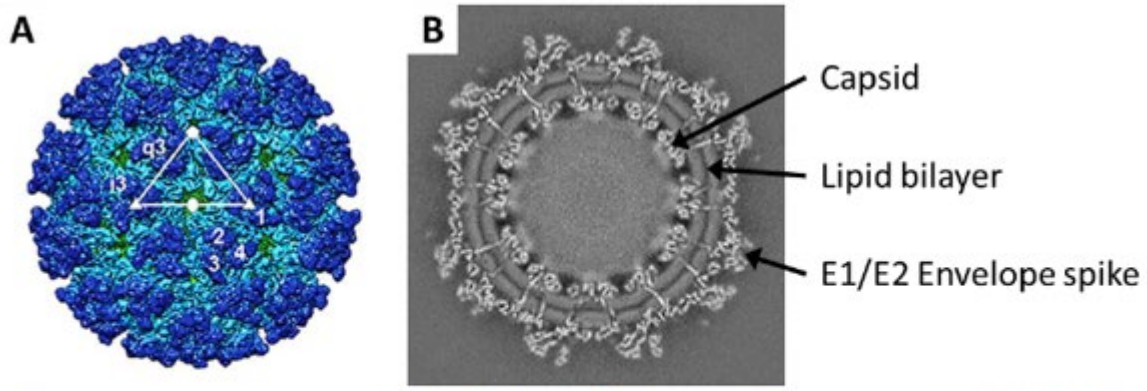
The vaccine is supplied as a pre-filled syringe (PFS) with 0.80 mL deliverable dose volume, to be administered intramuscularly (IM) in the deltoid muscle. A Notified Body Opinion confirming compliance of the device part of the PFS to the relevant General Safety and Performance Requirements in Annex I of Regulation (EU) 2017/745 is available.

2.4.2. Active Substance

2.4.2.1. General information

The chikungunya virus virus-like particle (CHIKV VLP) active substance (AS) is comprised of three (3) recombinant CHIKV structural proteins derived from the CHIKV Senegal strain 37997: capsid (C, ~35 kDa), envelope 1 (E1, ~55 kDa), and envelope 2 (E2, ~50 kDa), which self-assemble to form the VLP (Akahata, 2010). The spherical, highly ordered structure of the CHIKV VLP is shown in Figure 1. Cryo-electron microscopy shows the trimeric spikes that are each composed of three heterodimers of E1 and E2 (Figure 1A). The capsid core consists of hexamers around each 2-fold axis and pentamers at each 5-fold vertex of the nuclear capsid. The capsid core is separated from the E1 and E2 proteins by a ~15 Å-wide lipid membrane (Figure 1B). The membrane is traversed by pairs of α helices representing the E1 and E2 carboxyterminal regions. Comparison of the CHIKV VLP and the native virus by transmission electron microscopy and cryo-electron microscopy demonstrate that the particles are structurally indistinguishable.

Figure 1 Structure of the CHIKV VLP



(A) Colored surface representation of CHIKV VLP resulting from cryo-EM reconstruction. CHIKV VLP proteins are arranged in an ordered, spherical structure. The white triangle indicates one icosahedral asymmetric unit.
(B) Two-dimensional equatorial slice of CHIKV VLP captured in a cis-transmission electron micrograph. (Source: **Basore, 2019**)

The capsid protein consists of 261 amino acids with no glycosylation site. E1 glycoprotein of CHIKV is a 439 amino acid protein with an N-linked glycosylation site at residue Asn141 and E2 glycoprotein of CHIKV is 424 amino acids in length and has two N-linked glycosylation at positions Asn263 and Asn345. Based on sequence similarity with Semliki Forest Virus (SFV), both these glycoproteins are expected to have acylation sites which has also been shown for CHIKV VLP transiently expressed in the Human Embryonic Kidney 293 (HEK293) cells.

The mechanism of action of the CHIKV VLP is expected to elicit neutralising antibodies that will block multiple steps involved in infection of the target cells by the native virus, both entry and release. Two well characterised conformational antibodies are employed in the microfluidic immunoassay as an in vitro measure of the potency of each CHIKV VLP vaccine lot at release and during stability testing.

2.4.2.2. Manufacture, characterisation and process controls

The active substance manufacturer is Bavarian Nordic Berna GmbH, Oberriedstrasse 68, 3174 Thörishaus Switzerland. The manufacturers involved in testing the Chikungunya Virus Virus-Like Particle (CHIKV VLP) active substance (AS) and the specific testing conducted at each site are provided.

Description of manufacturing process and process controls

The AS manufacturing process follows a standard method for recombinant protein production. HEK293 cells (VRC293) are cultured in suspension and chemically transfected with the plasmid (VRC8900). The manufacturing process is divided into three main stages, (1) upstream process starting with thawing of a WCB vial, cell expansion in shaker flasks and bioreactors, (2) downstream process with harvest by depth filtration, followed by several purification steps and (3) AS manufacture (sterile filtration). The information on medium and buffer preparation, column cleaning and sanitisation procedures, and resin lifetime (up to 5 chromatography cycles) has been provided. Minimum and maximum AS batch sizes are stated. As indicated in the dossier, the CHIKV VLP AS manufacturing process does not involve reprocessing or refiltration. Flow diagrams including in-process controls are provided. In summary, the process has been sufficiently described and in-process controls are adequately set to control the process.

Control of materials

A list of compendial and non-compendial raw materials, their compendial status and use within the manufacturing process has been provided. Specifications for non-compendial materials are included in the dossier. Media are serum-free and chemically defined. The qualitative components of the expression media are registered in the dossier and confirmation has been provided that there is a quality agreement with the manufacturer in place to notify the costumer about relevant changes.

No material of animal origin is used in the manufacturing process of the CHIKV VLP vaccine active substance.

VRC8900 plasmid

The structure and generation of the VRC8900 plasmid is described in sufficient detail. The VRC8900 plasmid was transformed into competent *E. coli* NEB5α cells to generate a research cell bank (RCB), followed by manufacture of the master cell bank (MCB) and working cell banks (WCBs). MCB and WCBs were tested for identity, purity, plasmid presence and stability and viability. Sequence integrity (full plasmid) was confirmed for the MCB but not for the WCB. The VRC8900 plasmid MCB and WCB vials are stored at -70°C ±10°C. Stability testing of the VRC8900 plasmid WCB will be performed annually, while stability of MCB will be tested when thawed for preparation of a new WCB.

One vial of the VRC8900 plasmid WCB is used to manufacture the production plasmid. The manufacturing process of the production plasmid itself (which is used to transfect HEK293 cells in routine productions) is sufficiently outlined including in-process controls (IPCs) (culture purity prior inoculation and at harvest). Manufacture and release/stability testing of purified VRC8900 plasmid is performed. The purified VRC8900 plasmid is tested at release with appropriate acceptance criteria for content (A_{260}), purity ($A_{260/280}$), identity (restriction analyses), total circular and supercoiled forms (anion exchange high-performance liquid chromatography, AEX-HPLC), percent host-cell RNA (HIC

(hydrophobic interaction chromatography) -HPLC), percent host-cell protein (bicinchoninic acid, BCA assay), percent host-cell DNA (quantitative polymerase chain reaction, qPCR), endotoxin, osmolality, pH, appearance, degree of coloration and clarity, identity (sequencing), TAMC (total aerobic microbial count) / (total yeast mould count) TYMC, and mycoplasma. Identity testing is performed by sequence analysis covering the entire plasmid. The purified plasmids are stored at ≤ -20°C in polyethylene terephthalate glycol (PETG) bottles and periodically tested for appearance, pH, content, and % circular and supercoiled forms. The release and stability testing applied is found suitable also for qualification of future purified plasmid lots.

VRC293 MCB and WCB

The source, history and generation of the HEK293 MCB (VRC293 MCB) and current WCB (VRC293 WCB) is described in sufficient detail and includes at least identity, sterility, viability and adventitious agent testing. Additional tests are also performed for MCBs. Cell banks (MCB, and WCBs) were in the main, tested in compliance with Ph. Eur. 5.2.3 on "Cell substrates for the production of vaccines for human use" and ICHQ5A and ICHQ5D requirements. The MCB was not tested for mycobacteria, however, the respective test was performed on the WCB. Methods used for VRC293 MCB and WCB characterisation testing are described in sufficient detail. The controls and validity criteria are considered suitable to ensure proper performance of the individual assays.

The WCB is stored in the gas phase of liquid nitrogen tanks. WCB stability (IPC cell count and cell viability) is evaluated each time a AS manufacturing process is initiated or at least every 5 years in case no production takes place.

A protocol for preparation and qualification of future WCB is in place and found acceptable.

Cells at the limit of in vitro cell age

Cells at the limit of in vitro cell age (LIVCA) were evaluated following a two-tiered approach, since the transfected cells are not proliferating anymore and therefore cannot continue to be cultured. Safety with respect to adventitious viruses was evaluated on both EOP (Tier 1) and LIVCA cells (Tier 2), while cell substrate stability was assessed on LIVCA cells. The testing performed on EOP and LIVCA cells is acceptable and cell substrate integrity and safety with respect to adventitious viruses has been confirmed.

Control of critical steps and intermediates

The overall control strategy and definition/classification of terms is outlined in the dossier. Critical and non-critical process parameters (CPPs/ nCPPs) associated with the manufacturing process have been provided and discussed in Module 3. Justifications for criticality assignment of CPPs and definition of key process parameters (KPPs) have been provided. The targets, normal operating ranges (NORs) and proven acceptable ranges (PARs) are sufficiently justified based on process characterisation and validation data.

In-process controls (IPCs) and in-process tests (IPTs) are defined for each manufacturing step and testing and respective acceptance criteria are considered largely appropriate.

The post-use sterile filter integrity test and preceding microbial enumeration tests in the downstream are defined as IPTs and not IPCs since these results and the post-use sterile filtration results are typically unavailable before manufacture of BDP. Thus, IPC values are actively used to further calculate or directly control the process while IPT values are recorded but not used to guide the next step in the process. Confirmation that confirmed filter integrity failure of both sterilising filters will lead to batch rejection has been provided.

Process validation

Process performance qualification (PPQ) was performed following a classical validation approach with manufacture of three consecutive process validation batches at scale. The PPQ was executed according to the approved protocol under normal operating conditions covering all manufacturing steps from thaw of WCB VRC293 through sterile filtered AS. Quality and performance attributes complied with pre-defined acceptance criteria. The results of the three consecutive PPQ batches demonstrated that the upstream production (USP), downstream purification (DSP) through the complete AS process, operated within established parameters, performed effectively and reproducibly to produce product meeting the pre-determined requirements and quality attributes. All process parameters were within their defined NOR and the process critical quality attributes (CQAs) and KPAs met the defined acceptance criteria.

The criticality level of several performance and quality attributes was updated following PPQ and appropriate justifications have been provided in most instances. Criticality of residual host cell DNA has been re-defined from presumptive critical quality attribute (pCQA) to non-critical quality attribute (nCQA) given that PPQ demonstrated consistent clearance of host cell DNA to levels well below than the WHO guidance recommendation of ≤ 10 ng/dose. Furthermore, it is noted that residual plasmid DNA is reduced to consistently low levels with a fragment size below 200 bp. For N-glycosylation and protein composition (i.e. ratio of the three co-expressed proteins), it has been confirmed that these remain CQAs and are tested as part of extended characterisation studies when substantial changes in upstream processing are introduced.

Resin-life time studies were performed. Hold times are defined. The proposed intermediate hold times are sufficiently supported by process validation data of one engineering run held to the maximum process time and hold times for intermediate steps at scale (cumulative hold run), and a small-scale hold time study using all three PPQ batches. The proposed NOR and PAR for the hold time of sterile filtered AS at room temperature in the isolator (start of hold after filtration – start of BDP formulation), are sufficiently justified based on data. Hold time validation results have been provided for three batches of formulation buffer. Specified impurities were shown to be removed to consistently low levels during PPQ. The final step for AS manufacturing is sterile filtration. Sterile filter validation was performed using the same filter used in production. The validation was performed with three filter units from three unique batches. Filter integrity tests and challenge tests complied with acceptance criteria.

Manufacturing process development

Multiple changes were implemented between phase 1/2, phase 2, phase 3 and PPQ/commercial manufacturing. Changes implemented between clinical phases and manufacture of PPQ/commercial material are sufficiently detailed in the dossier. The main changes between phase 2 and phase 3 manufacturing include scaling up the manufacturing process and process optimization. After the manufacture of the phase 3 clinical trial material, the manufacturing site was redesigned for the commercial manufacturing and aseptic formulation of bulk active substance, and additional changes were implemented at different production stages to increase process productivity, robustness, and consistency.

Limited comparability data are available for early clinical phases, while the additional changes implemented following Phase 3 and prior to PPQ/commercial production were evaluated in an extensive comparability study. Selection of batches used for comparability studies has been sufficiently justified. To establish comparability between Phase 3 and PPQ/commercial material, 4 pre-change batches (three Phase 3 batches) were compared to 4 post-change batches (3 PPQ batches and one additional GMP batch) using release, in-process and extended characterisation testing

Stability data were not considered for comparability testing, since the AS is further progressed to BDP manufacture.

Provided data for release and in-process tests and extended characterisation testing indicate comparability. Levels for residual plasmid DNA are comparable between Phase 3 clinical trial material (CTM) and PPQ batches. Respective plasmid DNA sizes are well below 200 bp. Comparability of purity has been evaluated and pre- and post-change N-glycan chromatograms indicate comparability for all batches tested.

Characterisation

The CHIKV VLP structure and size were analysed by transmission electron microscopy (TEM) (1 Phase 2, 2 Phase 3, 3 PPQ batches), cryo-electron microscopy and dynamic light scattering (DLS), respectively. The VLPs for all the lots was approximately 60 nm in diameter, and their morphology and size correlates well with alphaviruses and native CHIKV.

Physicochemical characterisation was performed by western blot (identity, confirmed by peptide mapping (partial coverage)), Reversed-phase HPLC (RP-HPLC) (ratio of proteins and purity), hydrophilic interaction liquid chromatography (HILIC) and liquid chromatography mass spectrometry (LC-MS) (N-glycan analysis), LC/MS/MS for evaluation of post-translational modification, SDS-PAGE (purity), and quaternary amine anion exchange–size exclusion chromatography (Q-SEC) HPLC (concentration of intact CHIKV VLP). Western blot analysis was performed for one Phase 3 and the three PPQ batches for respective AS and the corresponding BDP/ DP (drug product i.e. unlabelled finished product).

The relative molar ratios of the capsid, E1, and E2 proteins of 1:1:1 observed by SDS-PAGE/Western blot were confirmed by RP-HPLC using a fluorescence detector for process development, Phase 3 and the PPQ batches. The LC-MS/MS results confirmed that the bands observed in the SDS-PAGE gel for the RP-HPLC fractions correspond to their respective E1 and E2 proteins, which is further supported considering their respective molecular weight which is in multiples of the monomeric proteins. As indicated by the Applicant, these high molecular weight species may be a result of their exposure to the HPLC conditions.

Comparability throughout development was confirmed including post-translational modifications.

Biological activity

As regards biological activity of CHIKV VLPs, the Applicant refers to published literature for studies in vaccinated mice, non-human primates and humans. Furthermore, the Applicant refers to the protection of non-human primates (NHPs) from high-dose CHIKV challenge observed following vaccination with CHIKV VLP. These studies are discussed in the respective non-clinical and clinical ARs.

In vitro potency (specific activity/potency) is determined as the ratio of immunoreactivity (microfluidic immunoassay) to total protein concentration (alum-µBCA). Immunoreactivity (microfluidic immunoassay) is determined by a nanolitre-scale sandwich immunoassay performed on the Gyrolab xPlore platform using two CHIKV neutralising monoclonal antibodies. For monoclonal antibody (mAb) characterisation, the Applicant refers to published literature showing that they are targeting conformational surface epitopes that are adjacent or overlapping the protein's receptor binding site and their capacity to neutralise CHIKV in vitro. Furthermore, results of studies conducted on CHIKV VLP treated either with reducing agent (dithiothreitol, DTT) or heated showing the ability of the immunoreactivity assay to appropriately detect changes in epitopes conformation have been provided.

In vitro immunoreactivity and mouse immunogenicity correlation studies

The relevance of the immunoreactivity assay for determination of potency was investigated in two studies comparing *in vitro* immunoreactivity results to mouse neutralising antibody titres (NT80) across a range of CHIKV VLP DP doses. *In vitro* and *in vivo* results were directly compared using the same samples. The *in vitro* assay is more sensitive to small decreases of vaccine dose than the *in vivo* mouse immunogenicity assay, which is not unexpected.

Stability-indicating properties of the *in vitro* potency assay and comparability with *in vivo* data were evaluated in two studies using aged material.

Impurities

Aggregates may form when the CHIKV VLPs are exposed to e.g. thermal stress. Aggregate formation is well controlled and tested by DLS at AS release. The degradation pathway of the CHIKV VLP was evaluated for one AS batch, which was subjected to heat treatment at 44˚C for 2-14 days. Respective samples were analysed using different analytical methods. Respective data show a progressive loss of protein structure proportional to length of heat treatment, while particle size increases.

Process-related impurities relevant at the AS stage have been discussed including plasmid DNA, host cell DNA, host cell proteins, extractables/leachables and adventitious agents. Host cell protein, adventitious viruses, sterility, mycoplasma and endotoxins are controlled as release assays at the stage of pre-filtered harvest and/or AS. Effective removal to limits below the limit of detection was demonstrated during DSP. The respective limits are justified based on risk assessments.

Following benzonase treatment, residual host cell DNA and plasmid DNA is well below a fragment size of <200 bp. Residual host cell DNA content is reduced to values well below 10 ng/per dose (0/1 ng/per dose), while residual plasmid DNA is reduced to consistently low levels. The risk as regards leachables/extractables originating from single-use manufacturing equipment and the AS primary container can be considered as low. Therefore, specified impurities have been present in product studied in clinical trials with the exception of leachables/extractables from single-use equipment.

2.4.2.3. Specification

The specifications for CHIKV VLP AS include appearance, description, identity, quantity, purity, impurities and potency as set out in ICH Q6B. The pre-filtered viral harvest is tested for microbial enumeration, *in vitro* adventitious viruses, and mycoplasma. The quality attributes tested are considered acceptable for the control of CHIKV VLP pre-filtered viral harvest and AS at release. No shelf-life specifications are proposed, since the AS is processed directly to bulk DP. The hold time of the AS was validated during PPQ.

Active substance specifications are justified based on product understanding and critical quality attributes (CQAs), safety aspects, clinical experience, toxicology studies, process capability and manufacturing history and relevant guidelines including ICH Q6B and ICHQ5A and Ph. Eur. 0153 General Monograph on Vaccines for Human Use. Release data of four Phase 3 clinical AS batches, two engineering non-GMP batches, three PPQ batches and two additional post-PPQ GMP batches were used to justify the proposed acceptance criteria. For quantitative release parameters, one-sided tolerance intervals (TI) with 95% probability and 99% coverage were calculated to guide setting of specification limits. As requested, the Applicant justified the 3-replicates approach used for justification of specifications of the attributes pH, particle size, purity, host cell proteins (HCP) and residual host DNA.

The proposed acceptance criteria for appearance, pH, identity, particle size and the safety-relevant tests on the pre-filtered harvest are agreeable. The acceptance criterion for total protein content was

tightened. Furthermore, the acceptance criterion for the immunoreactivity assay has been tightened in line with the one-sided tolerance intervals with 95% probability and 99% coverage. Batches administered in the Phase 3 clinical studies were included in the respective statistical analyses. The specification for purity by SDS-PAGE has been tightened following re-evaluation. The acceptance criterion for HCP is justified based on results from historical batches including batches from the Phase 3 clinical study.

The exclusion of specified tests for process-related impurities from AS release specifications is sufficiently justified based on characterisation studies and risk assessments.

Analytical methods

The non-compendial methods have been validated and compendial methods have been verified for their purpose (including required method suitability tests (growth promotion, maximum valid dilution) for sterility, bioburden and endotoxin testing, respectively). Method validation and verification summaries and full method validation reports are available in the dossier. The method validations and verifications demonstrated that the methods are suitable for their intended purpose.

Compendial methods are used for detection of mycoplasma and microbial enumeration at the level of pre-filtered harvest. The test for in-vitro adventitious agents on pre-filtered harvest is based on ICH Q5A Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products. Since HEK-293 cells were shown to be susceptible for infection by minute virus of mice (MMV), the pre-filtered harvest is tested for absence of MMV by qPCR as well (validation ongoing, **recommendation 1**).

The method descriptions including system suitability criteria have been provided for all non-compendial methods and are appropriate.

Batch analysis

Batch Analysis data have been provided for one toxicology batch, two Phase 1 and Phase 2 batches, seven Phase 3 batches, two engineering non-GMP batches, the three PPQ batches, and two additional GMP batches for DP PPQ and manufacturing support. The batches complied with pre-defined acceptance criteria valid at the time of testing (except one result from the triplicate determinations for one PPQ batch exceeded the limit for residual plasmid DNA; however, since two out of three determinations passed the test, this can be accepted) and indicate manufacturing process consistency.

Reference materials

A two-tiered reference standard (RS) system has been established for release and stability testing of commercial AS and DP.

A protocol for qualification of future secondary reference standards has been provided. The quality attributes and analytical methods used for qualification are appropriate. Respective acceptance criteria are more rigorous compared to AS release criteria, which is endorsed.

Historical reference materials are appropriately described including information on manufacturing process version and qualification testing, their respective use, and bridging between previous and current reference standards.

<u>Container closure</u>

The sterile filtered CHIKV VLP AS is held in an autoclaved glass bottle (Ph. Eur. 3.2.1 compliant) in a Grade A isolator. A custom-made tree port transfer cap is screwed on the top of the glass bottle with a rubber gasket in between. The tree port transfer components are composed of stainless steel, and the

coloured screw cap is constructed of polybutylene terephthalate (PBT). Certificates of Conformity for bottle and cap are included in the dossier.

Sterilisation is performed in-house in line with requirements outlined in GL EMA/CHMP/CVMP/QWP/850374/2015. The autoclave cycle validation approach used complies with Ph. Eur. 5.1.1. Extractables testing of the glass bottle was performed and included analysis for elemental and anionic extractables presented in and on the borosilicate glass bottle. The extractables levels obtained are below the analytical evaluation threshold (AET) and hence, no leachables testing was performed. The only additional components with product contact are the tree port transfers on the cap, which are made of stainless steel. It is agreed that the risk as regards extractables/leachables testing can be considered as negligible.

2.4.2.4. Stability

Active substance stability has been evaluated in a small-scale stability study on material derived from one Phase 3 lot, one engineering lot and three PPQ lots to support the proposed hold time of AS. The hold time was also validated. Stability samples are stored in glass bottles with polypropylene screw caps, which are considered representative for the commercial container. Acceptance criteria for the hold time study were identical at the time of testing for attributes tested at both AS release testing and hold time studies. Photostability of the AS was not specifically evaluated since the AS is not subjected to excessive light during the manufacturing process and no adverse effects linked to light exposure have been observed in AS release or stability testing.

No post-approval stability protocol and commitment has been proved, since the active substance is held up to the validated hold time before processing into bulk drug product (BDP). This can be agreed on.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Description of the product

The Chikungunya Virus Virus-Like Particle (CHIKV VLP) vaccine is a preservative-free, sterile suspension for intramuscular injection that is supplied as single dose of 0.8 mL in a Type I glass pre-filled syringe (PFS) closed with a rubber stopper and a rigid cap with rubber closure. The PFS is assembled with a finger flange and plunger rod. One dose contains 40 µg of CHIKV VLP antigen adsorbed to aluminium hydroxide. The suspension appears as a cloudy, white liquid.

The excipients, all of which are of Ph. Eur. grade, are well known for manufacture of human parenteral medicinal products including vaccines; the adsorbent aluminium hydroxide is commonly used as adjuvant for human vaccines. The excipients are purchased from qualified suppliers. The supplier of the aluminium hydroxide adjuvant is registered in the dossier and a representative certificate of analysis (CoA) is available.

Excipients of human or animal origin are not used in the manufacture of the CHIKV VLP vaccine. The function of all ingredients is shown in **Table 1**.

Table 1 Composition of the Dosage Form

Component	Function	Quality Standard
CHIKV VLP	Active Ingredient	In house

Component	Function	Quality Standard
Aluminium hydroxide gel (2% w/v)	Adjuvant and stabiliser	Ph. Eur.
Sucrose	Stabiliser	Ph. Eur./USP-NF
Dipotassium phosphate	Buffering agent	Ph. Eur./USP-NF
Potassium dihydrogen phosphate	Buffering agent	Ph. Eur./USP
Sodium citrate, dihydrate	Stabiliser	Ph. Eur./USP
Water for Injection (WFI)	Solvent	Ph. Eur./USP

Pharmaceutical development

The formulation development is sufficiently described. A combination of sucrose, sodium citrate, and potassium phosphate was initially selected based on their ability to stabilise the CHIKV VLPs and prevent aggregation, and their suitability for use in a frozen vaccine formulation intended for intramuscular injection. The formulation buffer remained unchanged throughout clinical development. For the early Phase 1 and 2 clinical studies a non-adjuvanted formulation was used. For the subsequent dose-finding Phase 2 study by Emergent BioSolutions/PaxVax Inc., aluminium hydroxide was added by bed-site mixing to enhance immunogenicity of the vaccine. Based on adsorption studies and regulatory considerations, aluminium hydroxide with a target of 300 µg Al per dose was chosen. For the Phase 3 studies, a non-frozen, ready-to-use CHIKV VLP vaccine adsorbed to aluminium hydroxide and supplied as suspension for injection in a PFS was introduced. The composition and presentation of CHIKV VLP vaccine administered in the Phase 3 study and the CHIKV VLP vaccine intended for marketing are identical. The temperature-dependent increase in pH observed during storage was attributed to dissolution of aluminium hydroxide by sodium citrate that generates sodium hydroxide. However, at long-term conditions only a slight pH increase is observed.

The manufacturing process changes implemented from early clinical development through commercial manufacture are sufficiently described and justified. The most significant change of the DP was the inclusion of the aluminium hydroxide adjuvant from the Emergent Phase 2 studies onwards. For the Phase 3 studies the container closure was changed from vial to PFS with a slightly increased fill volume, and the storage temperature was changed from frozen to refrigerated conditions. No changes of the DP were introduced between the Phase 3 study and process performance qualification (PPQ)/commercial manufacture. The process changes were mainly related to introduction of a bulk drug product (BDP) manufacturing stage to accommodate manufacture of a premixed formulation with adjuvant, changes of manufacturing sites, and change of the container closure system. Several process changes were implemented after manufacture of Phase 3 material that concern usage of an isolator for BDP manufacture, mixing of BDP, the storage container for BDP, transfer of DP manufacture, and a change of labelled finished drug product (FDP) manufacturer with introduction of a semi-automated final assembly process.

A reduced comparability exercise demonstrates comparability between CHIKV VLP vaccine administered in Phase 2 and vaccine used in Phase 3. Comparability of the Phase 3 process and the intended commercial manufacturing process was shown in a comprehensive comparability study performed in accordance with ICH Q5E. The risk assessment for CQAs as well as the statistical evaluation approach were discussed during the procedure. For all CQAs the individual batch results are tabulated and presented graphically. Consequently, no queries related to the evaluation approach for comparability are raised. No relevant analytical differences are present between material produced with the Phase 3 and the commercial process. The Phase 3 DP batches and the PPQ DP batches exhibit

comparable stability profiles. In conclusion, analytical comparability of the Phase 3 process and the intended commercial manufacturing process has been demonstrated.

According to the Applicant, the CQAs of CHIKV VLP BDP, DP, and FDP were established during product and process development and documented in the quality target product profile (QTPP). A summary listing the CQAs and justifications for their classification has been provided.

High level summaries of the process risk assessments performed to identify (non-) critical process parameters (CPPs), key process parameters (KPPs), material attributes, and key performance attributes (KPAs) for the BDP, the DP, and the FDP manufacturing processes are provided. Justifications for the classification of individual process parameters as CPPs, non-CPP, or KPP and their targets, NORs and PARs are presented. As homogeneity of BDP prior to QC sampling and filling is critical, additional CPPs for mixing of BDP have been implemented upon request. Process development studies performed to characterise the process steps and to establish the commercial manufacturing process are presented. The proposed parameter ranges are sufficiently supported by process development and validation data.

The development of the container closure systems is satisfactorily described. A single-use sterilised bag with a linear low-density polyethylene contact layer is used for storage of BDP. The bag is certified as BSE/TSE free and tested to meet USP <87>, USP <88> Class VI, USP <661>, endotoxin, subvisible particles and bioburden requirements. The product contact layer complies with Ph. Eur. 3.1.5. Suitability of the storage bag in terms of compatibility, container closure integrity, and safety has been satisfactorily demonstrated. The components of the PFS with direct product contact, i.e. Type I glass syringe barrel, synthetic rubber closure, and coated chlorobutyl rubber plunger stopper, as well as the silicon oil used as lubricant comply with requirements of Ph. Eur. 3.2.1, Ph. Eur. 3.2.9, and Ph. Eur. 3.1.8, respectively. A Notified Body Opinion confirming compliance of the device part of the PFS to the relevant General Safety and Performance Requirements in Annex I of Regulation (EU) 2017/745 is available. The DP container closure system appears suitable regarding compatibility, container closure integrity, and safety. The available data on extractables/leachables do not give raise to concerns. The Applicant committed to submit the final leachables study report including results for elemental impurities by Oct 2028 (**recommendation 2**).

2.4.3.2. Manufacture of the product and process controls

The finished product is release for commercial distribution at Bavarian Nordic A/S (Kvistgaard, Denmark). The batch size range has been defined.

At time of initial submission, multiple analytical methods intended for commercial release and stability testing had not been transferred to/validated at the intended commercial testing sites and hence, a **major objection** was raised at Day 90. In the meantime, the respective method transfers/validations have been successfully completed and the commercial supply chain is now fully established. Valid GMP certificates are available for the commercial manufacturing and testing sites.

The manufacturing process for CHIKV VLP vaccine is divided in three main sub-processes, i.e. manufacture of BDP, DP, and labelled finished drug product (FDP, assembled, labelled and packaged syringes) respectively, and is based on common pharmaceutical processing steps. Manufacture of BDP is a continuation of the AS manufacturing process and includes sterile filtration of the formulation buffer, sterilisation of the adjuvant, followed by aseptic formulation and filling into pre-sterilised single-use bags. The storage period for filled BDP is specified. Upon refrigerated transfer to the DP manufacturer, BDP is resuspended and aseptically filled into pre-sterilised syringes under continuous shaking and recirculation. Following 100% visual inspection and packaging into shipping cases, the PFS are stored at 2°C – 8°C until refrigerated shipment. The PFS are shipped to the FDP manufacturer,

assembled with plunger rod and finger flange, labelled, and packaged. The FDP is stored at 2°C – 8°C until distribution. No reprocessing is performed at any step of the manufacturing process.

A single AS batch is used to manufacture a BDP batch and one BDP batch is used to manufacture a DP batch. The DP batch size is described in terms of BDP volume and number of filled syringes.

The date of manufacture of DP is defined as the date of removal of BDP from 2°C-8°C storage and equilibration at RT at the DP manufacturer.

Overall, the process descriptions for manufacture of BDP, DP and FDP are in line with the requirements of the Guideline on manufacture of the finished dosage form EMA/CHMP/QWP/245074/2015. Narratives of the individual manufacturing process steps that are accompanied by flow charts showing the process steps, materials entering the process and in-process controls are provided in the dossier.

All CPPs and IPCs are listed together with their ranges and acceptance criteria, respectively. The established process controls are in line with regulatory expectations for this type of manufacturing process. The process parameter ranges are adequately supported by data.

Sterility of the formulation buffer and aluminium hydroxide prior to formulation of BDP is critical to ensure sterility of BDP which cannot be sterile filtered. Sterilisation and control of both components is adequately described. The container closures, i.e. bags for BDP and syringes with rubber stoppers for DP, are purchased pre-sterilised.

The defined long-term storage conditions for BDP, maximum filling time, and the maximum time out of refrigeration (TOR) for BDP, DP and FDP are justified by stability studies, process validation data and aseptic process validation.

The shipping processes for BDP, DP and FDP are sufficiently described.

Process validation

Three consecutive BDP, DP, and FDP batches were manufactured applying target setpoints. The presented PPQ results demonstrate that the proposed commercial BDP, DP, and FDP manufacturing process is reliable and delivers product of consistent and acceptable quality.

Manufacture of three BDP PPQ batches started within a day after manufacture of the corresponding AS PPQ batches. The BDP manufacturing size range is sufficiently covered by the available data. All CPPs were within their acceptable range. All release tests comply with their acceptance criteria (and the proposed commercial specifications).

Validation data supporting the maximum TOR for formulated BDP and the sterile hold times for formulation buffer and aluminium hydroxide are available.

Three DP PPQ batches were manufactured using two BDP PPQ batches and one post PPQ GMP BDP batch. The DP PPQ batch size covers the proposed batch size range. . Generally, CPP and non-CPPs complied to the specified ranges and deviations were suitably explained. All in-process controls and release tests complied with their acceptance criteria (and the proposed commercial specifications). Results are consistent across the three PPQ lots. Homogeneity throughout filling as well as adequacy of procedures for filling restart after line stoppages have been sufficiently demonstrated. The proposed maximum recirculation time and maximum TOR for DP are sufficiently supported by validation data.

Three consecutive assembled and labelled DP (ADP) batches FDP process were manufactured using the three DP PPQ batches. All CPPs conformed to the defined limits. For all three batches the release tests complied with their acceptance criteria. Results are consistent across the three PPQ lots. Additional CCI testing confirmed that the plunger rod insertion process does not compromise container closure integrity. The specified TOR has been evaluated in the PPQ campaign.

Shipping of BDP from BDP manufacturer to DP manufacturer has been sufficiently validated. Previous transportation studies covering shipment of DP from FDP manufacturer to packaging site and of shipment of FDP do not show any impact of the shipping process on product quality; the Applicant committed to submit the final results of the transport validation studies for DP and FDP by June 2025 (**recommendation 3**).

2.4.3.3. Product specification

The release and stability specifications for CHIKV VLP BDP, DP, and FDP are provided in the dossier. These tests include appearance (for BDP and DP), bacterial endotoxins (for BDP and DP), sterility (for BDP and DP), and identity (for BDP, DP and FDP).

The specifications and acceptance criteria are set based on a multifaceted approach which included product understanding and CQAs, safety concerns per regulations, clinical experience, toxicology studies, process capability and manufacturing history. In addition, relevant guidelines and compendial requirements were taken into account by the Applicant. Release and stability data of up to eleven BDP batches and nine DP batches including Phase 3 clinical batches, representative engineering runs, PPQ batches, and post PPQ batches were used to justify the proposed specification acceptance criteria. For quantitative release parameters a statistical approach was used (i.e. calculation of tolerance intervals with 95% confidence/99% coverage which might lead to widened acceptance criteria as compared to other approaches, such as the ±3SD one) to guide setting of specification limits for pH, aluminium content, osmolality, total protein concentration, immunoreactivity, container content and syringe functionality.

The release and stability specifications for BDP, DP, and FDP include the relevant attributes and are in line with regulatory expectations.

The release and stability acceptance criteria for protein concentration (BDP, DP) have been aligned upon request and are acceptable. Like for protein concentration, the acceptance criteria for immunoreactivity at DP release and stability have been aligned after re-evaluation and are agreeable. The proposed release and stability limits for BDP immunoreactivity are agreeable; however, taking into account that future release testing for immunoreactivity is subject to improved control, the BDP release criterion for immunogenicity should be re-evaluated upon manufacture of 30 commercial BDP batches (**recommendation 4**). Concerning *in vitro* potency, the revised tightened acceptance criteria for release and stability (BDP, DP) are agreeable. To ensure that a full dose of 40 µg is delivered to the vaccinee, the Applicant proposes to slightly increase the filling weight for commercial DP batches which is endorsed. As requested, the acceptance criterion for container content has been changed to include two decimal digits.

The identification numbers for the in-house methods and references to compendial monographs are included in the specification tables, method descriptions, and the method validation summaries.

Impurities

Risk assessments are provided for elemental impurities, nitrosamines, and extractables/leachables originating from single-use materials used during manufacture or container closure systems. Based on the information provided by the Applicant, the risk related to impurities appears adequately controlled and negligible.

Analytical methods

The analytical methods intended for testing of commercial product are sufficiently described.

At the time of initial submission for many of the non-compendial analytical methods intended for commercial release and stability testing, no validation data were available, or method transfer to the intended commercial release testing site had not been completed yet. Thus, at Day 90 a **major objection** (**MO**) was raised. Satisfactory analytical method validation and/or transfer data are now available for all analytical methods intended for release and stability testing of commercial product. The presented data demonstrate that the analytical methods have been successfully implemented at the commercial testing sites and are suitable for the intended purpose. In summary, the MO was considered resolved.

Batch analysis

Batch analyses results are shown for 9 FDP batches, 11 DP batches, and 13 BDP batches including non-clinical, clinical batches, engineering batches and PPQ batches. All batches comply with the release specifications valid at time of release; the Phase 3 (if respective test was performed) and PPQ batches also meet the proposed acceptance criteria for commercial product. Overall, the results indicate that the manufacturing process is capable of delivering drug product of consistent quality that meets its specifications.

Reference materials

The reference standards are described under 3.2.S.2.5.

Container closure systems

The BDP is stored in a pre-sterilised, single-use bag with linear low-density polyethylene contact layer which complies with Ph. Eur. 3.1.5 and USP tests for plastic containers/materials. The bag is pre-sterilised by gamma irradiation in accordance with ISO 11737. The name and address of the site of sterilisation are registered in the dossier.

The DP container closure consists of a lubricated Type I glass barrel equipped with a Luer lock adapter, a rigid cap containing a synthetic Isoprene-Bromobutyl rubber closure, and a coated chlorobutyl rubber stopper. The PFS is assembled with a plunger rod and a finger flange. The plunger rod and finger flange as well as the rigid cap have no direct product contact. The barrel, rubber closure and rubber stopper comply with Ph. Eur. The silicone oil used for lubrication conforms to Ph. Eur. The syringes are sterilised using ethylene oxide (ETO) according to ISO 11135. Ethylene oxide residuals and ethylene chlorohydrin are controlled at acceptable levels. Name and address of the site of sterilisation are registered in the dossier. Rubber stoppers are steam sterilised using a Ph. Eur. compliant sterilisation cycle; the site of sterilisation has been registered.

Representative certificates of analysis for the single-use bag, the syringe, and the rubber plunger stopper are available.

A Notified Body opinion confirms conformance of the device part to the relevant General Safety and Performance Requirements in Annex I of Regulation (EU) 2017/745 is provided.

2.4.3.4. Stability of the product

Bulk drug product

A shelf-life of is agreed for CHIKV VLP BDP when stored in specified bags. The claimed shelf-life is based on stability data from several BDP stability batches. All batches are considered representative for commercial BDP. For BDP stored at long-term conditions, all available results comply with the acceptance criteria valid at time of testing and the proposed commercial acceptance criteria. Accelerated stability data are also available.

Drug product For CHIKV VLP filled in Type I glass syringes, the Applicant proposes a shelf-life of 36 months at 2 - 8°C.

To support the shelf-life claim, stability data for six primary DP stability batches including three Phase 3 clinical batches and the three PPQ batches are presented. The Phase 3 batches are representative for commercial DP; comparability has been demonstrated. In the main, the studies follow ICH Q5C (for several attributes, testing frequency was reduced). The PFS are stored in vertical or horizontal position for up to 60 months. For the three primary Phase 3 DP batches, long-term data for up to 36 months are currently available. For the three PPQ DP batches, the available data cover 6 months at long-term conditions. In addition, data are available for supportive Phase 3 batches that were stored for up to 36 months (three batches) or 24 months (one batch) at long-term conditions, respectively. All results comply with the acceptance criteria valid at time of testing and the proposed commercial acceptance criteria.

Based on the available data, a shelf-life of 36 months at 2 - 8°C for CHIKV VLP DP is agreeable.

Under accelerated storage conditions, a more pronounced increase of the pH value is observed. Nevertheless, all results remain within the acceptance limits valid at time of testing and the proposed commercial acceptance criteria.

A representative engineering DP batch was manufactured with cumulative hold times throughout the manufacturing process from WCB thaw onwards and placed on stability to demonstrate end-to-end stability. The available stability at long-term conditions and at accelerated conditions results comply with the proposed commercial acceptance criteria. The results are consistent with the results obtained for the Phase 3 and PPQ DP batches and support the proposed hold times.

Temperature cycling studies demonstrate that exposure of DP for up to 7 days at room temperature (RT), repeated cycling for up to 4 times between 2 – 8°C and an 8-hour hold at RT, or exposure to 0°C for up to 24 hours has no detrimental effect on product quality. To prevent unnecessary disposal of product, information on product stability after temperature excursions has been included in the SmPC (see below).

The CHIKV VLP vaccine is sensitive to light exposure and thus, the syringe must be kept in the outer carton in order to protect the product from light. No negative effects due to light exposure were observed under on routine manufacturing operations.

Finished drug product

For FDP, a shelf-life of 36 months at 2 - 8°C from date of manufacture of DP is proposed.

Long-term stability data covering 36 months are presented for three Phase 3 clinical FDP batches. For the three PPQ FDP batches data covering 3 months at 2 - 8°C are available so far. Only appearance of packaging components, container content, CCI, syringe functionality, and sterility were monitored. Considering that DP attributes are not expected to be impacted by the assembly step, the reduced design of the studies is acceptable. All available results comply with their acceptance criteria and do not show any trend. As the FDP assembly process has no impact on DP attributes, the approach regarding start and duration of shelf-life (i.e. same shelf-life as DP, start date DP date of manufacture) is acceptable.

Stability data indicate that the vaccine is stable for 4 hours when stored at temperatures from 8°C to 25°C and for at least 24 hours when stored at 0°C to 2°C. At the end of this period VIMKUNYA should be used immediately or discarded. These data are intended to guide healthcare professionals in case of temporary temperature excursion only. This is included in the SmPC.

For FDP, a shelf-life of 36 months at 2 - 8°C from date of manufacture is accepted.

2.4.3.5. Adventitious agents

The measures set to ensure product safety with regard to non-viral and viral adventitious agents include selection of appropriate raw materials, testing of cell banks and process intermediates for microbial and viral contaminants, testing of microbial attributes as in-process controls and at release, and validation/evaluation of manufacturing steps contributing to virus reduction. In addition, microbial quality is ensured by process design (microbial reduction filtrations, sterile filtration, aseptic processing) and sanitisation procedures.

Absence of contamination due to bacterial and fungal adventitious agents is controlled at various stages of production via QC release testing, in-process control and in-process monitoring testing. The risk of microbial contamination is adequately controlled.

No raw materials of animal origin are used in the manufacturing processes of the VRC8900 plasmid and the VRC293 WCB other than the VRC293 cell substrate. The working cell bank was produced from VRC293 MCB with animal-component free medium. Certificates confirming compliance with the note for guidance EMA/410/01 Rev. 3. have been provided. In conclusion, the risk arising from potential TSE contaminants can be considered as negligible.

Absence of viruses in the MCB, WCB and/or EOP/LIVCA cells was determined by a battery of tests covering a broad range of viruses: *in vitro* adventitious agent assay, *in vivo* adventitious agent assays, and tests for retroviruses (TEM, fluorescent product enhanced reverse transcriptase (F-PERT assay). The MCB was additionally tested to confirm the absence of human viruses (human adenovirus (HAdV), human adeno-associated virus (HAAV), human cytomegalovirus (HCMV), Epstein Barr virus (EBV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), human herpes virus 6 (HHV-6), human herpes virus 7 (HHV-7), human herpes virus 8 (HHV-8), human immunodeficiency virus (HIV-1, HIV-2), human T-cell lymphotrophic virus HTLV-1, HTLV-2), bovine, porcine and equine viruses, and porcine circovirus Types 1 and 2 (PCV 1 and 2). The information as regards methods used for viral safety testing of cell banks is sufficient.

Unprocessed (pre-filtered) harvest is routinely tested for the absence of adventitious viruses by *in vitro* assay. The method is briefly described and was shown to be suitable for its intended purpose. Results for the Pre-filtered Harvests from the three PPQ batches complied with acceptance criteria (i.e. absence of adventitious viruses confirmed). Absence of MVM will be routinely tested by qPCR, the respective test will be performed on all batches intended for marketing within the EU and the respective validation report will be provided post-approval (**recommendation 1**). The absence of viral contamination was also demonstrated by next generation sequencing (NGS) of several at-scale Phase 3 batches. For instance, NGS testing was performed using a genomic approach. Sequences from human adenovirus type 5 were identified (consistent with the known integration of a hAd5 region fragment within chromosome 19 of HEK293 cells), as well as from human endogenous retroviral genes (inherent to the human HEK293 host cell line, highly defective and unable to produce infectious particles). No other significant viral sequence fragments were detected.

The capacity of the manufacturing process (AEX step) to clear adventitious viruses has been assessed in virus clearance studies using scale down models. Given that AEX is a contributing (and no dedicated) step to virus clearance, that it is only moderately effective in clearing virus, can be accepted. In conclusion, the measures set to ensure safety with respect to adventitious viruses (selection of appropriate raw materials, testing cell banks and pre-filtered harvest, AEX step contributing to viral clearance) are considered suitable to ensure safety with respect to adventitious viruses.

2.4.3.6. GMO

N/A

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Active Substance

The Chikungunya Virus Virus-Like Particle (CHIKV VLP) active substance (AS) is comprised of three recombinant CHIKV structural proteins derived from the CHIKV Senegal strain 37997: Capsid (C, ~35 kDa), Envelope 1 (E1, ~55 kDa), and Envelope 2 (E2, ~50 kDa), which self-assemble to form the VLP.

The CHIKV VLPs are produced in HEK293 cells (VRC293) cultured in suspension and transfected de novo with the plasmid encoding the structural proteins (VRC8900) for each manufacturing run. The AS is directly forward processed to yield the bulk drug product. The manufacturing process is described in sufficient detail. Targets and ranges for process parameters and criticality assignment, classification of IPCs/IPTs and hold times are sufficiently justified.

Raw and starting materials and their use in manufacture of active substance are appropriately described.

Relevant critical quality attributes (CQAs) and key performance attributes (KPA) are listed in the dossier including the rationale for their designation. The Applicant's justifications for criticality assignment appear reasonable.

Process validation was performed following a classical process validation approach with three PPQ batches manufactured at commercial scale. Results from QC release, in-process control and additional characterisation testing indicate a consistent manufacturing process. Process-related impurities were shown to be removed to consistently low levels during PPQ. Overall, it can be concluded that the AS manufacturing process reproducibly delivers AS of adequate quality meeting the predetermined quality attributes.

Multiple changes were implemented between Phase 1/2, Phase 2, Phase 3 and PPQ/commercial manufacturing. Changes implemented between clinical phases and manufacture of PPQ/commercial material are sufficiently detailed in the dossier.

The quality attributes and acceptance criteria proposed for pre-filtered harvest and AS release testing are acceptable. Batch analyses data are presented for multiple batches which complied with pre-defined acceptance criteria valid at the time of testing (except one result from the triplicate determinations for one which exceeded the limit for residual plasmid DNA) and indicate manufacturing process consistency.

A two-tiered reference standard system has been established and reference material is principally sufficiently characterised.

The hold time for the AS is sufficiently justified by available small-scale validation and media fill data.

Drug product

VIMKUNYA is a preservative-free, sterile suspension for injection for intramuscular injection containing CHIKV VLPs adsorbed to aluminium hydroxide. It is supplied in a lubricated Type I glass pre-filled syringe closed with a chlorobutyl rubber stopper and a rigid cap with a synthetic isoprene-bromobutyl rubber closure and contains a single dose of 40 µg total protein. Bulk drug product is stored in sterile single-use bags. The primary packing materials for both BDP and DP, comply with the respective Ph. Eur. requirements; their suitability in terms of compatibility, integrity, and safety has been

satisfactorily demonstrated. The final leachables report will be submitted post authorisation which is acceptable as available results give not raise to concerns (**recommendation 2**). A Notified Body opinion is available for the device part of the PFS.

All excipients are well-known for manufacture of parenteral medicinal products including human vaccines and comply with Ph. Eur. requirements. No excipients of animal or human origin are used.

The development information provided in the dossier is satisfactory. The control strategy is adequately described and in line with regulatory expectations.

Process changes implemented during development have been adequately described and justified. Drug product from the different development stages was demonstrated to be analytically comparable.

The manufacturing sites and their responsibilities are described satisfactorily. Of note, compared to release testing of PPQ batches, for release testing of commercial product multiple testing sites have been changed; the testing sites involved in testing of PPQ batches and future commercial testing sites are listed in the dossier. Valid GMP certificates are available for the commercial manufacturing and testing sites. The description of the manufacturing process, which is separated into BDP, DP, and FDP manufacturing and relies on standard pharmaceutical processing steps, and its control are sufficient. Hold times are described and supported by data. Sterilisation of the formulation buffer and aluminium hydroxide and related controls are sufficiently described as well. Process validation and batch analyses data for BDP, DP, and FDP demonstrate that the manufacturing process performs consistently and delivers product of adequate and consistent quality. Previous transport studies indicate that the shipping process for BDP, unlabelled PFS, and FDP is suitable. Final results of recently initiated transport validation studies for DP and FDP will be provided post authorisation (**recommendation 3**).

The set of parameters included in the release and stability specifications for BDP, DP, and FDP is in line with regulatory requirements. The proposed acceptance criteria are agreeable. The BDP release acceptance criteria for immunoreactivity should be re-evaluated upon manufacture of 30 commercial BDP batches (**recommendation 4**).

The analytical methods intended for testing of commercial product are adequately described. References to compendial monographs and unique identifiers for in-house methods are provided.

The **Major Objection** that has been initially raised regarding insufficient analytical method validation and transfer is resolved. The presented method validation/transfer data demonstrate that the analytical methods are suitable for the intended purpose. Bridging data between the AAS method (DP) and the complexometric titration method (BDP) for aluminium content are available. Reference standards are described in the AS part of the dossier.

Impurities including extractables/leachables, elemental impurities, and potential N-nitrosamines have been sufficiently addressed and do not give raise to concerns.

Stability studies were performed for BDP, DP, and FDP. The studies are generally in accordance with ICH Q5C.

For BDP, the available results support the proposed shelf-life in the specified bag. The available stability data support the claimed DP and FDP shelf-life of 36 months at 2 – 8°C when stored in the CCS described in 3.2.P.7. Considering that FDP manufacture has no impact on product quality, the approach for shelf-life assignment for FDP is acceptable.

Temperature cycling studies demonstrate that exposure of DP for up to 7 days at RT, repeated cycling for up to 4 times between 2 – 8°C and an 8-hour hold at RT, or exposure to 0°C for up to 24 hours has no relevant impact on product quality. Information on product stability following temperature excursions has been added to the SmPC/PL.

Photostability studies under ICH Q1B conditions revealed that CHIKV VLP vaccine is light sensitive and must be stored protected from light.

Satisfactory post-approval stability protocols for BDP and DP as well as a commitment to continue the ongoing studies according to protocol and inform the competent authority about OOS results, are available.

From the quality perspective, VIMKUNYA is approvable. Four recommendations for future quality development have been agreed with the Applicant and are detailed below.

The 'new active substance' claim is supported for this product.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The CHMP has identified the following measures necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product:

1. The validation report covering product-specific validation for MMV testing of CHIKV VLP pre-filtered harvest should be available by February 28, 2025, and should be submitted upon grant of an MA.
2. The final study report (DP) for leachables including elemental impurities should be submitted by October 2028.
3. The final results of the transport validation studies for unlabelled drug product (D) and labelled drug product (FDP) should be submitted by June 2025.
4. The bulk drug product (BDP) release acceptance criteria for immunoreactivity should be re-evaluated upon manufacture of 30 commercial BDP batches

2.4.6. Recommendation(s) for future quality development

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

1. The validation report covering product-specific validation for MMV testing of CHIKV VLP pre-filtered harvest should be available by February 28, 2025 and should be submitted upon grant of an MA.
2. The final study report (DP) for leachables including elemental impurities should be submitted by October 2028.
3. The final results of the transport validation studies for unlabelled drug product (D) and labelled drug product (FDP) should be submitted by June 2025.
4. The bulk drug product (BDP) release acceptance criteria for immunoreactivity should be re-evaluated upon manufacture of 30 commercial BDP batches.

2.5. Non-clinical aspects

2.5.1. Introduction

Chikungunya virus (CHIKV) virus-like particle (VLP) has been developed by Bavarian Nordic as a vaccine for the prevention of Chikungunya virus disease. The active substance is comprised of three recombinant CHIKV structural proteins which self-assemble to form the VLP. The final drug product contains 40 µg CHIKV VLP adsorbed on aluminium hydroxide in stabilizing buffer.

Nonclinical characterization of CHIKV VLP covered efficacy and safety aspects. Proof of efficacy was provided in mice and macaques, for different VLP formulations (including the clinical formulation) using protocols for active (protection via vaccination) and passive (protection via serum/IgG transfer) immunization. Moreover, the Applicant demonstrates that VLP-mediated protection is antibody-based. Serum neutralizing antibody (SNA) titres were correlated with the probability of protection from CHIKV disease. To this end, antibody titres sufficient to neutralize 80% (NT80) pseudotyped CHIKV were identified by use of an established luciferase neutralization assay. The corresponding protection from CHIKV-caused viremia was shown by quantification of CHIKV-genomes in test animal serum by RT-qPCR.

The toxicology studies conducted cover the intended human target dose and include an extended vaccination regimen with up to five vaccine administrations providing additional safety margin.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

The Applicant provided a sufficient package of pharmacological studies to demonstrate that the test article confers immunity against CHIKV in animals which are either (i) vaccinated with CHIKV VLP (active immunization) or (ii) recipient of sera or IgG from animals or humans which were vaccinated (passive immunization). For reasons of clarity and comprehensibility, the conducted studies should be categorized as "active immunization studies" and "passive immunization studies".

Active immunization studies

Report RPT062501: Immunogenicity and protective efficacy of non-adjuvanted CHIKV VLP vaccine in mice and non-human primates

Originally, the CHIKV VLP had been developed (in a non-adjuvanted formulation) by the National Institute of Health Vaccine Research Centre (NIH VRC), which were also the first to test this vaccine for its immunogenicity and protective efficacy. Throughout the discussion of this report, the NIH VRC CHIKV VLP vaccine will also be referred to as CHKVLP059. In total, 5 initial studies were conducted by the NIH VRC, which ought to be discussed in the following.

Study no. 1 investigated the "immunogenicity of NIH VRC CHIKV VLP in mice". For this study, research-grade VLPs (produced in 293F cells) were utilized for two injections (19 µg VLPs each, 28 days apart) into 5 BALB/c mice. Diluted sera from such mice were tested for their capacity to neutralize pseudotyped luciferase-encoding virus in a cell-culture assay. The study identified a half-maximal inhibitory concentration (IC_{50}) at titres of 9,600 (against strain 37997) and 12,031 (against strain LR2006 OPY-1). This study clearly shows that vaccination with CHKVLP059 has the potential to protect against CHIKV.

Study no. 2 investigated the "NIH VRC CHIKV VLP immunogenicity and protection from challenge in rhesus macaques". The rationale of this study was very similar to the mouse-study above but differed in testing Rhesus macaques (n = 6), included one additional immunization point (6 months after first) and included a "challenge"-step at which animals were tested for their immunity against CHIKV. A comprehensive study protocol is provided in the Table 2 below.

Table 2 Study design for assessment of immunogenicity of CHIK VLP in Rhesus Macaques

Test species/strain	Rhesus macaques
N	6 animals/group
Treatment groups	CHIKV VLPs (research grade) Phosphate buffered saline (PBS)
Immunization dose and regimen	20 µg CHIKV VLP (research grade) Immunizations on Day 0, 1 month, and 6 months Sera collected 10 days after each immunization
Challenge	1×10^{10} PFU of strain LR2006 OPY-1 administered intravenously (IV) 15 weeks after final immunization
Endpoints	Serum neutralizing antibodies by plaque reduction neutralization (PRNT) Serum neutralizing antibodies by CHIKV VLP pseudotyped lentiviral infection of 293F cells Viremia 24 hours after challenge Monocyte levels (% of white blood cells) 7 days after challenge
Results	Immunogenicity shown in Figure 3 and Figure 4 Animals immunized with CHIKV VLPs had no detectable viremia after challenge whereas control unimmunized animals had a viral load of 10^4-10^5 PFU/mL No change in monocyte counts 7 days after challenge in immunized animals versus a 5-fold increase in control animals

Of note, two approaches were employed to identify SNA-titres in this study: (i) the pseudotyped luciferase-encoding lentiviral vector system (mentioned in the mouse-study above) and (ii) a typical plaque reduction neutralization assay. Both assays clearly showed that vaccination triggered the emergence of CHIKV specific antibodies (especially after two immunizations). The numbers derived from the luciferase assay and PRNT were roughly comparable. The "challenge"-study (see table above for details)demonstrated that unvaccinated control NHPs had a marked viral load (10^4 - 10^5 PFU/mL) and increased monocyte counts upon CHIKV-challenge. In vaccinated NHPs neither viremia nor increased monocyte counts were detected. Hence, this study shows that, upon vaccination, NHPs develop CHIKV-specific antibodies and are protected from CHIKV-challenge.

Study no. 3 investigated the "protection against heterologous CHIKV challenge in immunodeficient mice by passive transfer of IgG from rhesus macaques". Here, immunodeficient mice were utilized, which are characterized by interferon-α/β receptor-1 knockout (*Ifnar* 1-/-) and for which CHIKV infection is lethal (Couderc et al., 2008). The study design is described in the report as follows: "Purified IgG from either control or CHIKV VLP-immunized NHPs was IV transferred to *Ifnar* -/- mice (2 mg IgG per mouse) 24 hours prior to a lethal challenge of 30 PFU via intradermal administration of CHIKV strain LR2006 OPY-1. Serum samples were collected from the mice to measure viral load, and mortality was followed for 15 days." Strikingly, *Ifnar* -/- mice which received IgG from control NHPs did not survive to day 4 post CHIKV challenge, whereas *Ifnar* -/- mice which received IgG from CHIKV VLP-immunized NHPs survived throughout the observation period and had no signs of viremia as measured by quantification of viral RNA copies in the blood. This study provides further evidence that IgG antibodies emerging from CHKVLP059 vaccination suffice to protect from CHIKV infection.

Studies no. 4 and no. 5 investigated the "comparative immunogenicity of research vs development CHIKV VLP lots in mice" as well as "comparative immunogenicity of development and clinical CHIKV VLP lots in mice", respectively. Thus, the scope of these studies was to compare the immunogenic efficacy of 3 different lots (research, development and clinical) in mice. Importantly, all these lots contain unadjuvanted CHIKV VLP (i.e., CHKVLP059). The term *clinical lot* should not indicate that this is the object of the present MAA (though two clinical trials VRC311 and VRC704 were based on this

formulation).
In each group, BALB/c mice (n=5) received two IM immunizations of VLPs at one of three dose levels (0.2, 2.0 and 20 µg of protein in study no. 4 and 0.02, 0.2, 2.0 and 20 µg of protein in study no. 5) at an interval of 28 days. Sera (10 days after 2nd immunization) were tested for VLP-binding antibodies by ELISA. CHIKV SNA titres which neutralize 80% pseudo virus (NT80) were assessed using the luciferase assay mentioned above. In studies no. 4 and no. 5 all tested lots of unadjuvanted NIH VRC CHIKV VLP triggered the development of similar amounts of antibodies which bind to CHIKV VLP (ELISA) and neutralize CHIK pseudo virus (luciferase assay). As conclusive dose response curves could not be derived (especially in study no. 5 where the immune response to the lowest dose roughly equals the one to the highest dose; see figs. 9 and 10), comparing the immunogenic potencies of the different formulations is difficult. However, this is considered a minor and issue and no concern is raised.

In summary, study report RPT062501 conclusively shows that (i) CHKVLP059 vaccination triggers the development of CHIKV specific antibodies, which (ii) neutralize CHIKV and (iii) protect from viremia upon CHIKV challenge.

PTR-TRD-CHIK-004: Immunogenicity Assessment of Chikungunya VLP Vaccine in Mice:
The purpose of study PTR-TRD-CHIK-004 was to evaluate the immunogenicity CHIKV VLP in the absence or presence of aluminium hydroxide adjuvanted formulations (alum adjuvant). Two lots (+/- alum adjuvant → 4 different conditions) were tested: CHIKV VLP , and CHKVLP059 . The study was conducted in CB6F1 hybrid mice and comprised 19 experimental groups (n=6 per group). At an interval of 35 days mice were immunized twice and blood was collected on days 14, 28 and 56 post prime immunization. The study was terminated on day 56. The table below provides an exhaustive study schedule:

Table 3 Study schedule

Study Schedule:

- Day 0 (31Mar17): Study start and prime immunization
- Day 14 (14Apr17): Blood collection
- Day 28 (28Apr17): Blood collection
- Day 35 (05May17): Boost immunization
- Day 56 (26May17): Blood collection and termination

		Dose					Study Schedule				
Group	Vaccine	VLP µg	Alum µg	Volume	Route	# animals	d0	d14	d28	d35	d56
1	Chik VLP	0.2	–	0.1 mL	i.m.	6 CB6F1	Vv	B	B	Vv	B/T
2	Chik VLP	2	–	0.1 mL	i.m.	6 CB6F1	Vv	B	B	Vv	B/T
3	Chik VLP	20	–	0.1 mL	i.m.	6 CB6F1	Vv	B	B	Vv	B/T
4	Chik VLP + alum	0.2	250	0.1 mL	i.m.	6 CB6F1	Vva	B	B	Vva	B/T
5	Chik VLP + alum	2	250	0.1 mL	i.m.	6 CB6F1	Vva	B	B	Vva	B/T
6	Chik VLP + alum	20	250	0.1 mL	i.m.	6 CB6F1	Vva	B	B	Vva	B/T
7	*Chik VLP + alum	2	250	0.1 mL	i.m.	6 CB6F1	* Vva	B	B	* Vva	B/T
8	* Chik VLP + alum	20	250	0.1 mL	i.m.	6 CB6F1	* Vva	B	B	* Vva	B/T
9	*Chik VLP + alum	2	250	0.1 mL	i.m.	6 CB6F1	* Vva	B	B	* Vva	B/T
10	* Chik VLP + alum	20	250	0.1 mL	i.m.	6 CB6F1	* Vva	B	B	* Vva	B/T
11	VRC Chik VLP	0.2	–	0.1 mL	i.m.	6 CB6F1	Vv	B	B	Vv	B/T
12	VRC Chik VLP	2	–	0.1 mL	i.m.	6 CB6F1	Vv	B	B	Vv	B/T
13	VRC Chik VLP	20	–	0.1 mL	i.m.	6 CB6F1	Vv	B	B	Vv	B/T
14	VRC Chik VLP + alum	0.2	250	0.1 mL	i.m.	6 CB6F1	Vva	B	B	Vva	B/T
15	VRC Chik VLP + alum	2	250	0.1 mL	i.m.	6 CB6F1	Vva	B	B	Vva	B/T
16	VRC Chik VLP + alum	20	250	0.1 mL	i.m.	6 CB6F1	Vva	B	B	Vva	B/T
17	Chik DNA	50	–	0.1 mL	i.m.	6 CB6F1	Dv	B	B	Dv	B/T
18	Alum only sham	–	250	0.1 mL	i.m.	6 CB6F1	Sv	B	B	Sv	B/T
19	Naïve	–	–	–	–	6 CB6F1	–	B	B	–	B/T

* groups to support Pharmacy Manual 4 and 24 hour hold time for VLP+alum

Chik VLP = Based on 37997 native Chik strain
Chik DNA = DNA plasmid expressing 37997 stuctural proteins
(the same plasmid used to generate VLP)
alum = Aluminum hydroxide adjuvant
(2% Alhydrogel, Brenntag, Denmark)
CB6F1 = BALB/c x C57BL/6, b x d MHC haplotype
Total mice = 114 CB6F1 hybrid mice

VLP = Virus Like Particle
i.m. = intramuscular
Vv = VLP vaccination
Vva = VLP-alum vaccination
Vva* = VLP-alum vaccination
(mix performed 4 or 24 hours in advance)
Dv = DNA vaccination
Sv = Sham vaccination (alum only)
B = Bleed
T = Termination

Mouse sera (pooled within group) were examined for the presence of VLP binding and CHIKV neutralizing antibodies by ELISA and CHIKV-Luc neutralization assay (this is the luciferase assay mentioned above), respectively. For both: PaxVax and VRC CHIKV VLP, the presence of alum adjuvant caused substantial increases (~ 3 – 10-fold) in the amounts of VLP binding and CHIKV neutralizing antibodies. In all cases, PaxVax and VRC CHIKV VLP lots seemed to trigger quite similar antibody responses. Preparation of adjuvanted formulations 4 or 24 h prior to immunization (groups 7 – 10) (instead of completely fresh) did not affect the extent of the immune response. Control groups (17 - 19) only displayed negligible amounts of binding antibody. As expected, NT80 antibody titres of sera post 2nd immunization greatly exceeded (~ 8-fold) NT80 antibody titres of sera post prime immunization.

Taken together, the main goal of this study was to demonstrate a dose sparing effect of alum adjuvant by increasing antibody response levels. This was achieved.

PAS-NHP-CHIK-001: Immunogenicity and Protective Efficacy of Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], Unadjuvanted or Alum adjuvanted against Chikungunya Virus Challenge in Cynomolgus Macaques.

(Supported by study: PAS-NHP-CHIK-001P: Pilot Study in Cynomolgus Macaques to Evaluate the Effect of Different Challenge Doses of Chikungunya Virus Strain LR2006-OPY1)

Study PAS-NHP-CHIK-001 sought to recapitulate previous mouse-data (Study PTR-TRD-CHIK-004) in Cynomolgus Macaques. That is, immunogenicity and efficacy testing of non-adjuvanted or alum-adjuvanted CHIKV-VLP (PXVX0317) lot (same lot was used in Phase 2 clinical trials PXVX-CV-317-001 and EBSI-CV-317-002) in cynomolgus monkeys. As this study includes CHIKV-challenge experiments, the Applicant conducted a preceding study (PAS-NHP-CHIK-001P) with the purpose to identify an optimal challenge dose of CHIKV strain LR2006-OPY1. This pilot study should be briefly discussed beforehand. Three groups of NHPs (n = 3/group) received either 10^6, 10^7 or 10^8 plaque-forming units (PFU) of the LR2006-OPY1 strain of CHIKV. After 10 days animals were euthanized and examined. Although the inoculation dose appears to correlate positively with the extent of viremia, clinical signs, requirement for supportive care, and joint pathology (a hallmark of CHIKV disease in human), none of these parameters differed significantly between treatment groups. In order to minimize the risk of fulminant infection (associated with 10^8 PFU),the dose of 10^7 PFU per animal in subsequent cynomolgus monkey studies is agreeable.
Having established a challenge dose of 10^7 PFU of CHIKV strain LR2006-OPY1, the Applicant proceeded to PAS-NHP-CHIK-001. Animals were immunized intramuscularly on Days 0 and 28 with a range of doses of CHIKV VLP (1.25, 6 or 20 µg) with alum (300 µg), CHIKV VLP alone (20 µg) or alum alone (300 µg; control group). Animals were challenged on approximately day 56 (depending on the cohort) intravenously with 10^7 PFU of CHIKV LR2006-OPY1. Animals were euthanized 10 days post challenge and examined. The experimental design is outlined in the Table 4 below.

Table 4. Experimental design of active immunization study

Study procedures are shown by study day.

Group	Dose VLP (mcg)/ Adjuvant (mcg)	No. NHP	Study Day Based on Staggered Start						
			0	14	28	42	56*	57-63	66
1	1.25/300	5	B V	B	B V	B	B CHIKV-C	B (daily)	TB E
2	6/300	5	B V	B	B V	B	B CHIKV-C	B (daily)	TB E
3	20/300	5	B V	B	B V	B	B CHIKV-C	B (daily)	TB E
4	20/- (VLP only)	5	B V	B	B V	B	B CHIKV-C	B (daily)	TB E
5	-/300 (alum only)	4	B V	B	B V	B	B CHIKV-C	B (daily)	TB E

B = blood collection
V= vaccination
CHIKV-C = chikungunya virus challenge administered intravenously
TB = terminal bleed
E = euthanasia. Euthanasia will be performed on Day 10 post challenge.
* Depending on the cohort (see section below for cohort assignments) the animals will receive challenge on days 56-64, and will be followed for 10 days post challenge (up to euthanasia on day 10).

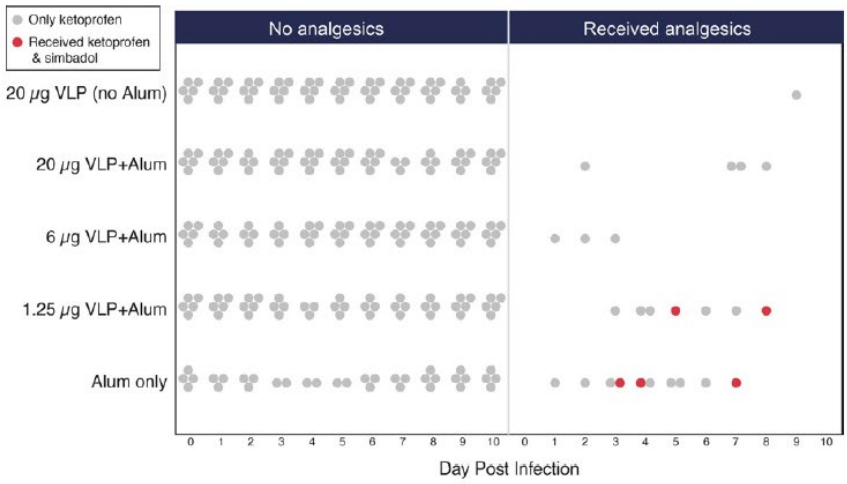
Examination included assessment of (i) viral RNA in plasma, (ii) infectious virus in plasma, (iii) body temperature and body weight, (iv) need of supportive care with analgesics, (v) joint circumference and temperature, (vi) lymphocyte counts, (vii) serum biochemistry, (viii) inflammatory cytokines and chemokines in plasma, (ix) serum neutralizing antibodies, (x) joint histology, (xi) viral RNA in tissues. This comprehensive study clearly demonstrates protection from CHIKV disease as a result of CHIKV VLP vaccination.

It is agreed that alum adjuvant (like in mouse study PTR-TRD-CHIK-004) significantly increases SNA-titres in serum in the following manner: 20 µg VLP only (geometric mean titre: 2333.2) < 1.25 µg VLP/Alum (3419.9) < 6 µg VLP/Alum (9305.0) < 20 µg VLP/Alum (12446.8). Although a correlation between SNA titer and disease outcome is plausible, the overall need of analgesics (Table 7) suggests that "20 µg VLP only" may clinically surpass "1.25 µg VLP/Alum", despite higher SNA titers in the latter group. A statistically significant advantage of "20 µg VLP + alum" over "20 µg VLP only" was not observed for any study parameter (except for SNA titers). Hence, a direct significant clinical benefit of alum adjuvant addition cannot be inferred from this study, though a trend was seen in terms of reduced CHIKV RNA detected in joints (Figure 2). As regards clinical dose selection, it is referred to the discussion on clinical efficacy.

Table 5. GMTs at time of challenge by treatment group

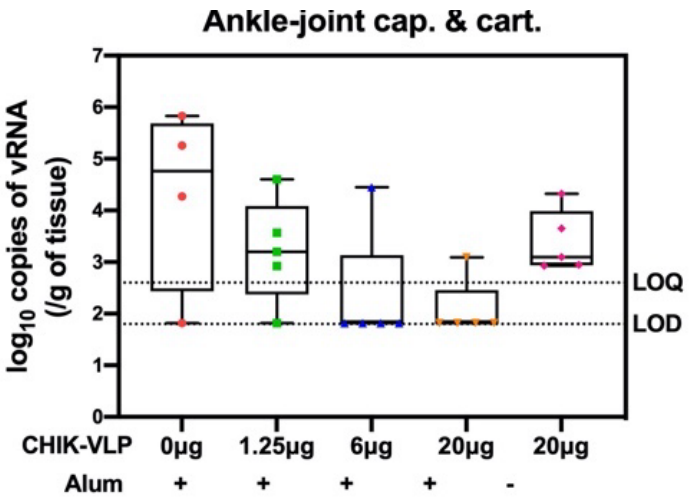
	Alum Only	1.25 µg VLP/Alum	6 µg VLP/Alum	20 µg VLP only	20 µg VLP/Alum
GMT	19.0	3419.9	9305.0	2333.2	12446.8

Table 6. Summary of treatments with analgesics



The left part of the figure represents, for each day after challenge, the number of animals that did not receive Ketoprofen nor simbadol. The right part of the graph summarizes when animals received Ketoprofen (grey) or Ketoprofen and simbadol (red)

Figure 2. Comparison of viral RNA in joint tissues



Samples with undetectable vRNA were given a value of the midpoint between the LoD and LLOQ for purpose of graphing and statistical analysis.

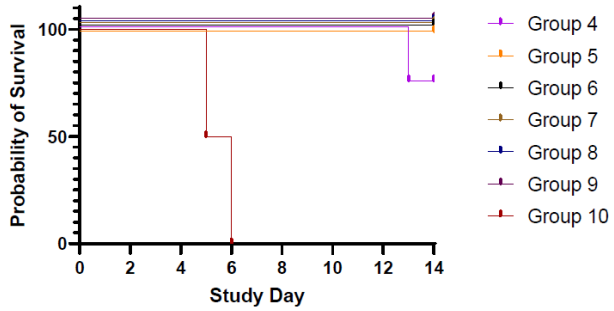
A statistically significant advantage of "20 µg VLP + alum" over "20 µg VLP only" was not observed for any study parameter (except for SNA titres). Hence, a direct clinical benefit of alum adjuvant addition cannot be inferred from this study.

Passive immunization studies

PAS-Mouse-CHIK-001: Testing normalized volumes of passively transferred human sera against Chikungunya Virus challenge in A129 mice.

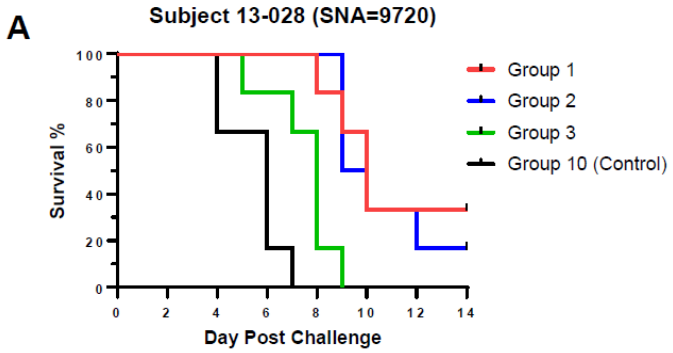
The purpose of study PAS-Mouse-CHIK-001 was to test the protective activity of sera derived from PXVX0317-vaccinated humans. Such sera were passively transferred to A129 mice followed by CHIKV challenge. This study consists of a first study (study 1) and a follow-up study (study 2). The follow-up study was initiated because high transferred serum volumes caused death in a substantial fraction (13 out of 40) of mice as a result of serum toxicity. More specifically, all mice in groups 1 and 2 as well as 3 out 4 mice in group 3 as well as 2 out of 4 mice in the control group died before viral challenge.

Hence, groups 1 -3 were excluded from the study. All mice in groups 5 – 9 survived subsequent challenge with 10^1 PFU of CHIKV strain LR2006-OPY19. Both mice in the control group which survived serum toxicity died on days 5 and 6 post viral challenge. If the transferred sera protected the animals from a lethal challenge dose cannot be deduced from the data, as it cannot be ruled out that the two remaining control mice died of delayed effects of serum toxicity. Hence, survival of any mice in this study should be interpretated with caution.



In the follow-up (passive immunization study 2) human sera volumes were administered which were unlikely to cause serum toxicity. However, the choice of 1 mL human serum in the control group is considered risky and a certain degree of serum toxicity cannot be excluded. Even more so since a similarly treated mouse died on day 13 in the previous study (see Kaplan-Meier plot above) and since the survival of mice in group 7 of this study is poor (1 of 6).

Still, the data are considered sufficient to show that passively transferred serum from CHIKV VLP vaccinated humans exerts a certain protective effect against CHIKV in mice. This is not only evident from absolute survival by the end of the observation period but also from a delay of death in each group compared to control (see representative Kaplan-Meier plot below).



Overall, it is agreed that this study does not suffice to conclude on the human SNA-titre to confer protection against lethal CHIKV-challenge.

PAS-NHP-CHIK-002: Protective Efficacy of passively transferred human IgG against Chikungunya Virus Challenge in Cynomolgus Macaques.
(Supported by study: PAS-NHP-CHIK-002PK: Report for Chikungunya Virus Neutralizing Antibody Titres in the NHP Pharmacokinetics (PK) Study)

The objective of study PAS-NHP-CHIK-002 was to test the protective effect of purified hyperimmune IgG from humans vaccinated with CHIKV VLP (PXVX0317). Such purified IgG was passively transferred to cynomolgus monkeys followed by CHIKV challenge. This present study was preceded by a supportive PK-study (PAS-NHP-CHIK-002PK) to inform the selection of IgG dose levels. This supportive study should be briefly discussed in the following. Cynomolgus monkeys (n=3 per dose level) were administered 10, 30 or 100 mg/kg purified human IgG (CHIKV NT80 = 32,532.8 at 100 mg/mL). Monkey sera were withdrawn regularly within the following 10 days and tested for CHIKV neutralizing antibodies by CHIKV-Luciferase neutralization assay. For the 10, 30 and 100 mg/kg groups the respective NT80 titres (geometric mean) were 51.6, 191.6 and 536.8 twenty-four hours post IgG-administration (which will be the time of CHIKV challenge).

Apparently, for the actual passive immunization study a different IgG-lot should be chosen with a NT80 of 17,881.7. As according to the Applicant: "Final doses were chosen to yield NT80 titres in recipients 24 hours post-administration of approximately 20 (near the lower limit of the assay and potentially allowing for breakthrough CHIKV disease), 60 (potentially a low dose that confers complete protection), and 370 (to provide maximum protection)." Consequently, the dose levels for the final IgG-lot were selected to be 5, 15 and 100 mg/kg. Apart from the fact that this calculation cannot be followed (e.g., 17881.7 / 32532.8 × 536.8 ≠ 370), the target titres 20, 60 and 370 appear arbitrary and the predictions in brackets lack corroboration. In fact, the subsequent study PAS-NHP-CHIK-002 demonstrated that IgG dose groups do not significantly differ in their protection against CHIKV disease. The experimental design for study PAS-NHP-CHIK-002 is outlined in the table below.

Table 7. Experimental design of active immunization study

Study procedures are shown by study day.

Group	Human IgG Dose	No. of Animals^	IgG administration (route)	Challenge 10^7 pfu IV (route)	Health Monitoring	Euthanasia & necropsy
1	100 mg/kg	6	Day 0 (IV in right saphenous vein)	Day 1 (IV in left saphenous vein)	Bleed daily from day 0-8, and day 11. Daily health checks; report any adverse events	Day 11
2	15 mg/kg	6				
3	5 mg/kg	6				
4	100 mg/kg*	6				

* IgG from PXVX0317-naïve subjects.
^ Each groups consists of 3 males and 3 females.

In brief, animals (n=6 per group) received purified IgG from humans that were either CHIKV VLP vaccinated or naïve (control group) at the indicated dose levels. Twenty-four hours later, animals were challenged with 10^7 PFU LR2006-OPY1. Animals were monitored (including blood withdrawal) for 11 days, after which animals were euthanized and examined. Examination included assessment of (i) viral RNA in plasma, (ii) infectious virus in plasma, (iii) body temperature and body weight, (iv) need of supportive care with analgesics, (v) joint circumference and temperature, (vi) haematology, (vii) serum biochemistry, (viii) inflammatory cytokines and chemokines in plasma, (ix) serum neutralizing antibodies, (x) joint histology, (xi) viral RNA in tissues. Most of these parameters clearly indicate that NHPs are protected from CHIKV as a result of passive transfer of IgG from human which were immunized with CHIKV VLP.

All doses were equally effective in eliminating infectious virus in the blood. All CHIKV dose groups were equally likely to require analgesics. Although there was trend for lower joint pathology scores with increasing CHIKV-IgG doses, the differences were not statistically significant.
A significant correlation was detected between SNA-titres and the presence of viral RNA in plasma. According to this, the Applicant states that "an 80% probability of preventing viremia ≥ LLOQ was associated with a day 1 serum antibody neutralization titre of 110.3, while a 90% probability of preventing viremia ≥ LLOQ was associated with a day 1 antibody neutralization titre of 139.5. Prevention of viremia ≥ LOD at 80% probability required a Day 1 SNA antibody titre of 417.7 and at 90% probability required a Day 1 SNA antibody titre of 711.5."
In conclusion, study PAS-NHP-CHIK-002 provides evidence that also seemingly low SNA-titres provide sufficient protection from CHIKV disease. Overall, the predictions made in PAS-NHP-CHIK-002PK did not hold true as for 5, 15 and 100 mg/kg the measured SNA titres (geometric mean) were 38, 101, 644, which are rather expected to offer good to very good protection. Hence, although a dose/effect

relationship could not be established (except for SNA-titres and plasma viral RNA), the study clearly demonstrates overall efficacy of passively transferred human IgG post CHIKV VLP vaccination.

PAS-NHP-CHIK-003: Efficacy of Serum from CIDKV VLP-Vaccinated Human Volunteers Against Chikungunya Virus in Cynomolgus Macaques.

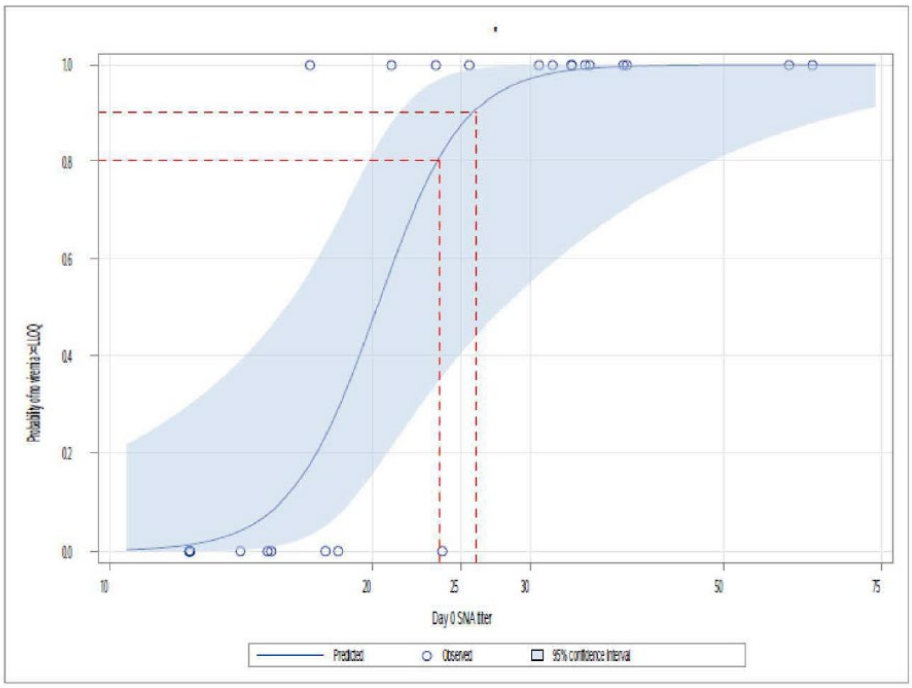
Study PAS-NHP-CHIK-003 is very similar to study PAS-NHP-CHIK-002, but used a subcutaneous CHIKV challenge rather than the intravenous route to better reflect a mosquito bite. In addition to the administration of one dose level of purified human CHIKV IgG (0.075 mL/kg of 79 mg/mL = 5.925 mg/kg; of note, this is the same IgG-Lot which was used in PAS-NHP-CHIK-002) cynomolgus monkeys received human CHIKV immune sera at 0.3, 0.6, 1.2 or 2.4 mL/kg. After 24 h animals were challenged with 10^5 PFU CHIKV strain LR2006 OPY-1. The present study was more focused on identifying a threshold circulating SNA titre required for protection following CHIKV challenge. The study design is outlined in the Table 8 below.

Table 8. Study design

Cohort	Group	N	Treatment Test Article	Test article volume (mL/kg)
1	1	2 (1 male, 1 female)	CHIKV IgG	0.075
	2	NA	NA	NA
	3	3 (1 male, 2 female)	CHIKV serum	1.2
	4	3 (2 male, 1 female)	CHIKV serum	0.6
	5	3 (1 male, 2 female)	CHIKV serum	0.3
	6	3 (2 male, 1 female)	Control serum	2.4
2	1	NA	NA	NA
	2	2 (1 male, 1 female)	CHIKV serum	2.4
	3	3 (2 male, 1 female)	CHIKV serum	1.2
	4	3 (1 male, 2 female)	CHIKV serum	0.6
	5	3 (2 male, 1 female)	CHIKV serum	0.3
	6	3 (1 male, 2 female)	Control serum	2.4

In contrast to treatment group 6, no viable virus was found post challenge in plasma of any of the treatment groups 1 – 5. However, viral RNA was found by RT-qPCR in plasma of animals in groups 4, 5 and 6. On Day 0 prior to challenge, all 6 of the 2.4 mL/kg control serum group titres remained <12.3. The geometric mean titre of the Group 1 (CHIKV IgG) group was 32.2, while mean titres for the CHIKV serum groups were 11.4, 21.3, 35.4, and 61.3 for Groups 5, 4, 3 and 2, respectively. Two of 6 NHPs in the 0.3 mL/kg CHIKV serum group (Group 5) had SNA titres of <12.3 on Day 0 at the time of challenge. A correlation (as performed in "PAS-NHP-CHIK-003 Statistical Report") between the presence of plasma viral RNA (as a measure of viremia) and serum neutralizing antibody titres came to the following conclusions. An 80% probability of preventing viremia ≥LOD/LLOQ was associated with a Day 0 SNA antibody titre of 23.6, while a 90% probability of preventing viremia ≥LOD/LLOQ was associated with a Day 0 SNA antibody titre of 25.9 (see logistic regression model below).

Figure 3. Logistic regression model of binary outcome (viremia/no viremia) as RT qPCR < LLOQ Days 1-5 and Day 10 predicted by log_{10} Day 0 SNA antibody titre



However, these data should be interpreted with caution as 95% confidence intervals (blue area in figure above) at the mentioned points are large, owing to the limited number of animals as well as animal-to-animal variability. On the other side, the provided analysis is considered conservative as it accounts for the total absence of plasma viral RNA, which is a very sensitive parameter for viremia.

2.5.2.2. Secondary pharmacodynamic studies

No specific nonclinical secondary pharmacology studies have been performed with CHIKV VLP, which is not a new molecular entity, and no off-target activities are expected due to its nature as protein-based vaccine. This is in accordance with WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO 2005).

2.5.2.3. Safety pharmacology programme

No safety pharmacology studies were conducted. In accordance with WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO 2005) and ICH Guideline (ICH S6 (R1) 2011) safety pharmacology assessments such as body temperature measurements and histopathology were covered in the repeated dose toxicology study. There were no findings from safety pharmacology evaluations that would indicate an unacceptable risk for administration of CHIKV VLP to individuals in accordance with the proposed indication.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were conducted with CHIKV VLP, which is in accordance with applicable guidelines (WHO 2005).

2.5.3. Pharmacokinetics

In accordance with WHO guidelines on non-clinical evaluation of vaccines (WHO 2005) and vaccine adjuvants and adjuvanted vaccines (WHO 2013), dedicated studies on absorption, distribution, metabolism, and excretion (ADME) were not performed.

Under this section the Applicant filed technical reports on (i) the luciferase-based chikungunya (CHIK) neutralization assay and (ii) the CHIKV RT-qPCR assay.

Technical documentation on the nonclinical luciferase-based chikungunya (CHIK-Luc) neutralization assay contained a comparison between the CHIK-Luc assay (NT80) (developed by the Applicant), a plaque reduction neutralization assay (PRNT80) (developed by the Applicant) and a flow-cytometry based CHIKV neutralization assay (EC80) (developed by an external laboratory). The purpose of the study was to determine whether these methods produce similar results in terms of antibody titre/neuralization correlation.

Figure 4. Comparison between the CHIK-Luc assay (NT80), a plaque reduction neutralization assay (PRNT80) and a flow-cytometry based CHIKV neutralization assay (EC80)

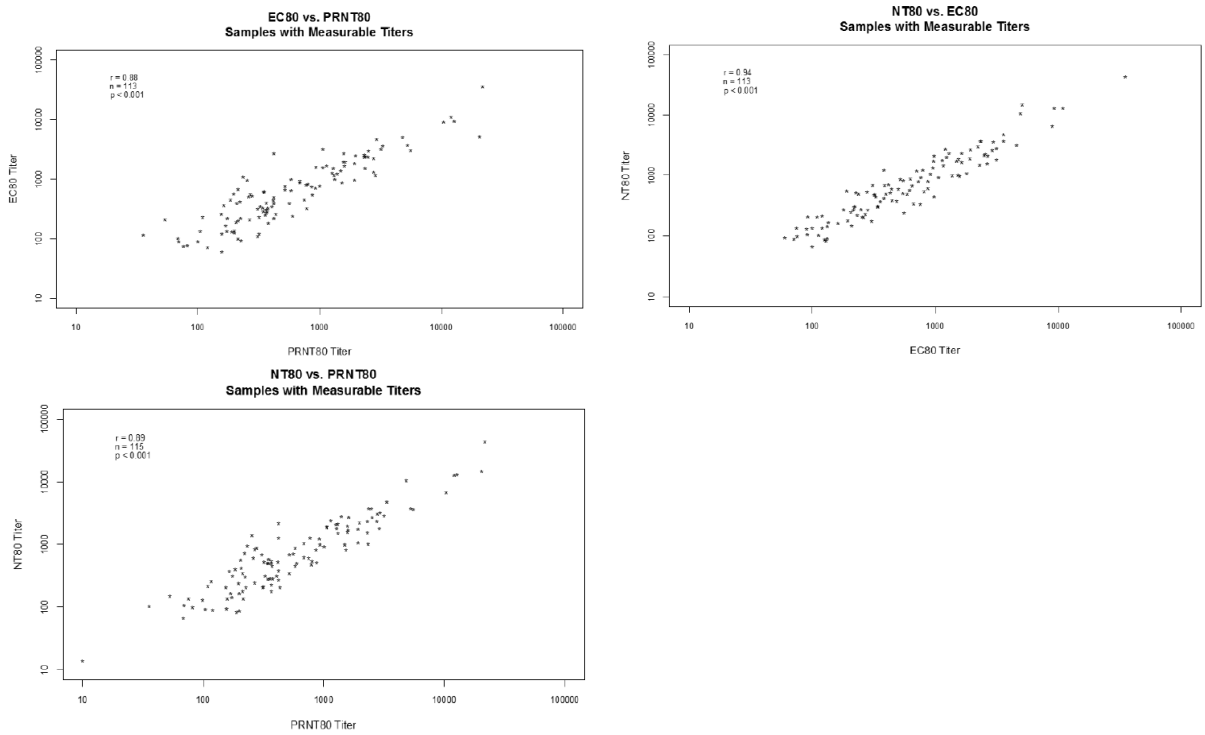


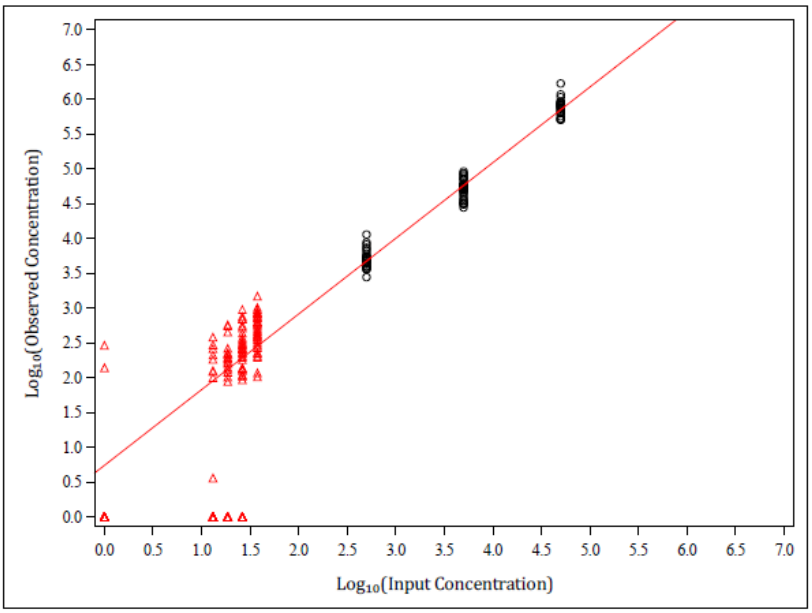
Table 9. Comparison of CHIKV-Specific Antibody Titres obtained from the PaxVax PRNT80, PaxVax CHIKV-Luc NT80, and NIH SFV/CHIKV OPY1 EC80 Assay Methods

Assay Comparison	n of Samples Quantifiable by Each Method	Correlation Coefficient	Median Bias (95% CI)
EC80 vs. PRNT80	113	0.88	-3% (-10%, 4%)
NT80 vs. EC80	113	0.94	15% (1%, 24%)
NT80 vs. PRNT80	115*	0.89	8% (-6%, 30%)

Figure 4 provides sufficient evidence that all three methods correlate well to justify the use of the luciferase-based chikungunya (CHIK-Luc) neutralization assay in nonclinical studies.

Technical documentation on the nonclinical NHP RT-qPCR contained a standard operating procedure for the isolation of RNA from non-human primate plasma, followed by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) quantitation of CHIKV non-structural protein (NSP4) gene.

Figure 5. Relationship between Log-transformed Observed and Input CHIKV concentration



- All data included in plot. Red triangles represent data from test samples with unacceptable precision. These data were not used to fit the regression model.
- The red solid line is a regression line fit to the observed data. The slope of the regression line was 1.09.

Figure 5 shows a clearly linear relationship between input CHIKV concentrations and observed concentrations, which justifies the nonclinical use of RT-qPCR for quantification of CHIKV RNA within the detection and quantification limits.

Supporting assays, such as a CHIKV VLP ELISA for the analysis of binding antibodies in vaccinated mice, NHPs and rabbits, were shown to be fit for their intended purpose, but were not validated.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

No single-dose toxicity studies with CHIKV VLP vaccine have been conducted, which is in line with the WHO guideline (WHO 2005). Standard toxicity parameters were evaluated as part of the repeat-dose toxicity study.

2.5.4.2. Repeat dose toxicity

The Applicant conducted a single repeat-dose toxicity study [MRI 110752.01.001] in NZW rabbits to investigate the safety of CHKVLP059. Unadjuvanted CHKVLP059 was used in this study, which is regarded acceptable as CHKVLP059 and adjuvanted CHIKV VLP vaccine materials were both produced using similar starting materials, manufacturing processes and formulations, and were demonstrated to be biochemically and biophysically comparable to each other in quality testing, including VLP content, identity, and purity, and aluminium hydroxide is widely used in human vaccines and has a demonstrated favourable safety profile. CHKVLP059 and CHIKV VLP vaccine were also shown to elicit

comparable neutralizing antibody titres in a head-to-head comparison in a mouse immunogenicity study (PTR-TRD-CHIK-004). This comparative study demonstrated that the inclusion of aluminium hydroxide-adjuvant in CHIKV VLP vaccine and CHKVLP059 formulations increased antibody titres and was dose-sparing, supporting the clinical development of aluminium hydroxide-adjuvanted formulations of CHIKV VLP vaccine.

2.5.4.3. Genotoxicity

No novel adjuvants or additives were included in the final vaccine formulation. Thus, in accordance with effective guidance, no dedicated genotoxicity studies were conducted.

2.5.4.4. Carcinogenicity

Consistent with the WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO 2005) and ICH guideline S6 (R1), carcinogenicity studies were not performed as a single-dose vaccine is not expected to be carcinogenic. The CHIKV VLP vaccine was formulated using well-characterized excipients and adjuvant and is administered using a pre-filled syringe.

2.5.4.5. Reproductive and developmental toxicity

Developmental and reproductive toxicology (DART) studies were conducted in the female rabbit and rat models to evaluate potential effects on fertility, foetuses, and pups. In the two DART studies (CHIK-DART-001, CHIK-DART-002), CHIKV VLP vaccine was administered intramuscularly up to five doses of 40 µg aluminium hydroxide adjuvanted vaccine each. In female rabbits, CHIKV VLP vaccine was administrated before mating, during gestation, and during lactation, and was tolerated with no indication of maternal systemic toxicity. In rat, up to five IM administrations of CHIKV VLP vaccine at a dose level of 40 µg/dose was well tolerated and yielded a robust neutralizing antibody response in female rats. Passive immunity was observed also in litter/pups in both species.

2.5.4.6. Toxicokinetic data

No toxicokinetic studies were performed.

2.5.4.7. Local Tolerance

Local tolerance was investigated as part of the repeat-dose toxicity and DART studies. No significant adverse findings related to administration of the CHIKV vaccine were observed.

2.5.4.8. Other toxicity studies

No novel adjuvants or excipients were used in the vaccine product. All excipient levels in the product are below the acceptable levels. Residual impurities that result from the DS manufacturing process were evaluated separately and toxicological assessments were conducted. None of the impurities were determined to pose unacceptable risk to people receiving a single or multiple doses of CHIKV VLP vaccine.

2.5.5. Ecotoxicity/environmental risk assessment

No dedicated ERA studies were conducted for CHIKV VLP vaccine, which is acceptable as according to the "Guideline on the environmental risk assessment of medicinal products for human use"- EMEA/CHMP/SWP/4447/00 corr 2 and the "Guideline on Influenza Vaccines - Non-clinical and Clinical Module" (EMA/CHMP/VWP/457259/2014). Vaccines based on recombinant proteins are exempted from the submission of specific ERA studies.

2.5.6. Discussion on non-clinical aspects

Pharmacology

The Applicant provided a sufficient package of pharmacological studies to demonstrate that the test article confers immunity against CHIKV in animals which are either (i) vaccinated with CHIKV VLP (active immunization) or (ii) recipient of sera or IgG from animals or humans which were vaccinated (passive immunization).
Active immunization studies provide ample evidence that CHIKV VLP vaccination triggers the emergence of CHIKV-neutralizing antibodies in mice (Report RPT062501 Study no. 1, no. 4 and no. 5, PTR-TRD-CHIK-004), in rhesus macaques (Report RPT062501 Study no. 2), and in cynomolgus macaques (PAS-NHP-CHIK-001). In addition, rhesus and cynomolgus macaques were protected from CHIKV challenge (10^{10} and 10^{7} PFU of LR2006-OPY1, respectively) after immunization with CHIKV VLP. Addition of alum adjuvant to CHIKV VLP was demonstrated to significantly increase serum neutralizing antibody titres in mice (PTR-TRD-CHIK-004) and cynomolgus monkeys (PAS-NHP-CHIK-001). However, although a correlation between SNA titre and disease outcome is plausible, a direct clinical benefit of alum adjuvant in this vaccine cannot be inferred from the nonclinical pharmacological studies.
Passive immunization studies clearly demonstrated the protective effect of passively transferred CHIKV immune sera or purified CHIKV IgG in *Ifnar*-KO (and hence immunodeficient) mice (Report RPT062501 Study no. 3), A129 mice (PAS-Mouse-CHIK-001) and cynomolgus monkeys (PAS-NHP-CHIK-002 and PAS-NHP-CHIK-003) against CHIKV challenge. Based on data from cynomolgus monkeys (PAS-NHP-CHIK-003) which received different amounts of sera or purified IgG from human CHIKV VLP vaccinated volunteers, a logistic regression model was established to identify SNA-titers of 23.6 and 25.9 conferring complete protection from CHIKV-associated viremia with respective probabilities of 80% and 90%.

No specific nonclinical secondary pharmacology studies have been performed with CHIKV VLP, which is not a new molecular entity, and no off-target activities are expected due to its nature as protein-based vaccine. This is in accordance with WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO 2005).

No safety pharmacology studies were conducted. In accordance with WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO 2005) and ICH Guideline (ICH S6 (R1) 2011) safety pharmacology assessments such as body temperature measurements and histopathology were covered in the repeated dose toxicology study. There were no findings from safety pharmacology evaluations that would indicate an unacceptable risk for administration of CHIKV VLP to individuals in accordance with the proposed indication. This is agreed.

No pharmacodynamic drug interaction studies were conducted with CHIKV VLP, which is in accordance with applicable guidelines (WHO 2005).

Pharmacokinetics

Described analytical methods (Luciferase-Based Chikungunya Neutralization Assay, CHIKV RT-qPCR) are considered suitable for utilization in nonclinical studies. In accordance with effective guidelines on non-clinical evaluation of vaccines, dedicated studies on absorption, distribution, metabolism, and excretion (ADME) were not performed.

Toxicology

No single-dose toxicity studies with CHIKV VLP vaccine have been conducted, which is in line with the WHO guideline (WHO 2005). Standard toxicity parameters were evaluated as part of the repeat-dose toxicity study (MRI 110752.01.001).

The Applicant conducted a single repeat-dose toxicity study [MRI 110752.01.001] in NZW rabbits to investigate the safety of CHKVLP059. Unadjuvanted CHKVLP059 was used in this study, which is regarded acceptable as CHKVLP059 and adjuvanted CHIKV VLP vaccine materials were both produced using similar starting materials, manufacturing processes and formulations, and were demonstrated to be biochemically and biophysically comparable to each other in quality testing, including VLP content, identity, and purity, and aluminium hydroxide is widely used in human vaccines and has a demonstrated favourable safety profile. CHKVLP059 and CHIKV VLP vaccine were also shown to elicit comparable neutralizing antibody titers in a head-to-head comparison in a mouse immunogenicity study (PTR-TRD-CHIK-004). This comparative study demonstrated that the inclusion of aluminium hydroxide-adjuvant in CHIKV VLP vaccine and CHKVLP059 formulations increased antibody titers and was dose-sparing, supporting the clinical development of aluminium hydroxide-adjuvanted formulations of CHIKV VLP vaccine. No safety signals were observed for CHIKV VLP vaccine with or without adjuvant in mice (PTR-TRD-CHIK-004) and NHPs (PAS-NHP-CHIK-001), nor with adjuvanted CHIKV VLP vaccine in two DART studies.

Forty µg of CHKVLP059 were administered four-times intramuscularly, providing an acceptable safety margin when compared to the single administration of 40 µg proposed in the clinics. Immunogenic response was shown in the animals confirming the rabbit to be a suitable toxicological animal model.

No morbidity or mortality or clinical signs indicative of systemic adverse effects were reported in rabbits. The administration of CHKVLP059 vaccine to rabbits via IM injection did not result in a treatment-effect on oedema or erythema at the injection site following four administrations of each test article to two sites on each administration day.

Among other haematological parameters, c-reactive protein was found statistically increased compared to control animals, however within the range of historical control data of this animal model, thus not regarded adverse.

In histopathological examinations, a statistically significant decrease in absolute thymic weight and thymic weight relative to either body weight or brain in the CHKVLP059-vaccinated male rabbits was observed. No corresponding pathological findings were reported in this treatment group.

Overall, the RDT toxicity study in NZW rabbits did not indicate any adverse findings relevant for human situation. Species selection and use of a single species only is in line with currently effective guidance and properly justified by showing an immunogenic response in rabbits. Route of administration and dose corresponds to the intended clinical use, thus accepted. Although only non-adjuvanted vaccine was administered in this study, the highest intended human dose of 40 µg was administered up to 4-times to individual rabbits, providing an acceptable safety margin. Further, the adjuvant aluminium hydroxide is well known und widely used in various vaccine formulations, and was also used by the Applicant in parts of the PD studies and in both DART studies, to provide further safety data also when using the adjuvanted product.

No novel adjuvants or additives were included in the final vaccine formulation. Thus, in accordance with effective guidance, no dedicated genotoxicity studies were conducted. This is accepted.

Consistent with the WHO Guidelines on Nonclinical Evaluation of Vaccines (WHO 2005) and ICH guideline S6 (R1), carcinogenicity studies were not performed as a single-dose vaccine is not expected to be carcinogenic. The CHIKV VLP vaccine was formulated using well-characterized excipients and adjuvant and is administered using a pre-filled syringe.

The Applicant has conducted two studies covering potential effects on female fertility, embryo-foetal (rabbit only) and postnatal development in rats and rabbits. According to ICH S5(R3), it is sufficient to conduct developmental toxicity studies in a single species. Therefore, the package can be considered as acceptable overall while questionable from a 3Rs perspective. No dedicated study was performed to investigate any effect on male fertility. The Applicant indicates that integrity of reproductive organs was demonstrated at histopathological examination of reproductive organs in the rabbit repeated dose toxicity study. This is acceptable.

In the rabbit DART study, a total of 44 females were included in each of the control and treatment groups, with half females C-sectioned on GD29 to assess potential effects on embryo-foetal development (cohort 1) and the other half allowed to deliver their pups for evaluation of maternal behaviour and pup development up postnatal day 29 (cohort 2). On each dosing occasion, animals received an intramuscular injection of either control article or a full human dose of the alum-adjuvanted CHIKV VLP vaccine (0.8 mL/injection). Cohort 1 animals underwent a total of 4 injections, on days 28 and 14 premating and on days 7 and 21 of gestation. Cohort 2 animals were treated similarly with an additional injection on lactation day 7. The parameters and endpoints evaluated in maternal animals, foetuses and offspring were suitable for evaluating any treatment-related effect on fertility, embryo-foetal, and postnatal development up to weaning. The overall study design is considered acceptable. In the treated group, a sustained immune response to CHIKV VLP was seen in maternal animals from study day 22 to GD 29 with CHICKV 80% neutralizing antibody titres (NT80) peaking on GD14. In foetuses from treated dams, NT80s measured on GD29 were similar to those measured in dams on that day, whereas approximately 10-fold lower titres were reported in offspring on LD29. A significant immune response was not detected in the control group. These data confirm the rabbit as an acceptable species for DART evaluation, with transfer of maternal antibodies to foetuses and offspring.

In maternal animals of both cohorts, there was no adverse treatment-related death or clinical signs, body weight and food consumption change, or gross macroscopic change. At C-section of cohort 1 females, statistically significant decreases in numbers of corpora lutea and implantation sites were noted. It is agreed that this should not be considered as treatment-related given the historical control data and lack of reproducibility in cohort 2 females. No significant difference between control and treated groups were observed regarding other parameters monitored at ovarian and uterine examination, and foetal examination.

As regards postnatal development, a decrease in postnatal survival was noted based on increases vs. controls in the number of offspring loss between birth and day 4 pp (3.5 vs. 1.5 per litter) and between days 4 and 7 pp (4.6 vs. 2.2 per litter), and a decrease in offspring survival from days 4 to 28 pp (63.18% vs. 77.28%). The underlying cause was not identified. The Applicant considered this finding as unrelated to treatment based on historical control data in two rabbit strains used at the testing facility (survival as low as 57.4%) and absence of embryo lethality in cohort 1 animals. Actually, the survival index from days 4 to 28 pp in offspring from treated dams (63.18%) was outside the range reported at this testing facility in control animals of the same Crl:KBL NZW rabbits (71.0-82.6%; 3 studies, 2016-2020) and in HRA:NZW rabbits (86.3-96.3%; 7 studies, 2012-2020). In parallel, the survival index observed in the concurrent control group (77.28%) lied within the reported

historical control range. The use of the 57.4-95.5% historical control range as the main element to exclude any treatment-related effect in the decrease of postnatal survival is questionable since this relies on 8 studies conducted in HRA:NZW rabbits from 1997 to 2013, i.e. at least 8 years before initiation of treatment in the current study (12 Oct. 2021). Further discussion was requested to the applicant to conclude on the relevance of this finding. In response, detailed historical control data were provided for studies conducted from 1997 to 2024 at the testing facility. Although it is understood that there are situations wherein it is necessary to expand the historical database, those generated from 1997 to 2011 were not taken into consideration in order to not deviate exaggeratedly from the recommended typical 5-year period for use of such data. In addition, the comparison exercise was challenging as the parameters reported were not consistent across studies. Moreover, a comparison based on the absolute number of surviving kits per litter should also be interpreted in relation to the number of liveborn kits per litter which was high in the CHIKV VLP treated groups (% survival was not provided).

In general, the data do not support that the decreased postnatal survival (mostly PND 0-7) in the group treated with CHIKV VLP reflects normal variation. The applicant also indicated that the differences in postnatal viability observed in the treated group may be related to poor maternal behaviour, given also the absence of treatment-related embryo-foetal lethality or malformations (particularly in heart and lungs) which are considered to be other relevant indicators of survival in cohort 1 animals. However, intramuscular administration of CHIKV VLP vaccine before, during gestation, and during lactation was tolerated with no indication of maternal systemic toxicity in the rabbit DART study. Of note, maternal observations were also recorded in animals allowed to deliver and no vaccine-related effect was observed. Overall, the data available from the study report do not support the hypothesis that decreased postnatal survival observed in the CHIKV VLP-treated group was related to poor maternal behaviour. It is noted that rabbits received up to five more injections than humans, in line with the design recommended for such studies – e.g. ICH S5(R3) indicates that the vaccination regimen should maximize maternal antibody titers and/or immune response throughout the embryonic, foetal, and early postnatal periods. The absence of a similar finding on postnatal survival in the rat DART study is also well-noted and is reassuring but does not allow to rule out any concern highlighted by the rabbit data. Overall, as indicated by the applicant, there was a decreased postnatal survival index in rabbits (but not in rats) whose underlying cause is not known. The wording of SmPC sections 4.6 and 5.3 reflect these findings. In addition, the incidence of splayed limbs and decreased activity was increased compared to concurrent controls in offspring of the treated group on a litter basis (5 vs. 1 and 9 vs. 3, respectively). This finding is reported as being a common background change in rabbits whose exact aetiology is not known precisely; genetic (recessive trait) and environmental (e.g. smooth flooring during early postnatal period) factors have been discussed. Considering also that there was no foetus with malrotated limbs in the embryofoetal arm of the study, it was considered overall that the available data do not suggest a direct treatment-related effect.

In the rat DART study, a total of 22 females per group were used to evaluate potential effects of the CHIKV VLP vaccine on female fertility and postnatal development. On each dosing occasion, animals received an intramuscular injection of either control article or a full human dose of the alum-adjuvanted CHIKV VLP vaccine (0.8 mL/injection). Group 1 control and group 2 treated females were injected intramuscularly at 5 occasions on days 28 and 14 premating, GD 0, GD 14 and LD7. Group 3 treated females were treated intramuscularly with the test article on day 14 premating, GD 0 and GD14. The parameters and endpoints evaluated in maternal animals and offspring were suitable for evaluating any treatment-related effect on postnatal development up to weaning. CHICKV 80% neutralizing antibody titres (NT80) were measured pre-dose on D1 and GD0, and then on HD21 and LD21. A sustained immune response was seen in maternal animals with NT80s peaking on LD21 in maternal animals and with equivalent values in their offspring. These data confirm the rat as an acceptable species for DART evaluation, with transfer of maternal antibodies to offspring.

In group 2, female mating and pregnancy indices (77.3% and 68.2%, respectively) were significantly decreased compared to concurrent controls. They also fell outside of the historical control range (i.e. 95.5-100% and 90.9-100%, respectively, from 9 FEED studies; 100-100% and 95.5-100%, respectively, from 2 PPND studies). This was considered as due to a lower number of group 2 females actually mating as the fertility index was not affected (using the testing facility's definition of these indices). In fact, there were 0 and 5 non-pregnant females with no confirmed mating date in the groups 1 and 2, respectively. The underlying cause for this finding is not known. The applicant indicates that mating was not affected in group 3 animals treated once premating (total 3 doses), considered as more clinically relevant since patients will be injected once. This reasoning can be accepted considering some limitations of the rat study (short mating period and absence of estrous cycle evaluation) and the absence of effects on mating and fertility in rabbits injected twice premating and up to 6 times in total.

No dedicated juvenile toxicity studies were performed, which is in accordance with guidelines (EMA 2008; ICH M3(R2) 2010) and the agreed paediatric plan. This is in line with guidance and was already agreed in the paediatric plan.

Local tolerance was investigated as part of the repeat-dose toxicity and DART studies. No significant adverse findings related to administration of the CHIKV vaccine were observed.

No stand-alone studies on antigenicity have been performed with the vaccine. Immunogenicity was evaluated as part of the primary pharmacodynamic studies and immunogenic response to the vaccine confirmed in toxicological studies.

No stand-alone immunotoxicity studies were performed. Immunotoxicological endpoints (including histology, haematology) were collected as part of the repeat-dose toxicity study; there were no unexpected notable effects observed.

Dependence studies with CHIKV VLP vaccine have not been conducted, since the medicinal product is a vaccine with a well-defined administration schedule. No dependence effect is expected.

Studies on metabolites are not considered relevant for the CHIKV VLP vaccine and thus have not been performed.

No novel adjuvants or excipients were used in the vaccine product. All excipient levels in the product are below the acceptable levels. Residual impurities that result from the DS manufacturing process were evaluated separately and toxicological assessments were conducted. None of the impurities were determined to pose unacceptable risk to people receiving a single or multiple doses of CHIKV VLP vaccine. See section on Description and Composition of the Drug Product for more detailed descriptions of residual impurities and excipient levels, respectively.

No dedicated ERA studies were conducted for CHIKV VLP vaccine, which is acceptable as according to the "Guideline on the environmental risk assessment of medicinal products for human use" - EMEA/CHMP/SWP/4447/00 corr 2 and the "Guideline on Influenza Vaccines - Non-clinical and Clinical Module" (EMA/CHMP/VWP/457259/2014). Vaccines based on recombinant proteins are exempted from the submission of specific ERA studies.

The active substance is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment. Therefore, CHIKV VLP vaccine is not expected to pose a risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

The pharmacology and toxicology studies performed are in line with the WHO and EMA guidelines, supporting the dose, dosing schedule and route of administration of CHIK VLP to humans.

Nonclinical active and passive immunization studies in mouse and monkey conclusively demonstrate that CHIKV VLP vaccination elicits the emergence of CHIKV serum neutralizing antibodies, which are protective against CHIKV-associated disease.

Alum adjuvant in addition to CHIKV VLP caused a significant increase in SNA titers, however a direct significant clinical benefit of the adjuvant cannot be extrapolated from the nonclinical pharmacological studies.

The toxicology studies conducted cover the intended human target dose and include an extended vaccination regimen with up to five vaccine administrations providing additional safety margin. Findings on developmental and reproductive toxicity are reflected in the SmPC sections 4.6 and 5.3.

The nonclinical package is considered acceptable in support of the MAA.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

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

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

- **Tabular overview of clinical studies**

The current application comprises data from 7 completed studies, 2 studies from early clinical development (**VRC 311, VRC 704)** conducted in healthy adults, 3 studies for Phase 2 clinical development **(PXVX-CV-317-001, EBSI-CV-317-002, and EBSI-CV-317-010**) conducted in healthy adults (of which **PXVX-CV-317-001** represents the main dose-finding evidence), and 2 studies for Phase 3 clinical development conducted in healthy adolescents and adults (**EBSI-CV-317-004**) and elderly subjects (**EBSI-CV-317-005**).

Table 10. List of completed clinical studies

Study ID	Number of Study Centres Location(s)	Study Status/Dates Enrolment: Planned / Total	Design Control Type	Study Objectives	Study & Control Drugs Dose, Route & Regimen	No. Participants by Arm Enrolled/ Completed	Duration	Gender M/F Age Mean (SD) [Range]	Main Inclusion Criteria	Primary Immunogenicity/ Efficacy Endpoint(s)
VRC 311[a] (Chang, 2014)	1 site US	Completed 12-Dec-2011 to 04-Mar-2013 25 planned/ enrolled	Dose-escalation, open-label phase 1	Safety, tolerability and immunogenicity	Unadjuvanted VRC CHIKV VLP vaccine (CHKVLP059) 10, 20 or 40 µg CHIKV VLP IM 3 doses at wk 0, 4 and 20	**Total:** 25 enrolled/ 23 completed **10 µg:** 5/5 **20 µg:** 10/10 **40 µg:** 10/8	44 wk	10M/15F 31 (7) [18-47]	Healthy adults (18 to 50 years of age)	CHIKV-specific humoral immune responses (ELISA and neutralizing antibody assays).
VRC 704[a] (Chen, 2020)	6 sites Puerto Rico, Haiti, Dominican Republic, Martinique, Guadeloupe	Completed 18-Nov-2015 to 06-Mar-2018 400 planned/ enrolled	Randomized, multicentre, placebo-controlled, double-blind phase 2	Safety, tolerability and immunogenicity	Unadjuvanted VRC CHIKV VLP vaccine (CHKVLP059)/ placebo 20 µg CHIKV VLP IM 2 doses at wk 0 and 4	**Total:** 400 enrolled/ 394 completed **20 µg:** 201/196 **Placebo:** 199/198	72 wk	**20 µg:** 105M/96F 35 (12) [18-60] **Placebo:** 96M/103F 36 (12) [18-59]	Healthy adults (18 to 60 years of age)	Vaccine-specific immune response by neutralizing antibody assay at wk 8.

Study ID	Number of Study Centres Location(s)	Study Status/Dates Enrolment: Planned / Total	Design Control Type	Study Objectives	Study & Control Drugs Dose, Route & Regimen	No. Participants by Arm Enrolled/ Completed	Duration	Gender M/F Age Mean (SD) [Range]	Main Inclusion Criteria	Primary Immunogenicity/ Efficacy Endpoint(s)
PXVX-CV-317-001[a] (Bennett, 2022)	3 sites US	Completed 18-Apr-2018 to 21-Sep-2020 430 planned/ 415 enrolled (+30 in open-label groups)	Multicentre, parallel group, randomized, double-blind phase 2	Safety, tolerability and immunogenicity	CHIKV VLP vaccine ± adjuvant[b] IM **G1:** 20/0, 2 doses (D1, D29) **G2:** 6/300, 2 doses (D1, D29) **G3:** 10/300, 2 doses (D1, D29) **G4:** 20/300, 2 doses (D1, D29) + 40/300 at D547 **G5:** 6/300, 2 doses (D15, D29) **G6:** 10/300, 2 doses (D15, D29) **G7:** 20/300, 2 doses (D15, D29) **G8:** 40/300, 1 dose (D29) **G9:** 20/300, 2 doses (D1, D29) **G10:** 40/300, 1 dose (D1)	**Total:** 445 enrolled; 367 completed **G1:** 53/42 **G2:** 52/42 **G3:** 51/39 **G4:** 50/41 **G5:** 53/43 **G6:** 53/47 **G7:** 51/46 **G8:** 52/39 **G9:** 20/18 **G10:** 10/10	Up to 790 days	**Total:** 186M/259F 31.5 (7.9) [18-45]	Healthy adults (18 to ≤45 years of age)	GMT of anti-CHIKV neutralizing antibody at Day 57 (28 days after the last injection).
EBSI-CV-317-002	2 sites US	Completed 20-Nov-2019 to 19-Jan-2021 60 planned/ 60 enrolled	Parallel-group, age- and gender-matched, open-label phase 2	Safety, tolerability and immunogenicity	CHIKV VLP vaccine adjuvanted[b] 40/300 µg IM Single dose	**Total:** 60 enrolled; 60 completed (30 with prior alphavirus vaccination, 30 without)	182 days	**Prior alpha:** 20M/10F 47.1 (8.7) [27-64] **Naïve alpha:** 20M/10F 46.9 (8.6) [29-62]	Healthy adults (18 to ≤65 years of age) with prior alphavirus vaccination or not	Anti-CHIKV seroconversion rate[c] at Day 22

Study ID	Number of Study Centres Location(s)	Study Status/Dates Enrolment: Planned / Total	Design Control Type	Study Objectives	Study & Control Drugs Dose, Route & Regimen	No. Participants by Arm Enrolled/ Completed	Duration	Gender M/F Age Mean (SD) [Range]	Main Inclusion Criteria	Primary Immunogenicity/ Efficacy Endpoint(s)
EBSI-CV-317-010	1 site US	Completed 11-Oct-2021 to 05-May-2022 25 planned/ 25 enrolled	Open-label, single-arm phase 2	Safety and immunogenicity	CHIKV VLP vaccine adjuvanted[b] 40/300 µg IM Single dose	25 enrolled; 25 completed	182 days	11M/14F 34.1 (5.8) [25-44]	Healthy adults (18 to 45 years of age)	Anti-CHIKV SNA seroresponse rate[d] at Day 22. Anti-CHIKV SNA GMT at Day 22.
EBSI-CV-317-004	47 sites US	Completed 29-Sep-2021 to 03-Apr-2023 3150 planned/ 3258 enrolled	Randomized, placebo-controlled, double-blind, parallel-group phase 3	Safety, immunogenicity, and clinical lot consistency	CHIKV VLP vaccine adjuvanted[b] or placebo 40/300 µg (*three different lots using in G1-G3 respectively) IM Single dose **G1:** CHIKV VLP vaccine* **G2:** CHIKV VLP vaccine* **G3:** CHIKV VLP vaccine* **G4:** placebo	**Total:** 3258 enrolled; 2902 completed **G1:** 919/803 **G2:** 948/837 **G3:** 927/832 **G4:** 464/430	183 days	**G1-G3:** 1358M/1436F 39 (14.3) [12-64] **Placebo:** 233M/231F 39 (14.4) [12-64]	Healthy adults and adolescents (12 to <65 years of age)	Anti-CHIKV SNA seroresponse rate[e] at Day 22. Anti-CHIKV SNA GMT at Day 22. Anti-CHIKV SNA GMT ratio between all three pairs of vaccine lots at Day 22.
EBSI-CV-317-005	10 sites US	Completed 12-May-2022 to 05-Jun-2023 400 planned/ 413 enrolled	Randomized, placebo-controlled, double-blind, parallel-group phase 3	Safety and immunogenicity	CHIKV VLP vaccine adjuvanted[b] or placebo 40/300 µg IM Single dose	**Total:** 413 enrolled; 388 completed **Vaccine:** 206/200 **Placebo:** 207/188	183 days	**Vaccine:** 81M/125F 71 (5.3) [65-95] **Placebo:** 90M/117F 71 (4.5) [65-84]	Healthy adults (65 years of age and older)	Anti-CHIKV SNA seroresponse rate[e] at Day 22. Anti-CHIKV SNA GMT at Day 22.

CHIKV = chikungunya virus; CHIKV VLP = chikungunya virus virus-like particle; D = day; ELISA = enzyme-linked immunosorbent assay; F = female; G = group; GMT = geometric mean titre; IM = intramuscular; ISE = integrated summary of efficacy; M = male; SD = standard deviation; SNA = serum neutralizing antibody; TBD = to be determined; US = United States of America; VRC = Vaccine Research Centre; wk = week.

[a] Data not included in the ISE.
[b] Doses shown as µg CHIKV VLP/µg aluminium as aluminium hydroxide adjuvant.
[c] Defined as a 4-fold rise over baseline in anti-CHIKV neutralizing antibodies.
[d] The analysis of this study was completed assuming a seroresponse rate of ≥40. Subsequently, a more conservative SNA titre of ≥100 was used for the ISE analyses.
[e] Seroresponse rate (considered the presumptive seroprotection rate) was defined as the percentage of participants who achieve an anti-CHIKV SNA titre ≥100.

Moreover, the Applicant plans to conduct a post-approval effectiveness study (EBSI-CV-317-007) for which a clinical study protocol (Version 4.0, dated 22 Jan 2025) has been provided. It is referred to respective section in this regard.

The CHIKV VLP vaccine was in-licensed by PaxVax Inc. from the US National Institutes of Health (NIH) Vaccine Research Centre (VRC), which initiated the development of an unadjuvanted formulation of CHIKV VLP vaccine (designated CHKVLP059 at that time). PaxVax, Inc. was acquired by Emergent BioSolutions, Inc. in October 2018, and the vaccine (designated PXVX0317 at that time) was further developed under a fully owned subsidiary, Emergent Travel Health, Inc. On 15-May-2023, Bavarian Nordic A/S acquired the CHIKV VLP vaccine development program from Emergent Travel Health, Inc. As the new sponsor, Bavarian Nordic, A/S assumed responsibility for the completion of the Phase 3 studies and for all planned CHIKV VLP vaccine clinical studies.

Consequently, several formulations were used during the clinical development, as depicted in Table 20

Table 11. Formulations used during the clinical development program of CHIK VLP vaccine

Formulation (Company code)	Doses	Sponsor	Clinical Studies
Formulation 1 (CHKVLP059)	10, 20 or 40 µg doses of unadjuvanted CHIKV VLP	NIH VRC	VRC 311 (phase 1) VRC 704 (phase 2)
Formulation 2 (PXVX0317)	6, 10, 20 or 40 µg[a] CHIKV VLP adjuvanted[b] with aluminum hydroxide; supplied as a sterile aqueous buffered solution filled into 3 mL single-dose glass vials with a 0.8 mL fill volume. Field formulated[c]	PaxVax/ Emergent	PXVX-CV-317-001 (phase 2) EBSI-CV-317-002 (phase 2) EBSI-CV-317-010 (phase 2)
Formulation 3 (PXVX0317)	40 µg CHIKV VLP adjuvanted with aluminum hydroxide; supplied as a single ready-to-use dose in a 1 mL glass pre-filled syringe, with 0.85 mL of vaccine to allow 0.8 mL delivered dose volume. Pre-filled syringe	Emergent/ Bavarian Nordic	EBSI-CV-317-004 (phase 3) EBSI-CV-317-005 (phase 3)

CHIKV = chikungunya virus; NIH = National Institutes of Health; VLP = virus-like particle; VRC = Vaccine Research Center.

[a] The 6, 10, and 20 µg doses were only used in study PXVX-CV-317-001.

[b] An unadjuvanted formulation of CHIKV VLP vaccine (PXVX0317) was also used in study PXVX-CV-317-001 as reference group (Group 1).

[c] Field formulated with three components: CHIKV VLP, diluent as required for each dose level, and aluminum hydroxide. These three components were combined in prespecified volumes to achieve the formulations and different dose levels.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Consistent with the WHO *Guidelines on Nonclinical Evaluation of Vaccines (WHO TRS N°927)*, the EU *Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1)* and US Guidance Documents such as *Development and Licensure of Vaccines to Prevent COVID-19 (Oct 2023)*, traditional pharmacokinetic studies, including biodistribution studies, have not been conducted with CHIKV VLP vaccine in humans as the kinetic properties of vaccines do not provide information to establish dosing.

2.6.2.2. Pharmacodynamics

The pharmacodynamic profiles of vaccines are defined by their immunogenicity, as stated in the WHO *Guidelines on Nonclinical Evaluation of Vaccines (WHO TRS N°927)*, the EU *Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1)* and US Guidance Documents such as *Development and Licensure of Vaccines to Prevent COVID-19 (Oct 2023)*. The safety and immunogenicity studies conducted with CHIKV VLP vaccine assessed the dose-response relationship and informed on optimal dose and dosage schedule by characterizing the immune response. Immunogenicity data are presented in the Efficacy Section.

Mechanism of action

Vimkunya is an adjuvanted VLP recombinant protein vaccine. The exact mechanism of protection against CHIKV infection and/or disease has not been determined. It is thought that Vimkunya can induce protection from CHIKV infection and thus prevent disease by eliciting antibodies against the viral proteins C, E1, and E2 contained in Vimkunya resulting in neutralisation of the infective activity of live virus. C is the capsid protein, E1 is a class II fusion protein that mediates membrane fusion during virus infection of cells, and E2 is a type I transmembrane glycoprotein responsible for receptor binding to cells during viral replication (Bréhin et al., Virology. 2008). E2 has been shown to be the primary target of CHIKV neutralizing antibodies produced as a result of natural infection (Kam et al., J Virol. 2012). An adjuvant is added to increase the magnitude of vaccine-mediated immune responses.

There is no established immunological correlate of protection against disease caused by CHIKV and the level of protection afforded by the CHIKV VLP vaccine is unknown. Clinical efficacy is inferred from a surrogate immunogenicity parameter in form of an anti-CHIKV SNA titre of at least 100 using the CHIKV-luc NT_{80} assay that can be considered as reasonably likely to predict protection (presumptive seroprotection threshold titre). The evidence to support this titre threshold stems from a prospective sero-epidemiologic study (published by Yoon et al., PLoS Negl Trop Dis. 2015) in subjects with prior exposure to CHIKV and from a non-human primate passive transfer/challenge study (PAS-NHP-CHIK-003) using pooled sera from humans vaccinated with CHIKV VLP vaccine and recommended in the final CHMP SA (EMA/SA/0000115136).

Bioanalytical methods

The bioanalytical methods used to analyse the humoral immune response induced by the CHIKV VLP vaccine are listed in the following table.

Table 12 Summary of bioanalytical methods used to analyse the humoral immune response induced by the CHIKV VLP vaccine

Method	Sponsor Study No.
Fit-for-use Human SNA Assay with (SFV)-CHIKV-GFP reporter virus	VRC 311
Fit-for-use Human SNA Assay with CHIKV-*luc* reporter virus	VRC 311
	VRC 704
Qualified Human SNA Assay with CHIKV-*luc* reporter virus	PXVX-CV-317-001
	EBSI-CV-317-002
	EBSI-CV-317-010
Validated Human SNA Assay with CHIKV-*luc* reporter virus	EBSI-CV-317-004
	EBSI-CV-317-005

Method	Sponsor Study No.
VRC Human IgG Titer ELISA using CHIKV strain 37997 VLP coated plates	VRC 311
CHIKjj DetectTM IgG ELISA	PXVX-CV-317-001
CHIKjj DetectTM IgG ELISA	PXVX-CV-317-010
CHIKjj DetectTM IgM ELISA	PXVX-CV-317-001
	PXVX-CV-317-010
Anti-CHIKV IgG endpoint ELISA	EBSI-CV-317-010
	EBSI-CV-317-002
Anti-CHIKV IgM endpoint ELISA	EBSI-CV-317-010
	EBSI-CV-317-002
Alphavirus PRNTs	EBSI-CV-317-002

CHIKV = chikungunya virus, ELISA = enzyme-linked immunosorbent assay, IgG = immunoglobulin G, IgM = immunoglobulin M, PRNT = plaque reduction neutralization test, SFV = Semliki Forest virus, SNA = serum neutralizing antibody, SOP = standard operating procedure

The serum neutralizing antibody response against Chikungunya virus (CHIKV) served as the primary measure of vaccine efficacy. Serum neutralising antibodies against CHIKV were quantitated by a CHIKV-*luc* based neutralisation assay in a 96-well plate format. Serial dilutions of the serum samples are incubated in triplicate with a fixed amount of recombinant CHIKV-*luc* (strain 181/25 engineered to express firefly luciferase) and subsequently inoculated onto Vero cells. The luciferase reporter gene activity correlates with the level of virus infection. Upon addition of luciferin reagent, the resulting luminescence is quantitated using a luminometer. The neutralising antibody titre 80% (NT_{80}) is determined by linear regression analysis and expressed as the reciprocal of the serum dilution conferring 80% reduction of luciferase activity. Assay controls include a virus control per plate (VC, virus only no serum) and cell control (CC, no virus no serum), positive human serum controls, and a negative human serum control per assay run.

The assay was qualified for testing of samples from pre-clinical and early clinical studies (VRC 311 and VRC 704) and subsequently for use in subsequent Phase 2 clinical studies. Prior to testing of samples from Phase 3 studies, the assay was validated. The following performance characteristics of the SNA assay were assessed during method validation:

Table 13. Summary of validation parameters, acceptance criteria and results

Parameter	Experimental Design	Acceptance Criteria	Results	Evaluation
Intra-Assay Precision (Repeatability)	6 positive anti-CHIKV human serum samples covering the titer range of the assay were tested 9 times in the same run. Two technicians tested 1 of the 6 samples on each of three days.	Overall intra-assay precision CV ≤25%	Overall intra-assay precision CV=13.6%	Pass
Inter-Assay Precision (Intermediate Precision)	35 anti-CHIKV human serum samples covering the titer range of the assay were tested a total of 8 times (4 separate days by 2 technicians per day)	Overall inter-assay precision CV ≤35%	Overall inter-assay precision CV=20.4%	Pass
Dilutional Linearity	3 positive anti-CHIKV human serum samples were tested undiluted and diluted in negative human serum (2.5-fold dilutions across the range of the assay with last titer expected to be negative) in duplicate (2 separate days by a different technician per day)	90% CI of the slope of 1st order linear regression analysis of log transformed titers between -1.3 and -0.7. R^2 ≥95%. Each sample was independently analyzed.	All 90% CIs of slopes are within -1.3 and -0.7 Minimal R^2=0.989 ≥0.95 (95%)	Pass
LoD/LLoQ	10 low anti-CHIKV human serum samples (titers between 10 and 100) and 10 negative human serum samples were tested a total of 8 times (4 days by 2 technicians per day) as part of Inter-Assay Precision. In addition, dilutions at or near the lowest reportable titer of 10 were included as part of Linearity.	LoD is the lowest titer in which 95% of the tests resulted in a reportable titer >10. LLoQ is the lowest titer that meets acceptance criteria for both intermediate precision and linearity and is at or above the LoD.	LoD=10 100% of negative samples yielded titers <10 100% of positive samples yielded titers >10 LLoQ=15 For samples with NT_{80} >15, max CV was 31.2% (<35%) and passed linearity	Pass
Robustness (Freeze/Thaw)	3 positive anti-CHIKV human serum samples were freeze/thawed 1, 2, 3, and 4 times and tested on 2 separate days by a different technician per day.	90% CI of the ratio of each test condition to the reference condition (a single freeze/thaw) is within 0.7 to 1.4.	All 90% CIs of ratios for freeze/thaws 2 to 4 to a single freeze/thaw within 0.7 and 1.4.	Pass
Robustness (Sample Stability)	3 positive anti-CHIKV human serum samples were maintained at room temperature overnight, and 2-8°C for 7, 14, and 28 days and tested on 2 separate days by a different technician per day along with the reference condition (a sample thawed and used within 3 hours).	90% CI of the ratio of each test condition to the reference condition is within 0.7 to 1.4.	All 90% CI of ratios for all test conditions to normal (reference) within 0.7 and 1.4.	Pass

Additional validation experiments were performed to further assess assay precision. Based on the results for 10 very high titre human serum samples, the ULOQ of the assay was determined.

Assay accuracy was characterized prior to assay qualification by testing clinical serum samples from the VRC 311 phase 1 clinical trial side-by-side on 3 different assay platforms (CHIKV SNA, SFV-CHIKV-GFP flow cytometry-based method, PRNT). Results of the comparison demonstrated that the human SNA Assay was comparable to both the PRNT and SFV-CHIKV-GFP methods.

Regarding specificity, the ability to detect unequivocally the target pathogen in the assay, was shown in pre-qualification experiments and following qualification of the assay where CHIKV-specific antibody titers were only detectable after vaccination with CHIKV VLP vaccine and were undetectable in pre-immune sera.

Matrix interference (selectivity) of haemolysed serum, lipemic serum, and plasma samples as well as further robustness with regard to incubation times (neutralisation, infection, Steady-Glo) was evaluated in addenda to the method validation

Enzyme-linked immunosorbent assays (ELISA) and plaque reduction neutralization assays (PRNT) were used to support secondary and exploratory study endpoints. The ELISA methods were used for qualitative or quantitative determination of IgG or IgM directed against CHIKV. The neutralising antibody responses against other CHIKV genotypes and other alphaviruses was analysed using different PRNT assays adapted to the different viruses.

2.6.3. Discussion on clinical pharmacology

Pharmacokinetic

According to the Guideline on Clinical Evaluation of New Vaccines (EMEA/CHMP/VWP/164653/2005), "*pharmacokinetic studies are usually not required for vaccines. However, such studies might be applicable when new delivery systems are employed or when the vaccine contains novel adjuvants or excipients"*. Since neither of those apply (no new delivery system is applied and the aluminium hydroxide adjuvant is well-established), pharmacokinetic studies are indeed not required.

Pharmacodynamic

According to the Guideline on Clinical Evaluation of New Vaccines (EMEA/CHMP/VWP/164653/2005), *"in relation to vaccines, pharmacodynamic studies are essentially comprised of the immunogenicity studies that characterise the immune response to the vaccine"*. The design and outcome of these studies are described in the clinical efficacy section.

Bioanalytical methods

Serum neutralising antibodies (SNA) against CHIKV were quantitated by a CHIKV based neutralisation assay on Vero cells and reported as NT_{80}. The principal acceptability of this method for determination of vaccine efficacy has been discussed in previous EMA Scientific Advice. A detailed method description as well as detailed qualification and validation reports have been submitted. The implemented assay controls are considered appropriate to ensure validity of each analytical run and assay plate.

For testing of samples from Phase 2 studies, the SNA assay was qualified. Prior to testing the samples from the pivotal Phase 3 studies EBSI-CV-317-004 and EBSI-CV-317-005 the SNA assay has been validated at the testing site for the samples of the pivotal Phase 3 studies.

Method validation covers the performance characteristics intra-/inter-assay precision, dilutional linearity, LoD, LLOQ, and ULOQ, as well as robustness in terms of freeze/thaw and storage stability of samples at RT or 2-8°C. Matrix interference by haemolytic or lipemic samples as well as robustness regarding incubation times was evaluated in validation addenda. An additional study investigated long term stability of clinical samples when stored at -80°C. The validation samples are considered sufficiently representative for the study samples and covered the analytical range. Acceptable precision and linearity were demonstrated over the specific analytical range. Haemolysis was demonstrated to have no effect on sample analysis. For low titre samples, significant interference by highly lipemic samples was observed. However, for samples with less than 50% lipaemia the level of interference was

limited and acceptable. Method performance during validation and preceding qualification was comparable.

Two important performance parameters, i.e. specificity and accuracy, were not investigated during method qualification or validation. Concerning **specificity,** the Applicant´s position that specificity is demonstrated by pre-qualification experiments and by testing of Phase 2 samples where CHIKV-specific neutralising antibodies were only detectable after vaccination with CHIKV VLP vaccine can be followed. Regarding **accuracy,** it is acknowledged that no international standard was available at time of validation. The Applicant´s approach to show accuracy by testing more than 100 samples from clinical studies side-by-side using three different assay platforms (CHIKV-*luc* SNA, PRNT, and SFV-CHIKV-GFP flow cytometry format) is acceptable. The results show high concordance and correlation between the different platforms (please refer to the pre-clinical AR). The 1st World Health Organization International Standard reference for anti-CHIKV immunoglobulin G 1502/19 was tested in the Applicant´s CHIKV SNA assay during development of the WHO IS, as part of the WHO Collaborative Study WHO/BS/2022.2434 to evaluate a candidate WHO IS for antibodies to Chikungunya Virus. More recently, the Applicant started to generate a secondary working standard that is calibrated against the WHO IS and that will be used for further clinical testing. The Applicant´s efforts regarding standardisation of the SNA against the WHO IS by implementation of the secondary working standard are highly endorsed and in line with Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1). Short-term sample stability, long term sample stability at -80°C throughout the storage period of clinical samples, and the impact of freeze/thaw cycles has been satisfactorily investigated.

In addition to the SNA assay, several ELISA methods and PRNT assays were used to support analysis of immunogenicity endpoints. No information on their performance characteristics is presented. Considering that the ELISA assays were not used in the pivotal Phase 3 studies and do not support any conclusions regarding vaccine efficacy no queries are raised on the ELISA methods. Regarding the PRNT assays used to analyse cross-reactivity against other CHIKV strains and other alphaviruses, performance characteristics like specificity, accuracy, precision, dilutional linearity, and assay range have not been formally evaluated. Considering that the assays were used to support secondary/exploratory endpoints in an uncontrolled Phase 2 study this can be accepted. However, relevant controls (i.e. virus control, negative serum control, positive serum control) were included during testing. For the VEEV $PRNT_{80}$ assay that supported a secondary endpoint the controls were evaluated against acceptance criteria. A retrospective evaluation of the performance of the assay controls has been provided. Overall, the observed inter-assay precision is in line with the variability that can be expected for such assays. In conclusion, the assays appear sufficiently controlled and suitable for the intended purpose. For supporting secondary endpoints in future studies, it is recommended to further evaluate relevant performance characteristics of the method.

2.6.4. Conclusions on clinical pharmacology

Overall, the CHIKV-luc based SNA, which was used to measure CHIKV-neutralising antibody titres in human serum as surrogate for determination of vaccine efficacy, is deemed suitable for its intended purpose and has been considered acceptable in previous SAs.

For additional immunogenicity assays, no information on assay performance characteristics has been provided. Considering that the ELISA assays were not used in the pivotal Phase 3 studies and do not support any conclusions regarding vaccine efficacy no queries are raised on the ELISA methods. Regarding the PRNT assays used to analyse cross-reactivity against other CHIKV strains and other alphaviruses, only limited information on method control and performance is available. However, the assays appear sufficiently controlled and suitable for the intended purpose.

Since no new delivery system is applied and the aluminium hydroxide adjuvant is well-established, pharmacokinetic studies are indeed not required. Concerning pharmacodynamic studies, it is referred to the clinical efficacy section.

2.6.5. Clinical efficacy

2.6.5.1. Dose response

The main dose finding evidence is provided by study PXVX-CV-317-001. Two studies from early clinical development (VRC 311, VRC 704) conducted in healthy adults with the 1st formulation are also referred in this section for historical reasons. VRC 311 and VRC 704 are further detailed in supportive studies section.

Study PXVX-CV-317-001

Study design & methods

Study PXVX-CV-317-001 was a multi-centre, parallel-group, randomized, double-blind, Phase 2 study, conducted in the US, to determine formulation (with or without aluminium hydroxide adjuvant), dose (6, 10, 20 or 40 µg) and schedule (variations of 1, or 2 IM doses, or placebo, given at Days 1, 15, or 29, or boost dose at Day 547) of the CHIKV VLP vaccine (designated PXVX0317) for further development with regard to safety, tolerability, and immunogenicity in healthy adults 18 to ≤45 yoa. At least 430 subjects were planned to be enrolled who met the eligibility criteria. A total of 415 subjects were randomized and an additional 30 subjects were added in the open-label groups.

Table 14 Study Treatment Groups

Treatment	N	IM Administered Dose of VLP in µg/Alhydrogel in µg or Diluent Placebo Control			
		Day 1	Day 15	Day 29	Day 547
Group 1	50	20/unadjuvanted	Placebo	20/unadjuvanted	N/A
Group 2	50	6/300	Placebo	6/300	N/A
Group 3	50	10/300	Placebo	10/300	N/A
Group 4	50	20/300	Placebo	20/300	40/300
Group 5	50	Placebo	6/300	6/300	N/A
Group 6	50	Placebo	10/300	10/300	N/A
Group 7	50	Placebo	20/300	20/300	N/A
Group 8	50	Placebo	Placebo	40/300	N/A
Group 9[1]	20	20/300	N/A	20/300	N/A
Group 10[1]	10	40/300	N/A	N/A	N/A
Total	**430**				

N, number of subjects; N/A, not applicable; IM, intramuscular; VLP, virus-like particle.
[1]Open label group.

Study duration

For Groups 1–9, the study consists of a 30-day screening period, a treatment and observation period from Day 1 through Day 57 and:

- For Groups 2, 3, 5, 6, and 7: a follow-up period through Day 365

- For Groups 1 and 8: a follow-up period through Day 760 without receiving a boost dose at Day 547
- For Group 4: a follow-up period through Day 760 after receiving a boost dose of 40/300 µg at Day 547
- For Group 9: a follow-up period through Day 182

For Group 10, the study consists of a 30-day screening period, a treatment and observation period from Day 1 through Day 22 and safety follow-up through Day 29.

The maximum possible study duration for an individual subject was 790 days.

Selection of Doses in the Study

The dose of CHIKV VLP in this Phase 2 study ranged from 6 µg to 40 µg. These doses are below or approximately equivalent to those used previously in the supportive early development NIH studies VRC 311 and VRC 704 (refer to supportive studies).

The Alhydrogel dose of 300 µg was within the range of doses of alum adjuvants used in many licensed vaccines, including Engerix-B. This dose also creates a concentration ratio to CHIKV VLP that achieves high (~90%) levels of adsorption, thought to enhance both immunogenicity and short-term stability. The IM route of administration in this study was consistent with the NIH clinical studies VRC 311 and VRC 704. Based on the findings from those studies and the addition of Alhydrogel, these doses were expected to be safe and immunogenic.

The Group 9 dose of 20 µg CHIKV VLP adjuvanted with 300 µg Alhydrogel on Days 1 and 29 was selected following the interim data analysis based on all Group 1 to Group 8 doses and their impact on subject seroconversion rates (at Days 29 and 57), GMT levels, and safety-related events.

The Group 10 single dose of 40 µg CHIKV VLP adjuvanted with 300 µg Alhydrogel on Day 1 was selected for future development following the Day 182 interim data analysis based on all Group 1 to Group 8 doses and their impact on subject seroconversion rates, GMT levels, and safety-related events.

The boost dose for Group 4 was 40 µg CHIKV VLP adjuvanted with 300 µg Alhydrogel, based on the final selected dose for the PXVX0317 development program.

Sampling Time Points

Neutralizing antibody levels were assessed using the CHIKV-luc NT80 assay at Day 1 (pre-vaccination) and at Days 8, 15, 22, 29, 36, and 57 (Group 9 did not have a Day 15 or Day 22 visit and Group 10 did not have a Day 36, Day 57, and 182 visit). For Groups 1 through 8, follow-up visits were performed, and blood was collected at Days 182 and 365. For Group 9 follow-up visit was only performed on Day 182. For Groups 1, 4, and 8, additional follow-up visits and blood was collected at Days 547 and 760. For the booster dose group 4, neutralizing antibody levels were also assessed at Day 575 post boost. Antibodies were also assessed by a licensed anti-CHIKV ELISA at Day 1 (pre-vaccination) and Day 57 for Groups 1 to 9.

Data Sets Analysed

Table 15. Overview of Analysis Dataset Populations (All Subjects)

Disposition	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10	Total
All Screened Subjects											512
Randomized Subjects	53	52	51	50	53	53	51	52	20	10	445
Exposed[1]	53 (100)	52 (100)	50 (98.0)	51 (102)	53 (100)	53 (100)	51 (100)	52 (100)	20 (100)	10 (100)	445 (100)
Safety[2]	53 (100)	52 (100)	50 (98.0)	51 (102)	53 (100)	53 (100)	51 (100)	52 (100)	20 (100)	10 (100)	445 (100)
mITT[3]	53 (100)	51 (98.1)	51 (100)	50 (100)	53 (100)	53 (100)	50 (98.0)	52 (100)	20 (100)	10 (100)	443 (99.6)
IE[4]	47 (88.7)	44 (84.6)	44 (86.3)	45 (90.0)	48 (90.6)	48 (90.6)	47 (92.2)	50 (96.2)	20 (100)	10 (100)	403 (90.6)
IE Boost[5]	N/A	N/A	N/A	39 (78.0)	N/A	N/A	N/A	N/A	N/A	N/A	39 (8.8)
Safety Boost[6]	N/A	N/A	N/A	42 (84.0)	N/A	N/A	N/A	N/A	N/A	N/A	42 (9.4)

N/A = not applicable.
Note: Percentages are based on the number of randomized subjects in each treatment arm.
[1]Randomized subjects who received at least one study vaccination. Subject 33-148 was randomized in Group 3 but received the vaccination in Group 4 due to the dispensing error.
[2]Exposed subjects who provide at least 1 safety assessment data.
[3]Modified Intent-to-Treat. It includes the randomized subjects who were treated and have evaluable immunogenicity results for baseline (Day 1) and at least 1 on-study sample.
[4]Immunogenicity Evaluable Population includes correctly treated subjects with evaluable serum sample results at Day 1 and Day 57 who have no major protocol deviation.
[5]Immunogenicity Evaluable Boost Population includes subjects who are in IE Population and received a Day 547 vaccination in Group 4.
[6]Subjects who are in safety population and received a Day 547 vaccination in Group 4.

Objectives

Primary Safety Objective

- The primary safety objective was to evaluate the safety and tolerability of eight formulation/schedule combinations of PXVX0317 in healthy adults and to select one or more formulation(s) for further vaccine development. Safety was assessed by measuring the incidence of local and systemic solicited adverse events (AEs), unsolicited AEs, and serious adverse events (SAEs).

Primary Immunogenicity Objective

- The primary immunogenicity objective was to assess the induction of anti-CHIKV neutralizing antibody responses by different formulations and schedules, as measured at 28 days after the last injection (Day 57).

Immunogenicity results

Only results of key immunogenicity endpoints are presented here below.

Primary immunogenicity endpoint: The primary immunogenicity endpoint was the GMT of anti-CHIKV neutralizing antibody determined by CHIKV-luc NT80 luciferase-based assay at Day 57 (28 days after the last injection).

Table 16. Geometric Mean Titers (NT80) of Neutralizing Antibodies Against CHIKV at Day 57 (Immunogenicity Evaluable Population)

Study Day Statistic	Group 1 (N=47)	Group 2 (N=44)	Group 3 (N=44)	Group 4 (N=45)	Group 5 (N=48)	Group 6 (N=48)	Group 7 (N=47)	Group 8 (N=50)	Group 9 (N=20)
Day 57									
N	47	44	44	45	48	48	47	50	20
GMT	2057.0	1116.2	1465.3	2023.8	920.1	1206.9	1562.8	1712.5	4197.4
95% CI	[1584.8, 2670.0]	[852.5, 1461.4]	[1119.1, 1918.4]	[1550.5, 2641.7]	[710.9, 1190.9]	[932.4, 1562.2]	[1204.1, 2028.3]	[1330.0, 2205.0]	[2515.8, 7003.2]
P-value	N/A	0.0015	0.0761	0.9317	<0.0001	0.0045	0.1437	0.3216	N/A

GMT=geometric mean titer; NA=not applicable.

Secondary immunogenicity endpoint: Geometric Mean Titre of anti-CHIKV neutralizing antibody determined by CHIKV-luc NT80 at all time points other than Day 57.

Table 17. Geometric Mean Titers (SNA) of Neutralizing Antibodies Against CHIKV Over Time (Immunogenicity Evaluable Population)

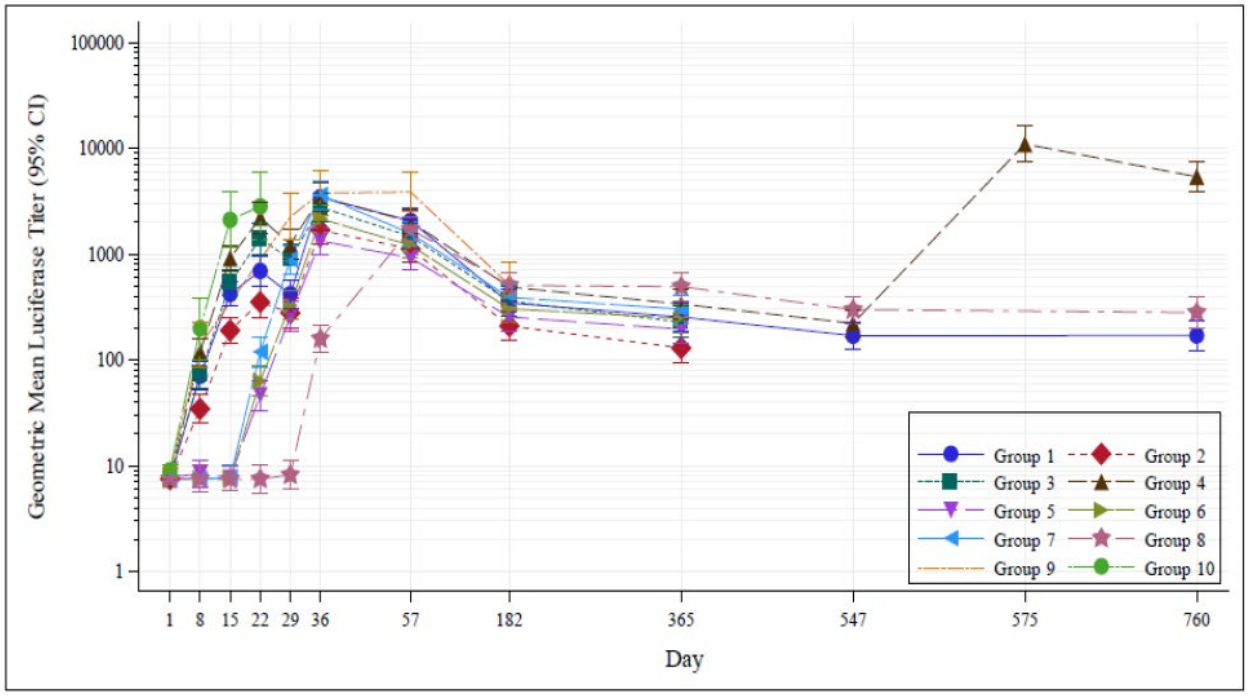


Table 18. Geometric Mean Titers (NT80) of Neutralizing Antibodies Against CHIKV (Immunogenicity Evaluable Population)

Study Day Statistic	Group 1 (N=47)	Group 2 (N=44)	Group 3 (N=44)	Group 4 (N=45)	Group 5 (N=48)	Group 6 (N=48)	Group 7 (N=47)	Group 8 (N=50)	Group 9 (N=20)	Group 10 (N=10)
Day 1										
N [a]	47	44	44	45	48	48	47	50	20	10
GMT [b]	7.5	7.5	7.5	7.5	7.8	7.5	7.5	7.5	8.6	9.0
95% CI [b]	[7.3, 7.7]	[7.3, 7.7]	[7.3, 7.7]	[7.3, 7.7]	[7.6, 8.0]	[7.3, 7.7]	[7.3, 7.7]	[7.3, 7.7]	[6.5, 11.5]	[6.0, 13.6]
P-value [b]		0.9875	0.9742	0.9831	0.0483	0.9829	0.9872	0.9745	NA	NA
Day 8										
N [a]	47	43	44	44	47	47	47	49	20	10
GMT [b]	70.6	34.3	72.6	117.6	8.4	7.5	7.5	7.5	131.1	189.4
95% CI [b]	[53.2, 93.7]	[25.6, 46.1]	[54.2, 97.2]	[87.8, 157.4]	[6.3, 11.1]	[5.7, 10.0]	[5.7, 10.0]	[5.7, 9.9]	[63.8, 269.6]	[47.6, 753.2]
P-value [b]		0.0006	0.8957	0.0140	<0.0001	<0.0001	<0.0001	<0.0001	NA	NA
Day 15										
N [a]	47	44	44	45	48	48	47	50	NA	10
GMT [b]	419.3	189.4	530.2	912.7	7.7	7.5	7.7	7.5	NA	1946.1
95% CI [b]	[323.6, 543.4]	[144.9, 247.6]	[405.6, 693.0]	[700.4, 1189.5]	[6.0, 10.0]	[5.8, 9.7]	[5.9, 9.9]	[5.8, 9.6]	NA	[524.9, 7215.7]
P-value [b]		<0.0001	0.2167	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NA	N/A
Day 22										
N [a]	47	44	44	45	48	48	47	50	NA	10
GMT [b]	691.5	351.3	1400.2	2198.1	46.4	63.1	119.0	7.5	NA	2687.3
95% CI [b]	[499.2, 957.9]	[250.9, 492.0]	[999.9, 1960.8]	[1575.6, 3066.5]	[33.6, 64.1]	[45.7, 87.1]	[85.9, 164.9]	[5.5, 10.3]	NA	[815.9, 8851.6]
P-value [b]		0.0047	0.0033	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NA	NA
Day 29										
N [a]	47	44	44	45	48	48	47	50	20	NA
GMT [b]	417.2	277.3	890.5	1238.1	258.0	328.8	886.2	8.2	2014.7	NA
95% CI [b]	[305.4, 570.0]	[200.9, 382.8]	[645.1, 1229.2]	[900.2, 1702.9]	[189.5, 351.3]	[241.5, 447.6]	[648.7, 1210.5]	[6.0, 11.0]	[1102.6, 3681.5]	NA
P-value [b]		0.0743	0.0010	<0.0001	0.0319	0.2863	0.0009	<0.0001	NA	NA
Day 36										
N [a]	47	44	44	45	48	48	46	50	20	NA
GMT [b]	3442.8	1675.8	2772.4	3435	1327.7	2155.4	3609.0	157.9	4191.7	NA
95% CI [b]	[2533.9, 4677.8]	[1220.9, 2300.2]	[2019.8, 3805.4]	[2511.4, 4698.1]	[980.4, 1797.9]	[1591.6, 2919.0]	[2647.7, 4919.3]	[117.3, 212.6]	[2661.8, 6601.1]	NA
P-value [b]		0.0014	0.3346	0.9918	<0.0001	0.0333	0.8317	<0.0001	NA	NA
Day 57										
N [a]	47	44	44	45	48	48	47	50	20	NA
GMT [b]	2057.0	1116.2	1465.3	2023.8	920.1	1206.9	1562.8	1712.5	4197.4	NA
95% CI [b]	[1584.8, 2670.0]	[852.5, 1461.4]	[1119.1, 1918.4]	[1550.5, 2641.7]	[710.9, 1190.9]	[932.4, 1562.2]	[1204.1, 2028.3]	[1330.0, 2205.0]	[2515.8, 7003.2]	NA
P-value [b]		0.0015	0.0761	0.9317	<0.0001	0.0045	0.1437	0.3216	NA	NA
Day 182										
N [a]	46	40	39	43	44	46	47	48	18	NA
GMT [b]	345.1	209	358.4	486.6	253.4	303.0	388.1	505.7	405.3	NA
95% CI [b]	[258.1, 461.3]	[153.1, 285.2]	[261.6, 491.2]	[360.5, 656.9]	[188.4, 340.9]	[226.7, 405.0]	[291.3, 517.1]	[380.7, 671.8]	[235.2, 698.4]	NA
P-value [b]		0.0211	0.8619	0.1062	0.1444	0.5334	0.5715	0.0649	NA	NA

Day 365										
N [a]	42	38	36	42	41	43	43	46	NA	NA
GMT [b]	243.4	137.5	226.7	336.6	195.2	246.9	302.0	491.7	NA	NA
95% CI [b]	[176.8, 334.9]	[98.3, 192.3]	[160.6, 320.0]	[244.6, 463.2]	[141.3, 269.6]	[180.1, 338.4]	[220.3, 414.0]	[362.5, 667.0]	NA	NA
P-value [b]		0.0159	0.7668	0.1585	0.3395	0.9502	0.3448	0.0019	NA	NA
Day 547‡										
N [a]	41	NA	NA	41	NA	NA	NA	40	NA	NA
GMT [b]	169.2	NA	NA	215.7	NA	NA	NA	297.9	NA	NA
95% CI [b]	[126.2, 226.8]	NA	NA	[160.9, 289.1]	NA	NA	NA	[221.4, 400.8]	NA	NA
P-value [b]		NA	NA	0.2484	NA	NA	NA	0.0082	NA	NA
Day 575†										
N [a]	NA	NA	NA	39	NA	NA	NA	NA	NA	NA
GMT [b]	NA	NA	NA	10941.1	NA	NA	NA	NA	NA	NA
95% CI [b]	NA	NA	NA	[7378.0, 16225.1]	NA	NA	NA	NA	NA	NA
P-value [b]		NA	NA	NA	NA	NA	NA	NA	NA	NA
Day 760‡										
N [a]	40	NA	NA	39	NA	NA	NA	38	NA	NA
GMT [b]	169.8	NA	NA	5365.1	NA	NA	NA	279.7	NA	NA
95% CI [b]	[122.4, 235.5]	NA	NA	[3853.2, 7470.1]	NA	NA	NA	[200.1, 391.1]	NA	NA
P-value [b]		NA	NA	<0.0001	NA	NA	NA	0.0369	NA	NA

Note: Values < the LLOQ (15) were assigned the value LLOQ/2 (=7.5). NA = Not Applicable; GMT=geometric mean titer.
[a] N Analyzable is the number of subjects with an available sample result at the indicated visit.
[b] Geometric mean titer estimates, together with their 95% CIs and the p-values, are derived from ANOVA model that includes site and treatment group as fixed effects assuming normality of the log titers. Comparisons of Group 1 vs Groups 2-8, respectively, are shown. No adjustment for Groups 9 and 10. Their geometric mean estimates and 95% CIs are calculated from Proc Univariate.
All doses of PXVX0317 were adjuvanted, unless otherwise specified.
† Participants in group 4 received a booster dose (40 µg PXVX0317) on day 547 (18 months after the first vaccination). Day 575 assessment represents 28 days after the booster dose of PXVX0317.
‡ Only participants in groups 1, 4, and 8 were followed through day 760.

Secondary immunogenicity endpoint: The GMTs and GMFIs (relative to Day 547 for Group 4 only) based on antibody titers measured at all other protocol-specified time points from before to after boost dose.

Table 19. Geometric Mean Titre and Fold Increase (Pre-Boost to Post-Boost) in Luciferase Titre against CHIKV (IEP)

	Group 4 (N=39)	
Study Day Statistics	**Actual Value**	**Fold Increase**
Day 547		
N Analyzable [a]	39	
Geometric Mean [b]	223.6	
95% CI [b]	[166.7, 299.9]	
Day 575		
N Analyzable [a]	38	38
Geometric Mean [b]	12325.8	54.6
95% CI [b]	[8951.4, 16972.4]	[34.9, 85.4]
Day 760		
N Analyzable [a]	38	38
Geometric Mean [b]	5874.2	26.0
95% CI [b]	[4269.4, 8082.3]	[17.1, 39.5]

Note: Values < the LLOQ (15) were assigned the value LLOQ/2 (=7.5) for actual value of Titers and the value LLOQ (=15) for Folder Increase calculation.
Note: The fold increase at this table is compared to Day 547.
[a] N Analyzable is the number of subjects with an available sample result at the indicated visit.
[b] Geometric mean titer estimates, together with their 95% CIs are derived from ANOVA model that includes site and treatment group as fixed effects assuming normality of the log titers.

For safety results, it is referred to the safety section.

2.6.5.2. Main studies

The current application is supported mainly by the data from two completed Phase 3 studies, namely EBSI-CV-317-004 and EBSI-CV-317-005.

2.6.5.2.1. EBSI-CV-317-004

Methods

Study design

EBSI-CV-317-004 was a randomized, placebo-controlled, double-blind, parallel-group Phase 3 study to investigate safety, immunogenicity, and clinical lot consistency of a single intramuscular (IM) 40 µg dose CHIKV VLP vaccine adjuvanted with 300 µg aluminium hydroxide given per pre-filled syringe (PFS) in 3258 healthy adults and adolescents 12 to <65 yoa, of which 3254 were treated (2790 CHIKV VLP vaccine, 464 placebo).

Participants were randomized in a 2:2:2:1 ratio within each of the three age strata (ages 12 to <18 years, 18 to <46 years, and 46 to <65 years) to receive one of three consecutively manufactured lots of CHIKV VLP vaccine or placebo. With 3150 participants planned for enrolment, the treatment group totals were estimated as follows:

- Group 1- CHIKV VLP vaccine (PXVX0317) lot 104: n=900
- Group 2- CHIKV VLP vaccine (PXVX0317) lot 105: n=900

- Group 3- CHIKV VLP vaccine (PXVX0317) lot 106: n=900
- Group 4- Placebo: n=450

A nested lot consistency sub study was performed in adult participants 18 to <46 years of age in the CHIKV VLP vaccine treatment groups (lot 104, lot 105, and lot 106).

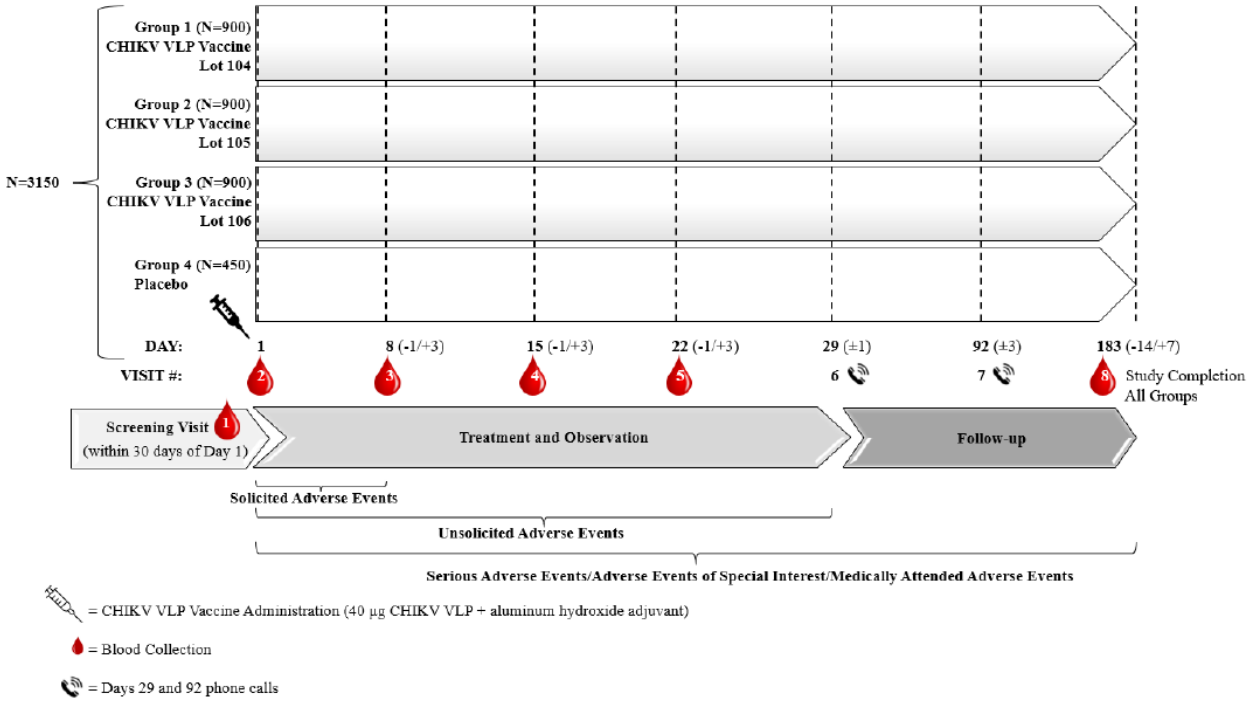
Number of participants analysed:

A total of 3258 participants were enrolled as follows:

- Group 1- CHIKV VLP vaccine (PXVX0317) lot 104: n=919
- Group 2- CHIKV VLP vaccine (PXVX0317) lot 105: n=948
- Group 3- CHIKV VLP vaccine (PXVX0317) lot 106: n=927
- Group 4- Placebo: n=464

The per participant estimated total study duration was 212 days. The screening window was no greater than 30 days prior to Day 1. Day 1 included randomization and administration of investigational product (IP) (either CHIKV VLP vaccine or placebo). The immune response to CHIKV VLP vaccine was measured by anti-CHIKV SNA up to Day 183 End of Study (EOS) Visit.

Figure 6. EBSI-CV-317-004: study schema



- **Study Participants**

This multicentre study was conducted in the US, using 47 sites.

Inclusion criteria were standard as for all clinical trials and included generally healthy males and females 12 to <55 years of age.

Exclusion criteria were also standard and included, among others, prior receipt of a CHIK vaccine or investigational vaccines, congenital or acquired immunodeficiency that could impact the response to vaccination or anticipated use of systemic immunomodulatory or immunosuppressive medications from six months prior to screening through Day 22. Pregnant women were excluded from the clinical trial: a

negative serum/urine test were to be met before screening and dosing at D1, respectively. Women of childbearing potential were to use adequate contraception methods throughout their participation in the study.

- **Treatments**

Participants received either CHIKV VLP vaccine (one of three consecutive lots 2861-104, 2861-105, and 2861-106: referred to in this report as lot 104, 105, or 106) as a single dose of 40 µg CHIKV VLP combined with 300 µg aluminium hydroxide or placebo (lot 101), a sterile aqueous solution with the same excipient composition as the drug product without CHIKV VLP and aluminium hydroxide adjuvant on Day 1 by intramuscular (IM) injection in the deltoid muscle.

CHIKV VLP vaccine and placebo were supplied as a single dose 0.8 mL pre-filled syringe. The pre-filled syringe containing the IP was delivered in kits to the study sites for administration. Each kit comprised one pre-filled syringe, labelled with an orange tinted label, packaged in a carton; each syringe and its outer carton were labelled with a unique kit number that did not reveal the identity of the IP (CHIKV VLP vaccine or placebo). The injection was to be administered within 2 hours of removal of the pre-filled syringe from 2 to 8°C storage.

- **Prior and Concomitant Therapy**

At the Screening Visit, the details of prior and concomitant medication usage were collected (through 30 days prior to screening, or 90 days for blood products, or six months prior to screening for systemic immunomodulatory or immunosuppressive medications). Concomitant medications were collected at each onsite visit or by phone interview (or early discontinuation/ withdrawal) for all groups. The details of all concomitant medications including those associated with solicited AEs and unsolicited AEs were collected. Concomitant medications associated with SAEs, AESI, and MAAEs were collected through EOS.

- **Objectives and endpoints**

Primary objectives

- To evaluate the safety of CHIKV VLP vaccine in healthy adult and adolescent participants 12 to <65 years of age.
- To compare the anti-CHIKV SNA response to CHIKV VLP vaccine and placebo at Day 22, as measured by geometric mean titre (GMT) and clinically relevant difference in seroresponse rate (CHIKV VLP vaccine minus placebo).

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA titre ≥100.

- To demonstrate the consistency of the anti-CHIKV SNA response across three consecutively manufactured lots of CHIKV VLP vaccine at Day 22 as measured by GMT.

Primary endpoints

Safety endpoints:

- Incidence of solicited adverse events (AEs) through Day 8 for CHIKV VLP vaccine and placebo.
- Incidence of unsolicited AEs through Day 29 for CHIKV VLP vaccine and placebo.
- Incidence of AEs of special interest (AESI), medically attended AEs (MAAEs), and serious AEs (SAEs) through Day 183 for CHIKV VLP vaccine and placebo.

Coprimary immunogenicity endpoints:

- Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) and associated 95% confidence interval (CI) at Day 22.
    - Success criterion: Statistical superiority to placebo and lower bound of the two-sided 95% CI on the difference in seroresponse rates between CHIKV VLP vaccine and placebo groups ≥70% (considered clinically significant).

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA titre ≥100.

- Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for CHIKV VLP vaccine and placebo.
    - Success criterion: Significant difference across all age groups combined using an analysis of variance (ANOVA) model with logarithmically transformed anti- CHIKV SNA titers (log10) as the dependent variable and treatment group and study site as the fixed effects with a two-sided significance level of 0.05.

- Anti-CHIKV SNA GMT ratio between all three pairs of CHIKV VLP vaccine lots (104:105, 105:106, 104:106) (adults 18 to <46 years of age) at Day 22.
    - Success criterion: Pairwise GMT ratios (104:105, 105:106, 104:106) each with a two-sided 95% CI within [0.667; 1.5] for the IEP in the 18 to <46 years of years of age stratum using an ANOVA model with log10-transformed anti-CHIKV SNA titers as the dependent variable and vaccine lot and study site as the fixed effects to derive the GMT ratios.

Secondary objectives

- To compare the anti-CHIKV SNA response to CHIKV VLP vaccine and placebo at Day 15, Day 183, and Day 8, as measured by GMT and seroresponse rate.
- To compare the anti-CHIKV SNA response to CHIKV VLP vaccine and placebo in participants 12 to <18 years of age, participants 18 to <46 years of age, and participants 46 to <65 years of age as measured by GMT and seroresponse rate.

Secondary endpoints

- Key secondary endpoints: Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) with associated 95% CI at Day 15, Day 183, and Day 8, in that order.
    - Success criterion: Statistical superiority to placebo, and at Day 15 only, lower bound of the two-sided 95% CI on the difference in seroresponse rates between CHIKV VLP vaccine and placebo groups ≥70% (considered clinically significant).
- Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at Day 8, Day 15, and Day 183.
- Geometric mean fold increase (GMFI) from Day 1 to subsequent collection time points.
- Number and percentage of participants with an anti-CHIKV SNA titre ≥15 and 4-fold rise over baseline.

Exploratory objective

- To evaluate anti-CHIKV SNA response across three consecutively manufactured lots of CHIKV VLP vaccine as measured by seroresponse rate for each lot at Day 22 in participants 18 to <46 years of age.

Exploratory endpoint

- Anti-CHIKV SNA seroresponse rate difference with associated 95% CIs for each pair of CHIKV VLP vaccine lots (104 minus 105, 105 minus 106, 104 minus 106) (adults 18 to <46 years of age) at Day 22.

- **Sample size**

The primary statistical comparison between the PXVX0317 and placebo treatment groups is based on the difference between treatment groups in seroresponse rate (proportion of participants with anti-CHIKV SNA NT80 ≥100, as measured by the human SNA assay) at Day 22, which will be tested using a chi-square test with a two-sided alpha=0.05 in the IEP across all age groups combined. The analysis will be repeated for the mITT as a measure of the robustness of the result. Based on the data from the phase 2 study (protocol PXVX-CV-317-001) (16, 28), the seroresponse rate for PXVX0317 vaccine is expected to be approximately 90% vs <5% for the placebo participants at Day 22. With an assumed 10% rate of non-evaluable participants, the power to show superiority over placebo with 2430 PXVX0317 vaccine and 405 placebo evaluable participants is >99.9% for the combined ages groups. The power is 99% for each of the smaller 12 to <18 and 46 to <65 age groups with samples sizes of 189 vs 32 evaluable participants (PXVX0317: placebo).

The difference in seroresponse rate between PXVX0317 and placebo groups that is considered clinically relevant is 70%. With 2430 PXVX0317-treated participants and a target seroresponse rate of 90% vs a rate of 0% for placebo, the width of a two-sided 95% CI would be ±1.2%. The difference in seroresponse rate must be above 72% for the lower bound of the 95% CI for the difference to be ≥70%.

- **Randomisation and Blinding (masking)**

Participants were to be randomized on Day 1 following confirmation of eligibility and immediately prior to study vaccine administration. Study personnel were to randomize the participant within the electronic data capture (EDC) system, and a randomization number was to be generated from the EDC randomization module. The EDC randomization module was to match the randomization number to an IP kit available at the site and inform the study personnel of the assigned kit. Study personnel were to use the appropriate kit for administration to the participant. Participants were to be considered enrolled once a randomization number has been assigned within the EDC system. The study was conducted as a double-blind study through Day 183 EOS. The participants, site personnel (including the investigator) and the Sponsor did not know participants' individual treatment assignments until all participants had completed their participation in the study through the Day 183 EOS and the database had been cleaned and locked.

Study participants, the investigator, and study site personnel were to remain blinded to all randomization assignments throughout the study. The sponsor’s personnel who were in regular contact with the study site and/or involved with documentation associated with the study were to remain blinded to all participant randomization assignments.

Additional safeguards were employed to reduce the risk of inadvertent unblinding.

- **Statistical methods**

Planned analysis

The superiority of the immune response to CHIKV VLP vaccine over that to placebo was demonstrated at Day 22 by comparing seroresponse rates between the two treatment groups (the proportion of participants with a human SNA assay ≥100; considered the presumptive seroprotection rate). The difference in seroresponse rates between CHIKV VLP vaccine and placebo groups was calculated, along

with the 95% CI for the difference based on the Newcombe hybrid score method. The lower bound of the two-sided 95% CI on the difference in seroresponse rates between CHIKV VLP vaccine and placebo groups had to be ≥70%. Additionally, the null hypothesis of no difference between seroresponse rate proportions was tested using a chi-square test with alpha=0.05. No multiplicity adjustment was employed and no covariate adjustment was performed. The primary comparison was in the IEP across all age groups combined. Tests were repeated in the mITT population as a measure of robustness, along with tests for each population in the separate age strata. No multiplicity adjustment was made for the analysis of the separate age strata as the primary population was the combined age groups.

In addition, Day 22 GMTs were compared between CHIKV VLP vaccine and placebo treatment groups and were analysed via a linear model based on an alpha=0.05. The primary model was an ANOVA, with logarithmically transformed anti-CHIKV SNA titers (log10) as the dependent variable and treatment group and study site as the fixed effects in the model. The adjusted least square means and their 95% CIs calculated based on the ANOVA were back transformed and reported as the group GMT values. All tests were carried out at a two-sided significance level of 0.05 and no adjustment for multiplicity was applied.

The primary comparison was in the IEP across all age groups combined. Tests were repeated in the mITT population as a measure of robustness, along with tests for each population in the separate age strata. No multiplicity adjustment was made for the analysis of the separate age strata as the primary population was the combined age groups.

Participants were included in the analyses to the extent of their available data; missing immunogenicity data were not imputed. Participants with missing categorical data are counted in missing or unknown categories when appropriate. Participants with missing numeric data are treated as missing completely at random when calculating summary statistics. For likelihood-based analyses (e.g., regression), missing at random is assumed.

The familywise error rate was fixed at a two-sided alpha of 0.05 by the requirement that all coprimary immunogenicity endpoints had to be met for a successful outcome.

Planned subgroup analyses

The primary dataset used for analysis was the IEP. The IEP population was analysed by age, sex, race, and ethnicity subgroups for important immunogenicity endpoints.

Error probabilities, adjustment for multiplicity and interim analyses

The familywise error rate is fixed at a two-sided alpha of 0.05 by the requirement that all coprimary endpoints must be met for a successful outcome: seroresponse at a titre of 100 (HA1), GMT (HA2), and lot consistency pairwise GMT ratios (HA3). Seroresponse key secondary immunogenicity endpoints are tested using a prespecified hierarchical strategy to preserve the type I error rate without the need for further multiplicity adjustment.

The sequential order of the seroresponse rate difference key secondary endpoints (HA4) is Days 15, 183, and 8, each for the IEP across all age groups combined. At Day 183 and Day 8, only superiority to placebo using a two-sided chi-square test with alpha=0.05 is tested.

- **Study assessments**

Prior to study vaccine administration on Day 1 (baseline), eligibility, medical history and concomitant medications were reviewed, and a baseline anti-CHIKV SNA sample was drawn. Vital signs including blood pressure, heart rate, respiratory rate, and temperature were taken on Day 1 before and after study vaccine administration. Directed physical examinations were performed if indicated at Days 1, 15, 22, and Day 183 EOS Visit. No safety laboratory samples were collected as part of the study.

Blood samples were collected for immunogenicity assessments prior to vaccination on Day 1 and on Days 8, 15, 22, and 183. A final analysis of data collected throughout the study from all participants was performed after the last participant completed the study and the immunogenicity and safety data were locked.

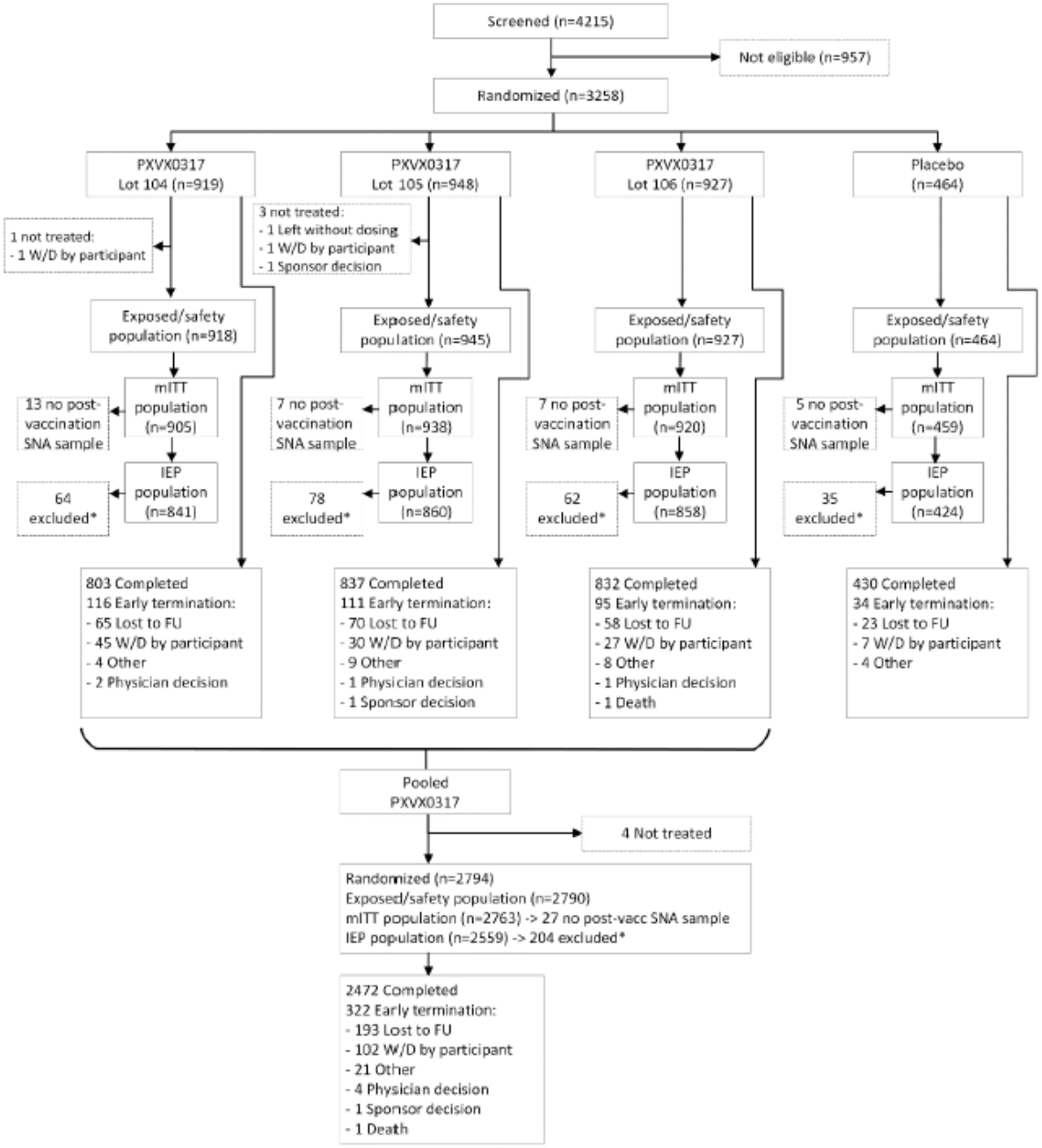
A planned independent Safety Monitoring Committee (SMC) blinded safety data review was conducted after the first 300 participants completed the Day 29 Visit, at which time no safety signals were identified. A second blinded SMC safety data review was conducted after the last participant completed the Day 29 visit, and again no safety concerns were raised and no unblinding took place. Detailed scope of the safety review was described in the SMC Charter and meeting outcomes were recorded in meeting minutes. No interim analysis of immunogenicity data was planned or conducted for the study.

Results

- **Participant flow and numbers analysed**

The first participant was enrolled on 29-Sep-2021 and the last participant completed on 03-Apr-2023, afterwards a final analysis of data collected throughout the study from all participants was performed and the immunogenicity and safety data were locked.

Figure 7. EBSI-CV-317-004: Disposition Flowchart of Participants



FU = follow up; IEP = Immunogenicity Evaluable Population; LLOQ = lower limit of quantitation; mITT = modified intent-to-treat; PXVX0317 = CHIKV VLP vaccine; SNA = serum neutralizing antibody; W/D = withdrawn .

*Exclusions from IEP were due to: No SNA result in Day 22 window, measurable (baseline positive, >LLOQ) Day 1 SNA, prohibited vaccine/medication use, eligibility criteria not met, missing Day 1 SNA sample, and/or serum processing error.

Exposed Population = Randomized participants who received study vaccination.

Safety Population = Exposed participants who provided safety assessment data.

mITT Population = Exposed participants who had at least one post-injection anti-CHIKV SNA result.

IEP = mITT population participants who had no measurable anti-CHIKV SNA at Day 1, had an evaluable Day 22 serum sample result within window (Day 19 through Day 27, inclusive), and had no important protocol deviation or other reason for exclusion as defined prior to unblinding.

Table 20. Summary of Participant Disposition (All Screened Participants)

Disposition	PXVX0317 Lot 104 n (%)	PXVX0317 Lot 105 n (%)	PXVX0317 Lot 106 n (%)	Pooled PXVX0317 n (%)	Placebo n (%)	Total n (%)
All screened	-	-	-	-	-	4215
Randomized	919	948	927	2794	464	3258
Treated	918 (99.9)	945 (99.7)	927 (100)	2790 (99.9)	464 (100.0)	3254 (99.9)
Completed study	803 (87.4)	837 (88.3)	832 (89.8)	2472 (88.5)	430 (92.7)	2902 (89.1)
Primary reason for not completing study:						
Death	0	0	1 (0.1)	1 (<0.1)	0	0
Lost to follow up	65 (7.1)	70 (7.4)	58 (6.3)	193 (6.9)	23 (5.0)	216 (6.6)
Physician decision	2 (0.2)	1 (0.1)	1 (0.1)	4 (0.1)	0	4 (0.1)
Sponsor decision	0	1 (0.1)	0	1 (<0.1)	0	1 (<0.1)
Withdrawn by participant	45 (4.9)	30 (3.2)	27 (2.9)	102 (3.7)	7 (1.5)	109 (3.3)
Other[a]	4 (0.4)	9 (0.9)	8 (0.9)	21 (0.8)	4 (0.9)	25 (0.8)
Study visit completion:						
Day 1	919 (100)	948 (100)	927 (100)	2794 (100)	464 (100)	3258 (100)
Day 8	895 (97.4)	928 (97.9)	908 (98.0)	2731 (97.7)	456 (98.3)	3187 (97.8)
Day 15	879 (95.6)	914 (96.4)	891 (96.1)	2684 (96.1)	449 (96.8)	3133 (96.2)
Day 22	878 (95.5)	904 (95.4)	904 (97.5)	2686 (96.1)	443 (95.5)	3129 (96.0)
Day 29	879 (95.6)	910 (96.0)	904 (97.5)	2693 (96.4)	445 (95.9)	3138 (96.3)
Day 92	861 (93.7)	897 (94.6)	882 (95.1)	2640 (94.5)	442 (95.3)	3082 (94.6)
Day 183	803 (87.4)	837 (88.3)	832 (89.8)	2472 (88.5)	431 (92.9)	2903 (89.1)

Data Source: Listing 16.2.1.1, Listing 16.2.4.1, Table 14.1.2, Table 14.1.3
PXVX0317 = CHIKV VLP vaccine
Note: Percentages are based on the number of randomized participants in each treatment group. An individual participant is considered completed based on the Day 183 visit and any required safety follow-up.
[a] Examples of "other" withdrawals include the participant moving, deployed, incarcerated.

Table 21. Timing of Blood Sample Collection Randomized Population

Time Point Blood Sample Collection In Window or Out of Window	PXVX0317 Lot 104 (N=919) n (%)	PXVX0317 Lot 105 (N=948) n (%)	PXVX0317 Lot 106 (N=927) n (%)	Pooled PXVX0317 (N=2794) n (%)	Placebo (N=464) n (%)	Total (N=3258) n (%)
Day 1 Blood Sample Collection						
Yes	918 (99.9)	944 (99.6)	927 (100)	2789 (99.8)	464 (100)	3253 (99.8)
Day 1 before Vaccination	917 (99.8)	944 (99.6)	925 (99.8)	2786 (99.7)	464 (100)	3250 (99.8)
Out of Window (before Day 1)	1 (0.1)	0	2 (0.2)	3 (0.1)	0	3 (0.1)
Day 8 Blood Sample Collection						
Yes	892 (97.1)	927 (97.8)	899 (97.0)	2718 (97.3)	455 (98.1)	3173 (97.4)
Day 7 to Day 11	872 (94.9)	898 (94.7)	869 (93.7)	2639 (94.5)	445 (95.9)	3084 (94.7)
Out of Window	20 (2.2)	29 (3.1)	30 (3.2)	79 (2.8)	10 (2.2)	89 (2.7)
No	2 (0.2)	0	2 (0.2)	4 (0.1)	0	4 (0.1)
Day 15 Blood Sample Collection						
Yes	856 (93.1)	893 (94.2)	873 (94.2)	2622 (93.8)	429 (92.5)	3051 (93.6)
Day 14 to Day 18	855 (93.0)	891 (94.0)	871 (94.0)	2617 (93.7)	428 (92.2)	3045 (93.5)
Out of Window	1 (0.1)	2 (0.2)	2 (0.2)	5 (0.2)	1 (0.2)	6 (0.2)
No	1 (0.1)	1 (0.1)	2 (0.2)	4 (0.1)	1 (0.2)	5 (0.2)
Day 22 Blood Sample Collection						
Yes	899 (97.8)	928 (97.9)	925 (99.8)	2752 (98.5)	463 (99.8)	3215 (98.7)
Day 19 to Day 27 (Analysis Window)	888 (96.6)	902 (95.1)	906 (97.7)	2696 (96.5)	454 (97.8)	3150 (96.7)
Out of Analysis Window	11 (1.2)	26 (2.7)	19 (2.0)	56 (2.0)	9 (1.9)	65 (2.0)
Day 21 to Day 25 (Protocol Window)	839 (91.3)	866 (91.4)	849 (91.6)	2554 (91.4)	425 (91.6)	2979 (91.4)
Out of Protocol Window	60 (6.5)	62 (6.5)	76 (8.2)	198 (7.1)	38 (8.2)	236 (7.2)
No	1 (0.1)	0	3 (0.3)	4 (0.1)	2 (0.4)	6 (0.2)

Table 22. Summary of All Enrolled Participants by Study Analysis Sets

Participant Population	**PXVX0317 Lot 104 (N=919) n (%)**	**PXVX0317 Lot 105 (N=948) n (%)**	**PXVX0317 Lot 106 (N=927) n (%)**	**Pooled PXVX031 (N=2794) n (%)**	**Placebo (N=464) n (%)**	**Total (N=3258) n (%)**
Randomized	919 (100)	948 (100.0)	927 (100)	2794 (100)	464 (100)	3258 (100)
Exposed Population[a]	918 (99.9)	945 (99.7)	927 (100)	2790 (99.9)	464 (100)	3254 (99.9)
Safety Population[b]	918 (99.9)	945 (99.7)	927 (100)	2790 (99.9)	464 (100)	3254 (99.9)
mITT Population[c]	905 (98.5)	938 (98.9)	920 (99.2)	2763 (98.9)	459 (98.9)	3222 (98.9)
IEP[d]	841 (91.5)	860 (90.7)	858 (92.6)	2559 (91.6)	424 (91.4)	2983 (91.6)

Data Source: Listing 16.2.3, Table 14.1.2

IEP = Immunogenicity Evaluable Population; N = number of enrolled participants in the study; mITT = Modified Intent-to-treat; n = number of participants per parameter; % = n/N*100; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody

[a] Exposed Population = Randomized participants who received study vaccination.

[b] Safety Population = Exposed participants who provided safety assessment data.

[c] mITT Population = Exposed participants who have at least one post-injection anti-CHIKV SNA result.

[d] IEP = mITT population participants who have no measurable anti-CHIKV SNA at Day 1, have an evaluable Day 22 serum sample result within window (Day 19 through Day 27, inclusive), and have no important protocol deviation deemed exclusionary or other reason for exclusion as defined prior to unblinding.

Table 23. Reasons for Exclusion from Analysis Populations Randomized Population

Parameter	PXVX0317 Lot 104 (N=919) n (%)	PXVX0317 Lot 105 (N=948) n (%)	PXVX0317 Lot 106 (N=927) n (%)	Pooled PXVX0317 (N=2794) n (%)	Placebo (N=464) n (%)	Total (N=3258) n (%)
Participants Excluded from the Exposed Population [a]						
Randomized, not treated	1 (0.1)	3 (0.3)	0	4 (0.1)	0	4 (0.1)
Participants Excluded from the Safety Population [b]						
Randomized, not treated	1 (0.1)	3 (0.3)	0	4 (0.1)	0	4 (0.1)
Participants Excluded from the mITT Population [c]						
No post-vaccination SNA result	13 (1.4)	7 (0.7)	7 (0.8)	27 (1.0)	5 (1.1)	32 (1.0)
Randomized, not treated	1 (0.1)	3 (0.3)	0	4 (0.1)	0	4 (0.1)
Participants Excluded from the IEP [d]						
Day 1 sample collected post vaccination	0	1 (0.1)	0	1 (0.0)	0	1 (0.0)
Eligibility criteria not met	2 (0.2)	3 (0.3)	1 (0.1)	6 (0.2)	1 (0.2)	7 (0.2)
Important Protocol Deviation	32 (3.5)	25 (2.6)	25 (2.7)	82 (2.9)	11 (2.4)	93 (2.9)
Measurable or Missing Day 1 CHIKV SNA	23 (2.5)	17 (1.8)	21 (2.3)	61 (2.2)	5 (1.1)	66 (2.0)
Missing Day 1 sample	0	1 (0.1)	0	1 (0.0)	0	1 (0.0)
No Evaluable Day 22 CHIKV SNA Result in Analysis Window	10 (1.1)	8 (0.8)	5 (0.5)	23 (0.8)	6 (1.3)	29 (0.9)
No SNA result in the Day 22 window (Days 19-27)	28 (3.0)	45 (4.7)	34 (3.7)	107 (3.8)	18 (3.9)	125 (3.8)
No post-vaccination SNA result	13 (1.4)	7 (0.7)	7 (0.8)	27 (1.0)	5 (1.1)	32 (1.0)
Prohibited vaccine/medication use	3 (0.3)	5 (0.5)	0	8 (0.3)	5 (1.1)	13 (0.4)
Randomized, not treated	1 (0.1)	3 (0.3)	0	4 (0.1)	0	4 (0.1)
Serum processing error	0	0	3 (0.3)	3 (0.1)	0	3 (0.1)

Note: Percentages are based on the number of randomized participants in each treatment group.
Participants are counted once within each exclusion reason. Participants may have more than one reason for exclusion.
[a] Exposed Population = Randomized participants who received study vaccination.
[b] Safety Population = Exposed participants who provided safety assessment data.
[c] mITT Population = Exposed participants who have at least one post-injection CHIKV SNA result.
[d] IEP = mITT population participants who have no measurable CHIKV SNA at Day 1, have an evaluable Day 22 serum sample result within window (Day 19 through Day 27, inclusive), and have no important protocol deviation or other reason for exclusion as defined prior to unblinding.
Reference: Listing 16.2.3

- **Conduct of the study**

As per protocol deviations, no participants received the wrong treatment or incorrect dose, however there were 10 (0.3%) IP temperature deviations (IP temperature excursion prior to administration resulting from a single instance where the IP rose to a maximum temperature of 8.61°C for a duration of 10 minutes [storage conditions for IP were 2 to 8°C]), 4 (0.1%) participants who were randomized but not treated, and 2 (<0.1%) IP administration errors. There were 19 participants who received an excluded concomitant medication. No emergency or accidental unblinding took place during the conduct of the study.

Blood sample collection was performed in 3253 participants (99.8%) with 3 out of window (0.1%) on Day 1. On Day 8 blood sample collection was performed in 3173 (97%) participants with 89 (3%) out of window and 4 (0.1%) missed blood collections, on Day 15 blood sample collection was performed in 3051 (94%) participants with 6 (0.2%) out of window and 5 (0.2%) missed blood collections, on Day 22 (coprimary endpoints) blood sample collection was performed in 3215 (99%) participants with 236 (7%) out of protocol window (Day 21 to Day 25), 65 (2%) out of analysis window (Day 19 to Day 27), and 6 (0.2%) missed blood collections, on Day 183 blood collection was performed in 2882 (89%) participants with 165 (5%) out of window and 26 (0.8%) missed blood collections.

Overall, there were 1150 participants (35%) with at least one important protocol deviation (iPD) during the study, 829 participants (25%) who had study visit related iPDs including out of window or missed visits, 233 (7%) who had eligibility iPDs, 97 (3%) who had noncompliance iPDs if they missed at least half of the required solicited AE entries in the eDiary, 82 (3%) who had laboratory assessment iPDs including handling/ processing errors or missing/ lost samples, 57 (2%) who had study procedure iPDs including out of window or not done, 16 (0.5%) who had IP administration iPDs, 16 (0.5%) who had safety monitoring iPDs, and 2 (<0.1%) had an iPD categorized as other.

- **Baseline data**

Table 24. Summary of Study Population Demographics by Treatment Group (Randomized Population)

Parameter	PXVX0317 Lot 104 (N=919) n (%)	PXVX0317 Lot 105 (N=948) n (%)	PXVX0317 Lot 106 (N=927) n (%)	Pooled PXVX0317 (N=2794) n (%)	Placebo (N=464) n (%)	Total (N=3258) n (%)
Age (years)						
Mean (SD)	39 (14.4)	38 (14.3)	39 (14.0)	39 (14.3)	39 (14.4)	39 (14.3)
Median	38	38	38	38	38	38
Min, Max	12, 64	12, 64	12, 64	12, 64	12, 64	12, 64
Sex, n (%)						
Male	467 (50.8)	442 (46.6)	449 (48.4)	1358 (48.6)	233 (50.2)	1591 (48.8)
Female	452 (49.2)	506 (53.4)	478 (51.6)	1436 (51.4)	231 (49.8)	1667 (51.2)
CBP	253 (56.0)	305 (60.3)	288 (60.3)	846 (58.9)	136 (58.9)	982 (58.9)
Age, n (%)						
12 to <18 years	67 (7.3)	76 (8.0)	74 (8.0)	217 (7.8)	37 (8.0)	254 (7.8)
18 to <46 years	541 (58.9)	552 (58.2)	543 (58.6)	1636 (58.6)	270 (58.2)	1906 (58.5)
46 to <65 years	311 (33.8)	320 (33.8)	310 (33.4)	941 (33.7)	157 (33.8)	1098 (33.7)
Race, n (%)						
White	663 (72.1)	693 (73.1)	687 (74.1)	2043 (73.1)	341 (73.5)	2384 (73.2)
American Indian or Alaska Native	11 (1.2)	6 (0.6)	13 (1.4)	30 (1.1)	2 (0.4)	32 (1.0)
Asian	26 (2.8)	30 (3.2)	23 (2.5)	79 (2.8)	16 (3.4)	95 (2.9)
Black or African American	190 (20.7)	175 (18.5)	169 (18.2)	534 (19.1)	89 (19.2)	623 (19.1)
Native Hawaiian or Other Pacific Islander	2 (0.2)	4 (0.4)	0	6 (0.2)	4 (0.9)	10 (0.3)
Multiracial	23 (2.5)	32 (3.4)	23 (2.5)	78 (2.8)	8 (1.7)	86 (2.6)
Not reported	4 (0.4)	8 (0.8)	12 (1.3)	24 (0.9)	4 (0.9)	28 (0.9)
Ethnicity, n (%)						
Hispanic or Latino	165 (18.0)	166 (17.5)	175 (18.9)	506 (18.1)	71 (15.3)	577 (17.7)
Not Hispanic or Latino	735 (80.0)	760 (80.2)	731 (78.9)	2226 (79.7)	379 (81.7)	2605 (80.0)
Not reported	18 (2.0)	22 (2.3)	21 (2.3)	61 (2.2)	14 (3.0)	75 (2.3)
Unknown	1 (0.1)	0	0	1 (<0.1)	0	1 (<0.1)
Height (cm)						
Mean (SD)	171.4 (10.03)	170.4 (9.55)	170.7 (10.02)	170.8 (9.87)	171.2 (9.97)	170.9 (9.89)
Median	170.7	170.2	170.4	170.2	170.8	170.2
Min, Max	142.2, 201.9	146.3, 198.1	133.4, 203.2	133.4, 203.2	141.2, 201.2	133.4, 203.2
Weight (kg)						
Mean (SD)	78.5 (16.50)	78.4 (16.08)	78.3 (16.42)	78.4 (16.33)	77.6 (16.42)	78.3 (16.34)
Median	77.6	77.9	78.2	77.8	76.0	77.5
Min, Max	30.8, 132.6	35.7, 125.6	32.5, 130.2	30.8, 132.6	35.8, 128.2	30.8, 132.6
BMI (kg/m^2)						
Mean (SD)	26.63 (4.579)	26.89 (4.480)	26.73 (4.499)	26.75 (4.519)	26.38 (4.570)	26.70 (4.527)
Median	26.78	26.90	26.71	26.77	26.26	26.71
Min, Max	13.06, 35.39	14.84, 34.95	14.90, 34.92	13.06, 35.39	15.66, 34.93	13.06, 35.39
Baseline Anti-CHIKV SNA Serostatus, n (%)						
Negative (<LLOQ)	894 (97.3)	925 (97.6)	906 (97.7)	2725 (97.5)	458 (98.7)	3183 (97.7)
Positive (≥LLOQ)	24 (2.6)	18 (1.9)	21 (2.3)	63 (2.3)	6 (1.3)	69 (2.1)
Missing	1 (0.1)	5 (0.5)	0	6 (0.2)	0	6 (0.2)

Data Source: Listing 16.2.4.1; Table 14.1.4.1

BMI = body mass index; CBP = childbearing potential; LLOQ = lower limit of quantitation; Min = minimum; Max = maximum; PXVX0317 = CHIKV VLP vaccine; SD = standard deviation

Note: Percentages are based on the number of participants in each treatment group, except that percentages for childbearing potential are based on the number of women. Weight and body mass index are at screening visit.

Table 25. Summary of Study Population Demographics by Age Stratum (Randomized Population)

Parameter	12 to <18 years (N=254) n (%)	18 to <46 years (N=1906) n (%)	46 to <65 years (N=1098) n (%)	Total (N=3258) n (%)
Age				
Mean (SD)	15 (1.7)	32 (7.6)	55 (5.3)	39 (14.3)
Median	14	32	55	38
Min, Max	12, 17	18, 45	46, 64	12, 64
Sex, n (%)				
Male	143 (56.3)	951 (49.9)	497 (45.3)	1591 (48.8)
Female	111 (43.7)	955 (50.1)	601 (54.7)	1667 (51.2)
CBP	105 (94.6)	796 (83.4)	81 (13.5)	982 (58.9)
Race, n (%)				
White	207 (81.5)	1356 (71.1)	821 (74.8)	2384 (73.2)
American Indian or Alaska Native	5 (2.0)	16 (0.8)	11 (1.0)	32 (1.0)
Asian	3 (1.2)	76 (4.0)	16 (1.5)	95 (2.9)
Black or African American	20 (7.9)	371 (19.5)	232 (21.1)	623 (19.1)
Native Hawaiian or Other Pacific Islander	1 (0.4)	9 (0.5)	0	10 (0.3)
Multiracial	17 (6.7)	54 (2.8)	15 (1.4)	86 (2.6)
Not reported	1 (0.4)	24 (1.3)	3 (0.3)	28 (0.9)
Ethnicity, n (%)				
Hispanic or Latino	56 (22.0)	385 (20.2)	136 (12.4)	577 (17.7)
Not Hispanic or Latino	196 (77.2)	1471 (77.2)	938 (85.4)	2605 (80.0)
Not reported	2 (0.8)	49 (2.6)	24 (2.2)	75 (2.3)
Unknown	0	1 (0.1)	0	1 (<0.1)
Height (cm)				
Mean (SD)	167.6 (10.50)	171.5 (9.78)	170.5 (9.76)	170.9 (9.89)
Median	166.4	171.5	170.2	170.2
Min, Max	141.2, 196.3	133.4, 203.2	139.7, 201.9	133.4, 203.2
Weight (kg)				
Mean (SD)	64.3 (16.50)	78.5 (15.96)	81.1 (15.28)	78.3 (16.34)
Median	61.4	77.7	80.5	77.5
Min, Max	30.8, 122.8	35.7, 130.2	40.7, 132.6	30.8, 132.6
BMI (kg/m^2)				
Mean (SD)	22.69 (4.646)	26.60 (4.473)	27.81 (4.021)	26.70 (4.527)
Median	21.85	26.44	27.89	26.71
Min, Max	13.06, 34.82	14.84, 34.99	15.11, 35.39	13.06, 35.39
Baseline Anti-CHIKV SNA Serostatus, n (%)				
Negative (<LLOQ)	252 (99.2)	1859 (97.5)	1072 (97.6)	3183 (97.7)
Positive (≥LLOQ)	2 (0.8)	43 (2.3)	24 (2.2)	69 (2.1)
Missing	0	4 (0.2)	2 (0.2)	6 (0.2)

Data Source: Listing 16.2.4.1; Table 14.1.4.1

BMI = body mass index; CBP = childbearing potential; LLOQ = lower limit of quantitation; Min = minimum; Max = maximum; PXVX0317 = CHIKV VLP vaccine; SD = standard deviation

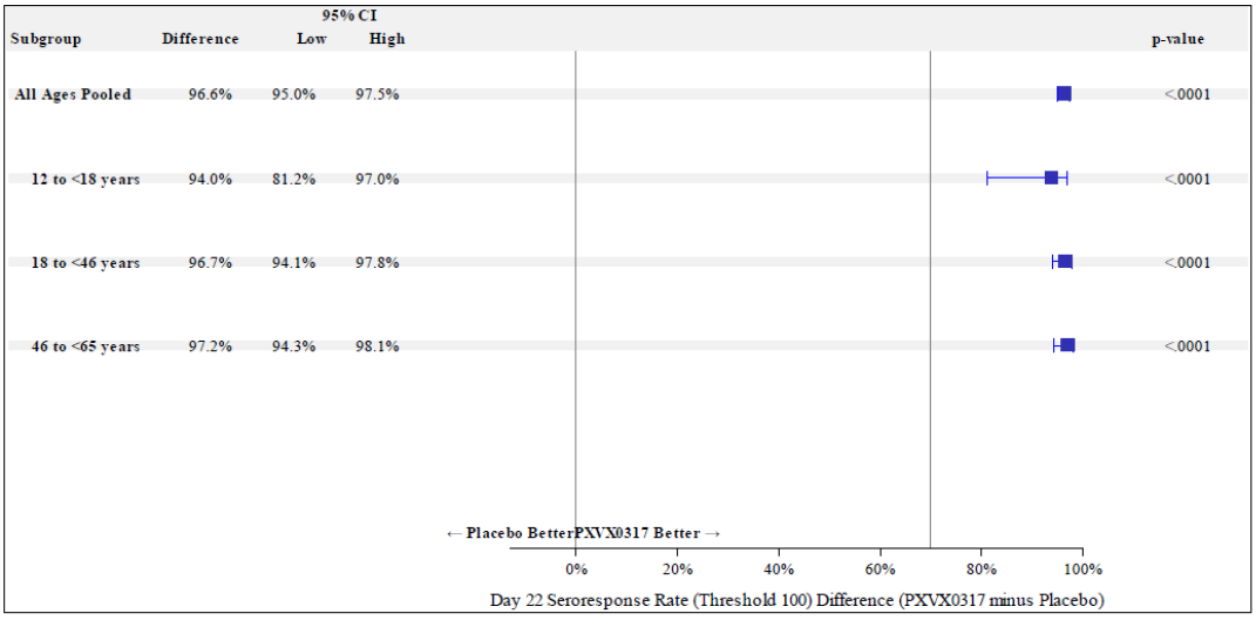
Note: Percentages are based on the number of participants in each treatment group, except that percentages for childbearing potential are based on the number of women. Weight and body mass index are at screening visit.

- **Outcomes**

Co-primary immunogenicity endpoints:

Co-primary endpoint: Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) and associated 95% confidence interval (CI) at Day 22.

Figure 8 Forest Plot of Seroresponse Rate Differences at Day 22 (Immunogenicity Evaluable Population, All Ages Pooled and by Age Group)



Note: Error bars are 95% CI based on the Newcombe hybrid score method; p-values are from a two-sided chi-square test of equality of seroresponse percentages between groups. CI = confidence interval; PXVX0317 = CHIKV VLP vaccine.

Table 26. Day 22 Difference in Anti-CHIKV SNA Seroresponse Rate (Immunogenicity Evaluable Population)

Group	Subgroup	Seroresponse Pooled PXVX0317 (N=2559) n/N (%)[c] [95% CI][d]	Seroresponse Placebo (N=424) n/N (%)[c] [95% CI][d]	Seroresponse Rate Difference [95% CI][a]	p-value[b]
All IEP	All IEP	2503/2559 (97.8%) [97.2%, 98.3%]	5/424 (1.2%) [0.5%, 2.7%]	96.6% [95.0%, 97.5%]	<0.0001
Sex	Male	1208/1241 (97.3%) [96.3%, 98.1%]	3/210 (1.4%) [0.5%, 4.1%]	95.9% [93.0%, 97.1%]	<0.0001
	Female	1295/1318 (98.3%) [97.4%, 98.8%]	2/214 (0.9%) [0.3%, 3.3%]	97.3% [94.8%, 98.2%]	<0.0001
Race	White	1847/1887 (97.9%) [97.1%, 98.4%]	2/315 (0.6%) [0.2%, 2.3%]	97.2% [95.4%, 98.0%]	<0.0001
	Non-White	636/652 (97.5%) [96.1%, 98.5%]	3/105 (2.9%) [1.0%, 8.1%]	94.7% [89.3%, 96.8%]	<0.0001
Age	12 to <18 years	195/201 (97.0%) [93.6%, 98.6%]	1/33 (3.0%) [0.5%, 15.3%]	94.0% [81.2%, 97.0%]	<0.0001
	18 to <46 years	1455/1480 (98.3%) [97.5%, 98.9%]	4/245 (1.6%) [0.6%, 4.1%]	96.7% [94.1%, 97.8%]	<0.0001
	46 to <65 years	853/878 (97.2%) [95.8%, 98.1%]	0/146 (0.0%) [0.0%, 2.6%]	97.2% [94.3%, 98.1%]	<0.0001

Data Source: Table 14.2.1.1.1, Table 14.2.1.1.2, Table 14.2.1.1.3, Table 14.2.1.1.4, Listing 16.2.5
CI = confidence interval; IEP = immune evaluable population; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody.
[a] Seroresponse rate difference is (PXVX0317 minus placebo); 95% CIs are based on the Newcombe hybrid score method.
[b] p-value is from a two-sided chi-square test of equality of seroresponse percentages between groups.
[c] n is the number of participants with seroresponse ≥ titer 100, divided by N, the total number of participants in the group.
[d] 95% CIs of seroresponse rates are based on the Wilson method.

Table 27. Day 22 Difference in Anti-CHIKV SNA Seroresponse and Seroresponse Rate (Immunogenicity Evaluable Population), Hispanic or Latino

Subgroup = Hispanic or Latino

Study Day Statistic	Pooled PXVX0317 (N=457) n (%)	Placebo (N=65) n (%)	Seroresponse Rate Difference [95% CI] [a]	p-value [b]
Day 22				
n [c]	457	65		
Participants with Titer >= 100 [95% CI] [d]	447 (97.8%) [96.0%, 98.8%]	0 (0.0%) [0.0%, 5.6%]	97.8% [92.0%, 98.8%]	<.0001

Table 37 Day 22 Difference in Anti-CHIKV SNA Seroresponse and Seroresponse Rate (Immunogenicity Evaluable Population), Not Hispanic or Latino

Subgroup = Not Hispanic or Latino

Study Day Statistic	Pooled PXVX0317 (N=2049) n (%)	Placebo (N=347) n (%)	Seroresponse Rate Difference [95% CI] [a]	p-value [b]
Day 22				
n [c]	2049	347		
Participants with Titer >= 100 [95% CI] [d]	2004 (97.8%) [97.1%, 98.4%]	5 (1.4%) [0.6%, 3.3%]	96.4% [94.3%, 97.4%]	<.0001

Co-primary endpoint: Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for CHIKV VLP vaccine and placebo

Table 28. Day 22 Difference in Anti-CHIKV SNA GMT (Immunogenicity Evaluable Population)

Group	Subgroup	Statistic	Pooled PXVX0317 (N=2559)	Placebo (N=424)	Ratio of GMTs [95% CI][a]	p-value[c]
All IEP	All IEP	n[b]	2559	424	206.12 [183.17, 231.95]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	1618.05 [1522.11, 1720.04] 1787.30 7.5, 60115.8	7.85 [6.98, 8.83] 7.50 7.5, 6007.3		
Sex	Male	n[b]	1241	210	168.79 [142.19, 200.37]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	1341.54 [1227.57, 1466.09] 1456.70 7.5, 60115.8	7.95 [6.71, 9.41] 7.50 7.5, 6007.3		
	Female	n[b]	1318	214	244.24 [207.55, 287.40]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	1968.33 [1799.36, 2153.17] 2230.35 7.5, 39875.7	8.06 [6.83, 9.52] 7.50 7.5, 1010.8		
Race	White	n[b]	1887	315	219.39 [191.76, 251.00]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	1608.48 [1484.28, 1743.07] 1806.30 7.5, 39875.7	7.33 [6.37, 8.43] 7.50 7.5, 1010.8		
	Non-White	n[b]	652	105	169.01 [130.62, 218.67]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	1624.15 [1395.51, 1890.25] 1715.85 7.5, 60115.8	9.61 [7.33, 12.59] 7.50 7.5, 6007.3		
Age Group	12 to <18 years	n[b]	201	33	274.28 [164.55, 457.20]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	2501.57 [1845.81, 3390.32] 2971.60 7.5, 33880.3	9.12 [5.38, 15.46] 7.50 7.5, 6007.3		
	18 to <46 years	n[b]	1480	245	228.13 [196.91, 264.30]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	1877.61 [1727.16, 2041.16] 2018.20 7.5, 39875.7	8.23 [7.08, 9.56] 7.50 7.5, 1010.8		
	46 to <65 years	n[b]	878	146	163.17 [133.36, 199.64]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	1174.50 [1050.73, 1312.85] 1297.50 7.5, 60115.8	7.20 [5.89, 8.80] 7.50 7.5, 62.3		

Data Source: Table 14.2.2.1.1, Table 14.2.2.1.2, Table 14.2.2.1.3, Listing 16.2.5

CI = confidence interval; GMT = geometric mean titer; IEP = immunogenicity evaluable population; LLOQ = lower limit of quantitation; Max = maximum; Min = minimum; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody.

Note: Values below lower limit of quantitation (LLOQ=15) are assigned the value LLOQ/2=7.5.

[a] Ratio of GMTs is (PXVX0317:placebo).

[b] n is the number of participants with a sample result available at the indicated visit.

[c] GMT estimates, together with their 95% CIs, are derived from an ANOVA model that includes site and treatment group as fixed effects, assuming normality of the log titers. Ratio of GMTs and 95% CIs are derived from the same model. p-value tests equivalence of group GMTs on the log scale (ie, ratio of GMTs equal to 1).

[d] Median and range are based on raw values with imputation per note.

Figure 9 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Visit – Immunogenicity Evaluable Population, All Ages Pooled

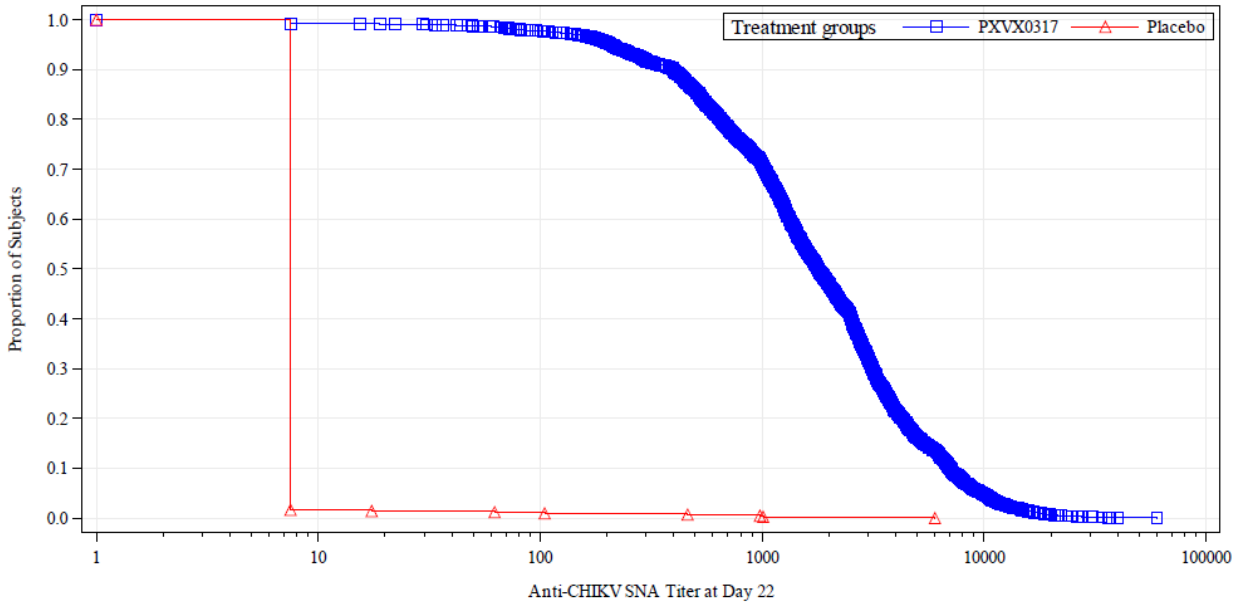


Figure 10 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Age Group – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

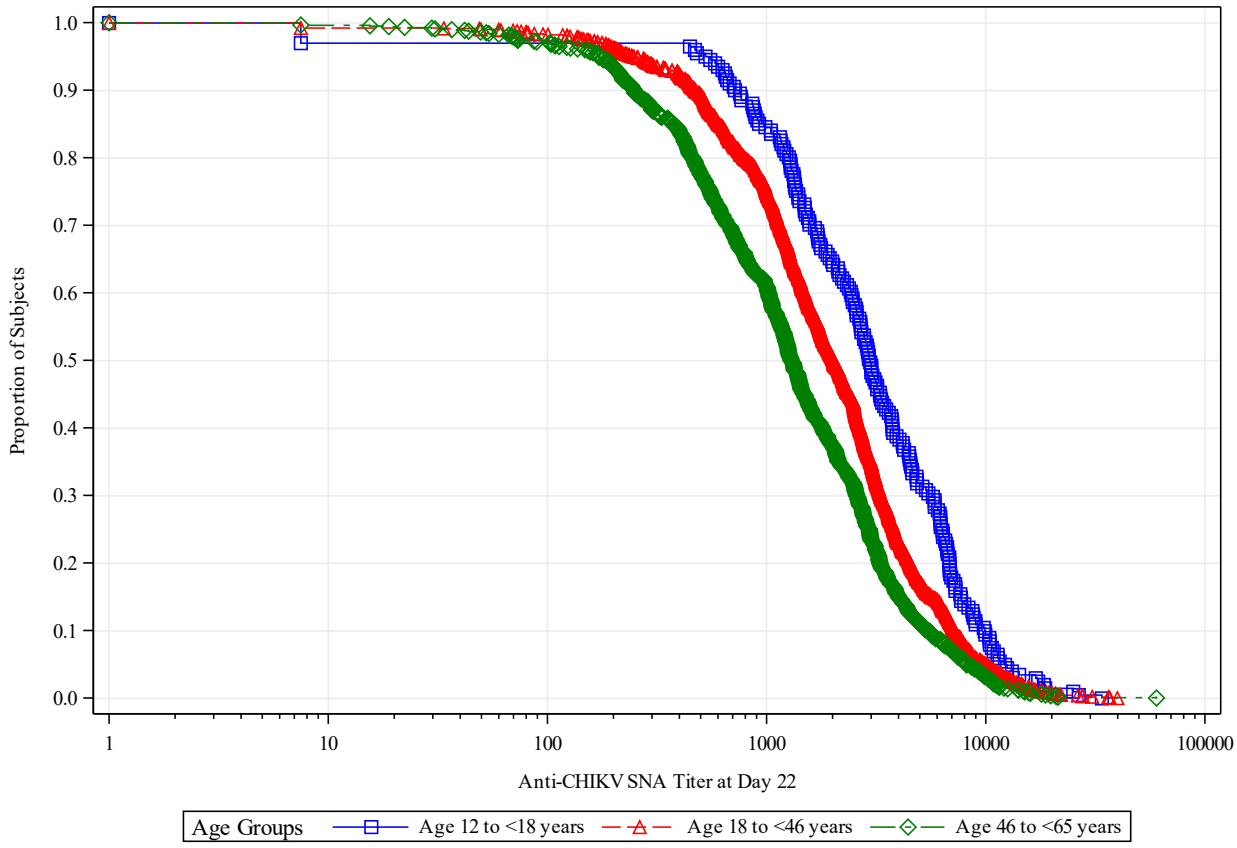


Figure 11 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Sex – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

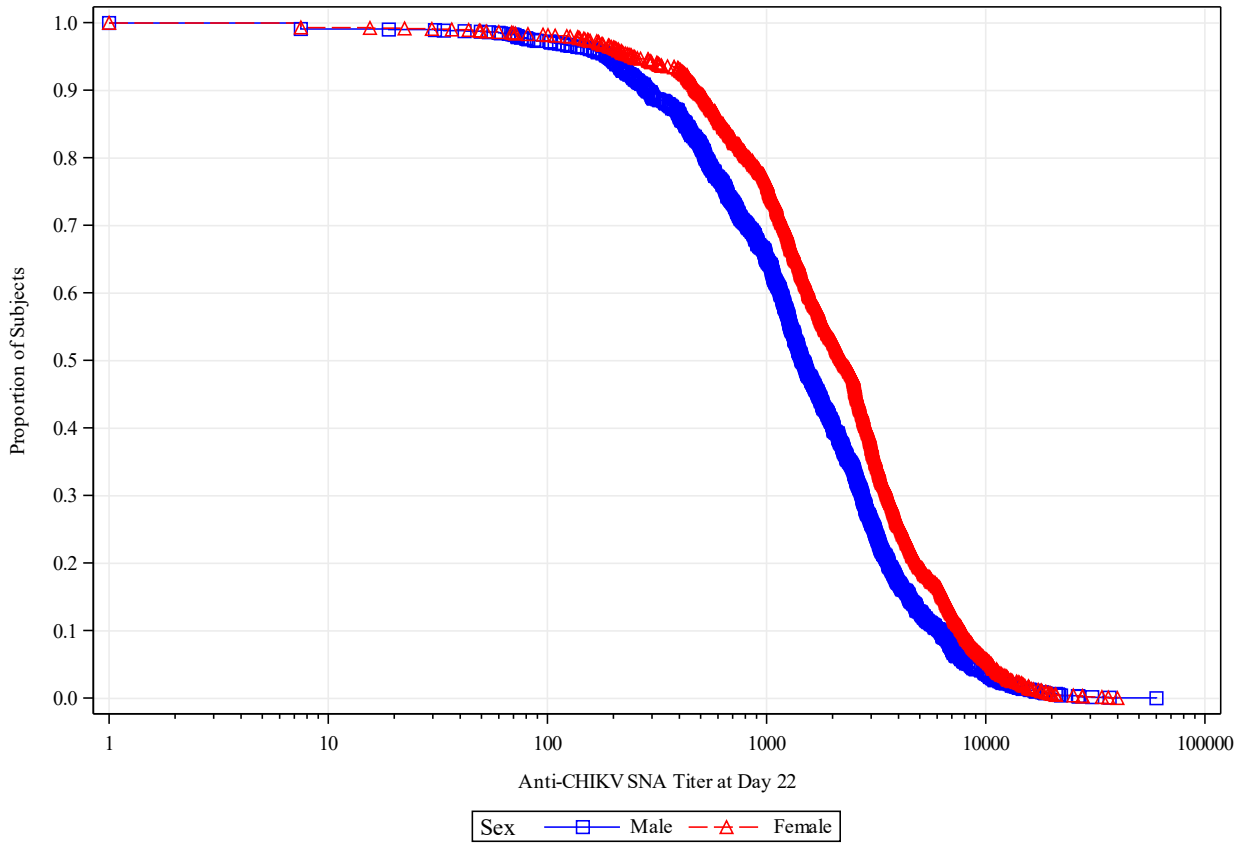


Figure 12 : Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Race – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

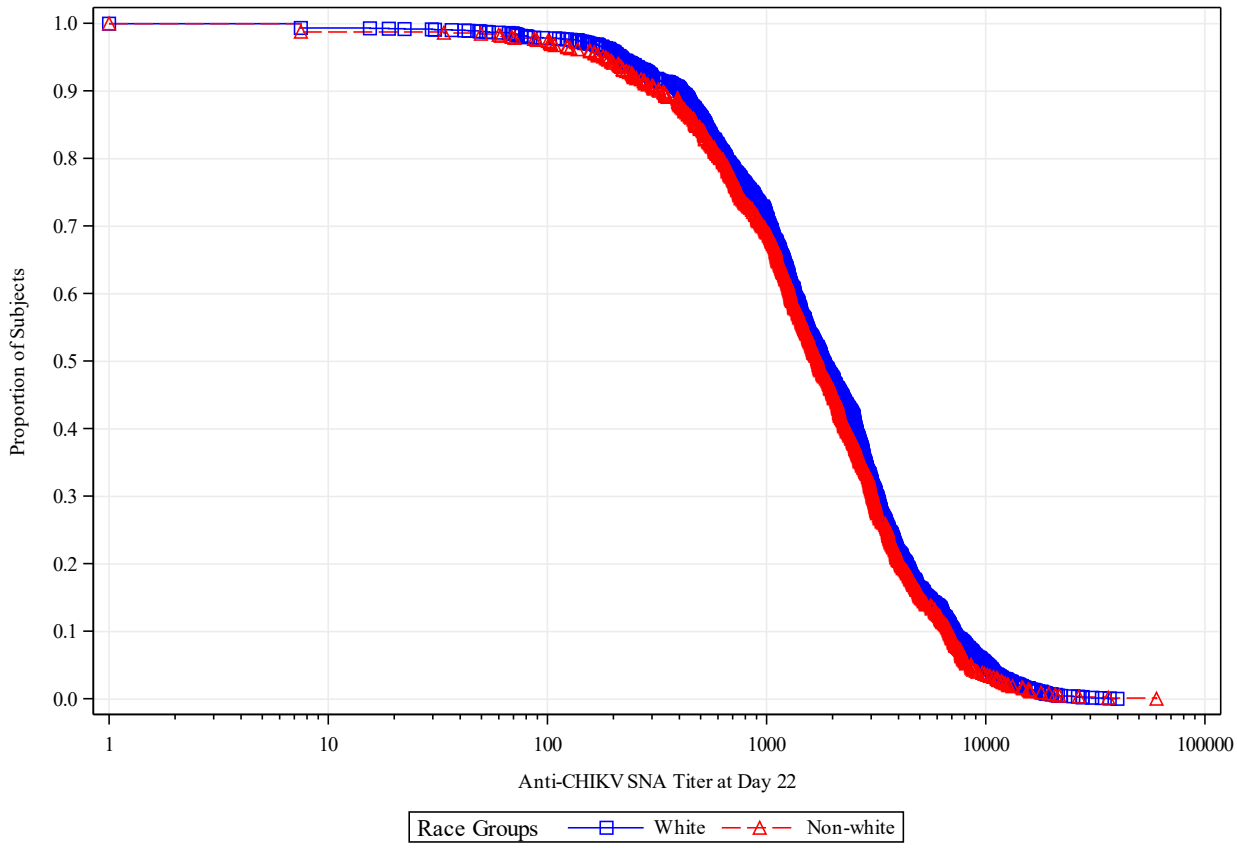


Table 29. Day 22 Difference in Anti-CHIKV SNA GMT (Immunogenicity Evaluable Population), Hispanic or Latino

Subgroup = Hispanic or Latino

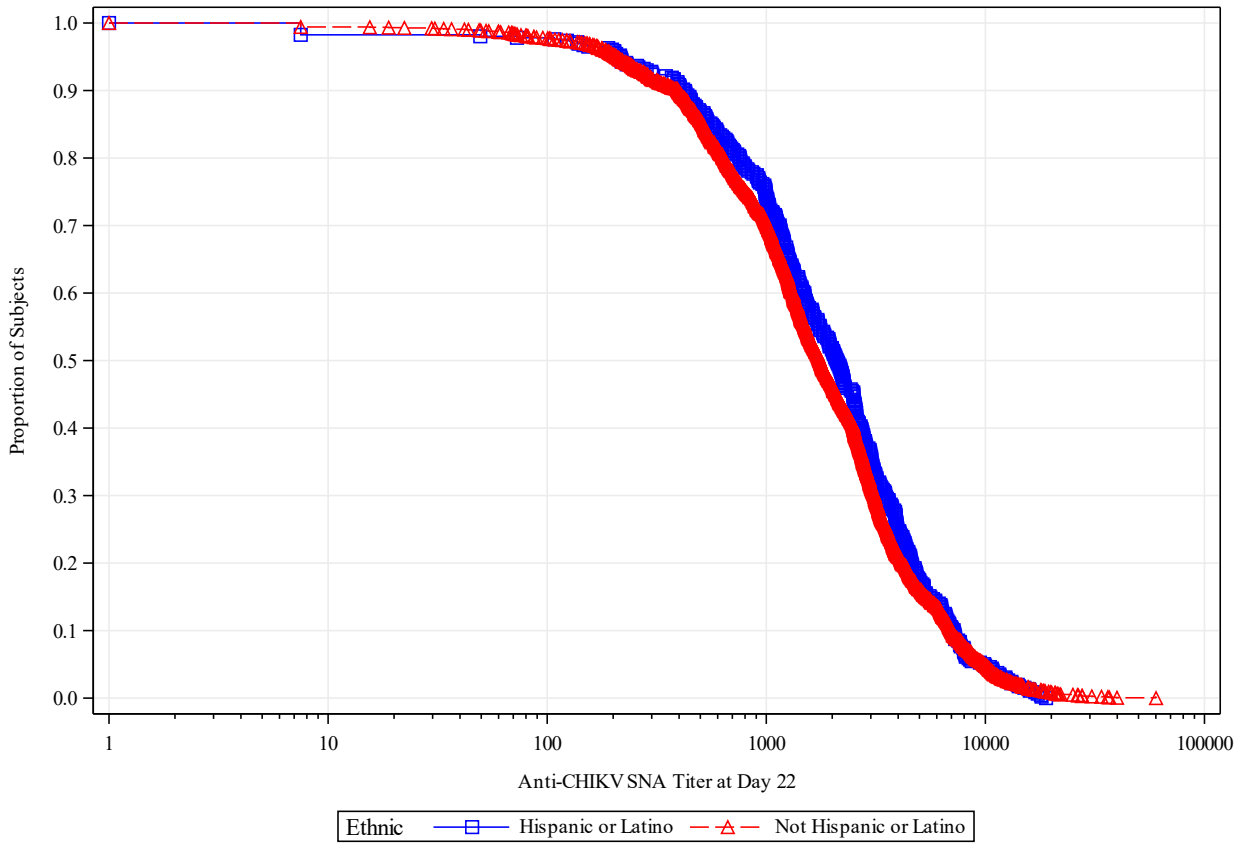
Study Day Statistic	Pooled PXVX0317 (N=457) n (%)	Placebo (N=65) n (%)	Ratio or GMTs [95% CI] [a]	p-value [c]
Day 22				
n [b]	457	65		
Geometric Mean Titer [95% CI] [c]	1747.97 [1445.23, 2114.13]	7.27 [5.17, 10.22]	240.44 [174.30, 331.68]	<.0001
Median [d]	2106.60	7.50		
Min, Max [d]	7.5, 18917.9	7.5, 7.5		

Table 30. Day 22 Difference in Anti-CHIKV SNA GMT (Immunogenicity Evaluable Population), Not Hispanic or Latino

Subgroup = Not Hispanic or Latino

Study Day Statistic	Pooled PXVX0317 (N=2049) n (%)	Placebo (N=347) n (%)	Ratio or GMTs [95% CI] [a]	p-value [c]
Day 22				
n [b]	2049	347		
Geometric Mean Titer [95% CI] [c]	1602.74 [1487.80, 1726.56]	7.89 [6.91, 9.01]	203.09 [178.26, 231.38]	<.0001
Median [d]	1751.10	7.50		
Min, Max [d]	7.5, 60115.8	7.5, 6007.3		

Figure 13 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Ethnicity – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only



Co-primary endpoint: Anti-CHIKV SNA GMT ratio between all three pairs of CHIKV VLP vaccine lots (104:105, 105:106, 104:106) (adults 18 to <46 years of age) at Day 22.

Table 31. Day 22 Pairwise Lot Comparison of Anti-CHIKV SNA GMT Ratio (Immunogenicity Evaluable Population, Adults 18 to <46 Years)

Study Day 22 Statistic	**PXVX0317 Lot 104 (N=488)**	**PXVX0317 Lot 105 (N=498)**	**PXVX0317 Lot 106 (N=494)**
n[a]	488	498	494
Geometric Mean Titer [95% CI][b]	1856.97 [1641.36, 2100.90]	1887.29 [1671.99, 2130.32]	1950.47 [1723.63, 2207.15]
Median[c]	2071.65	1944.95	2023.55
	GMT Ratio		
GMT Ratio Lot 104: Lot 105 [95% CI][b]	0.98 [0.85, 1.14]	-	-
GMT Ratio Lot 105: Lot 106 [95% CI][b]	0.97 [0.84, 1.12]	-	-
GMT Ratio Lot 104: Lot 106 [95% CI][b]	0.95 [0.82, 1.10]	-	-

Data Source: Listing 16.2.5, Table 14.2.3.1
CI = confidence interval; GMT = geometric mean titer; LLOQ = lower limit of quantitation; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody.
Note: Values below lower limit of quantitation (LLOQ=15) are assigned the value LLOQ/2=7.5.
[a] n is the number of participants with a sample result available at Day 22.
[b] Geometric mean titer estimates, together with their 95% CIs, are derived from an ANOVA model that includes site and product lot as fixed effects, assuming normality of the log titers. Geometric mean ratios and 95% CIs are derived from the same model.
[c] Median and range are based on raw values with imputation per note.

Key secondary immunogenicity endpoints:

Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) with associated 95% CI at Day 15, Day 183, and Day 8, in that order.

Table 32. Anti-CHIKV SNA Seroresponse Rate by Visit, Sex, Race, and Age (Immunogenicity Evaluable Population)

Day	Group	Subgroup	Seroresponse Pooled PXVX0317 (N=2559) n/N (%)[c] [95% CI][d]	Seroresponse Placebo (N=424) n/N (%)[c] [95% CI][d]	Seroresponse Rate Difference [95% CI][a,b]
Day 8	All IEP	All IEP	1169/2510 (46.6%) [44.6%, 48.5%]	2/419 (0.5%) [0.1%, 1.7%]	46.1% [43.8%, 48.1%]*
	Sex	Male	516/1216 (42.4%) [39.7%, 45.2%]	1/207 (0.5%) [0.1%, 2.7%]	42.0% [38.4%, 44.8%]*
		Female	653/1294 (50.5%) [47.7%, 53.2%]	1/212 (0.5%) [0.1%, 2.6%]	50.0% [46.5%, 52.7%]*
	Race	White	862/1853 (46.5%) [44.3%, 48.8%]	2/313 (0.6%) [0.2%, 2.3%]	45.9% [43.1%, 48.2%]*
		Non-White	297/638 (46.6%) [42.7%, 50.4%]	0/102 (0.0%) [0.0%, 3.6%]	46.6% [41.3%, 50.4%]*
	Age	12 to <18 years	141/199 (70.9%) [64.2%, 76.7%]	1/33 (3.0%) [0.5%, 15.3%]	67.8% [53.8%, 74.2%]*
		18 to <46 years	737/1440 (51.2%) [48.6%, 53.8%]	1/242 (0.4%) [0.1%, 2.3%]	50.8% [47.6%, 53.4%]*
		46 to <65 years	291/871 (33.4%) [30.4%, 36.6%]	0/144 (0.0%) [0.0%, 2.6%]	33.4% [29.4%, 36.6%]*
Day 15	All IEP	All IEP	2355/2434 (96.8%) [96.0%, 97.4%]	3/395 (0.8%) [0.3%, 2.2%]	96.0% [94.3%, 96.8%]*
	Sex	Male	1132/1182 (95.8%) [94.5%, 96.8%]	2/197 (1.0%) [0.3%, 3.6%]	94.8% [91.8%, 96.0%]*
		Female	1223/1252 (97.7%) [96.7%, 98.4%]	1/198 (0.5%) [0.1%, 2.8%]	97.2% [94.7%, 98.0%]*
	Race	White	1736/1799 (96.5%) [95.5%, 97.3%]	3/291 (1.0%) [0.4%, 3.0%]	95.5% [93.3%, 96.5%]*
		Non-White	599/615 (97.4%) [95.8%, 98.4%]	0/100 (0.0%) [0.0%, 3.7%]	97.4% [93.4%, 98.4%]*
	Age	12 to <18 years	187/191 (97.9%) [94.7%, 99.2%]	1/30 (3.3%) [0.6%, 16.7%]	94.6% [80.9%, 97.6%]*
		18 to <46 years	1375/1406 (97.8%) [96.9%, 98.4%]	2/228 (0.9%) [0.2%, 3.1%]	96.9% [94.5%, 97.8%]*
		46 to <65 years	793/837 (94.7%) [93.0%, 96.1%]	0/137 (0.0%) [0.0%, 2.7%]	94.7% [91.5%, 96.1%]*
Day 183	All IEP	All IEP	1967/2301 (85.5%) [84.0%, 86.9%]	6/401 (1.5%) [0.7%, 3.2%]	84.0% [81.7%, 85.6%]*
	Sex	Male	853/1097 (77.8%) [75.2%, 80.1%]	2/196 (1.0%) [0.3%, 3.6%]	76.7% [73.1%, 79.2%]*
		Female	1114/1204 (92.5%) [90.9%, 93.9%]	4/205 (2.0%) [0.8%, 4.9%]	90.6% [87.2%, 92.4%]*
	Race	White	1528/1727 (88.5%) [86.9%, 89.9%]	3/300 (1.0%) [0.3%, 2.9%]	87.5% [85.0%, 89.0%]*
		Non-White	423/556 (76.1%) [72.4%, 79.4%]	3/97 (3.1%) [1.1%, 8.7%]	73.0% [66.3%, 76.9%]*
	Age	12 to <18 years	182/192 (94.8%) [90.7%, 97.1%]	0/32 (0.0%) [0.0%, 10.7%]	94.8% [83.3%, 97.1%]*
		18 to <46 years	1098/1292 (85.0%) [82.9%, 86.8%]	4/229 (1.7%) [0.7%, 4.4%]	83.2% [79.9%, 85.4%]*
		46 to <65 years	687/817 (84.1%) [81.4%, 86.4%]	2/140 (1.4%) [0.4%, 5.1%]	82.7% [78.2%, 85.2%]*

Data Source: Table 14.2.1.1.1, Table 14.2.1.1.2, Table 14.2.1.1.3, Listing 16.2.5
CI = confidence interval; IEP = immunogenicity evaluable population; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody
*p-value <0.0001
[a] Seroresponse rate difference is (PXVX0317 minus placebo); 95% CIs are based on the Newcombe hybrid score method.
[b] p-value is from a two-sided chi-square test of equality of seroresponse percentages between groups.
[c] n is the number of participants with seroresponse ≥ titer 100, divided by N, the total number of participants in the group.
[d] 95% CIs of seroresponse rates are based on the Wilson method.

Seroresponse rate differences were analysed identically to the Day 22 analysis at Days 15, 183, and 8 (assessed in that order), except that the Day 22 success criterion (seroresponse rate difference 95% CI lower bound ≥70%) was only applied to Day 15. These endpoints were included in a prespecified type I error control hierarchy along with the coprimary immunogenicity endpoints.

Table 33. Difference in Anti-CHIKV SNA Seroresponse and Seroresponse Rate by Treatment Group and Visit (Immunogenicity Evaluable Population), Hispanic or Latino

Subgroup = Hispanic or Latino

Study Day Statistic	Pooled PXVX0317 (N=457) n (%)	Placebo (N=65) n (%)	Seroresponse Rate Difference [95% CI] [a]	p-value [b]
Day 1				
n [c]	457	65		
Participants with Titer >= 100 [95% CI] [d]	0 (0.0%) [0.0%, 0.8%]	0 (0.0%) [0.0%, 5.6%]		
Day 8				
n [c]	444	64		
Participants with Titer >= 100 [95% CI] [d]	217 (48.9%) [44.3%, 53.5%]	1 (1.6%) [0.3%, 8.3%]	47.3% [39.1%, 52.1%]	<.0001
Day 15				
n [c]	440	60		
Participants with Titer >= 100 [95% CI] [d]	427 (97.0%) [95.0%, 98.3%]	2 (3.3%) [0.9%, 11.4%]	93.7% [85.4%, 96.4%]	<.0001
Day 183				
n [c]	392	60		
Participants with Titer >= 100 [95% CI] [d]	339 (86.5%) [82.7%, 89.5%]	1 (1.7%) [0.3%, 8.9%]	84.8% [76.7%, 88.1%]	<.0001

Table 34. Difference in Anti-CHIKV SNA Seroresponse and Seroresponse Rate by Treatment Group and Visit (Immunogenicity Evaluable Population), Not Hispanic or Latino

Subgroup = Not Hispanic or Latino

Study Day Statistic	Pooled PXVX0317 (N=2049) n (%)	Placebo (N=347) n (%)	Seroresponse Rate Difference [95% CI] [a]	p-value [b]
Day 1				
n [c]	2049	347		
Participants with Titer >= 100 [95% CI] [d]	0 (0.0%) [0.0%, 0.2%]	0 (0.0%) [0.0%, 1.1%]		
Day 8				
n [c]	2013	343		
Participants with Titer >= 100 [95% CI] [d]	927 (46.1%) [43.9%, 48.2%]	1 (0.3%) [0.1%, 1.6%]	45.8% [43.2%, 48.0%]	<.0001
Day 15				
n [c]	1943	323		
Participants with Titer >= 100 [95% CI] [d]	1877 (96.6%) [95.7%, 97.3%]	1 (0.3%) [0.1%, 1.7%]	96.3% [94.6%, 97.1%]	<.0001
Day 183				
n [c]	1857	329		
Participants with Titer >= 100 [95% CI] [d]	1583 (85.2%) [83.6%, 86.8%]	5 (1.5%) [0.7%, 3.5%]	83.7% [81.1%, 85.5%]	<.0001

Secondary immunogenicity endpoints:

Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at Day 8, Day 15, and Day 183.

Table 35. Anti-CHIKV SNA Geometric Mean Titre and Geometric Mean Fold Increase from Baseline by Visit (Immunogenicity Evaluable Population)

Study Day Statistic	Pooled PXVX0317 (N=2559)	Placebo (N=424)	Ratio of GMTs [95% CI][a]	p-value[c,e]
Day 1				
n[b]	2559	424		
GMT[c] [95% CI] Median[d] Min, Max[d]	7.50 [7.50, 7.50] 7.50 7.5, 7.5	7.50 [7.50, 7.50] 7.50 7.5, 7.5	-	-
Day 8				
n[b]	2510	419		
GMT[c] [95% CI] Median[d] Min, Max[d]	93.36 [87.17, 99.99] 90.55 7.5, 48641.3	7.40 [6.49, 8.44] 7.50 7.5, 9949.3	12.62 [11.06, 14.41]	<0.0001
GMFI[e] [95% CI] Median[d] Min, Max[d]	12.45 [11.62, 13.33] 6.04 1.0, 3242.8	0.99 [0.86, 1.13] 1.00 1.0, 663.3	-	<0.0001
Day 15				
n[b]	2434	395		
GMT[c] [95% CI] Median[d] Min, Max[d]	1095.83 [1029.28, 1166.69] 1203.90 7.5, 52909.3	7.62 [6.76, 8.59] 7.50 7.5, 1267.5	143.88 [127.60, 162.24]	<0.0001
GMFI[e] [95% CI] Median[d] Min, Max[d]	146.11 [137.24, 155.56] 80.26 1.0, 3527.3	1.02 [0.90, 1.14] 1.00 1.0, 84.5	-	<0.0001
Day 22				
n[b]	2559	424		
GMT[c] [95% CI] Median[d] Min, Max[d]	1618.05 [1522.11, 1720.04] 1787.30 7.5, 60115.8	7.85 [6.98, 8.83] 7.50 7.5, 6007.3	206.12 [183.17, 231.95]	<0.0001
GMFI[e] [95% CI] Median[d] Min, Max[d]	215.74 [202.95, 229.34] 119.15 1.0, 4007.7	1.05 [0.93, 1.18] 1.00 1.0, 400.5	-	<0.0001
Day 183				
n[b]	2301	401		
GMT[c] [95% CI] Median[d] Min, Max[d]	337.73 [318.27, 358.37] 379.50 7.5, 9205.6	8.19 [7.33, 9.14] 7.50 7.5, 583.5	41.26 [36.95, 46.07]	<0.0001
GMFI[e] [95% CI] Median[d] Min, Max[d]	45.03 [42.44, 47.78] 25.30 1.0, 613.7	1.09 [0.98, 1.22] 1.00 1.0, 38.9	-	<0.0001

Data Source: Table 14.2.2.1.1, Table 14.2.4, Listing 16.2.5

CI = confidence interval; GMT = geometric mean titer; GMFI = geometric mean fold increase; LLOQ = lower limit of quantitation; Max = maximum; Min = minimum; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody.

Note: Values below lower limit of quantitation (LLOQ=15) are assigned the value LLOQ/2=7.5.

[a] Ratio of GMTs is (PXVX0317:placebo).

[b] n is the number of participants with a sample result available at the indicated visit.

[c] Geometric mean titer estimates, together with their 95% CIs, are derived from an ANOVA model that includes site and treatment group as fixed effects, assuming normality of the log titers. Ratio of GMTs and 95% CIs are derived from the same model. p-value tests equivalence of group GMTs on the log scale (ie, ratio of GMTs equal to 1).

[d] Median and range are based on raw values with imputation per note.

[e] Geometric mean fold increase from Day 1 (baseline) is (Day x/Day 1). GMFI estimates and 95% CIs are based on t-statistics assuming a normal distribution of the log fold increase in titer. p-value tests equality of log fold increase in titer between groups.

Table 36. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, subjects 12 to <18 years

Subgroup = Age 12 to <18

Study Day Statistic	Pooled PXVX0317 (N=201) n (%)	Placebo (N=33) n (%)	Ratio or GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	201	33		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	199	33		
Geometric Mean Titer [95% CI] [c]	164.41 [128.67, 210.10]	7.60 [4.97, 11.62]	21.64 [14.33, 32.67]	<.0001
Median [d]	181.30	7.50		
Min, Max [d]	7.5, 8242.6	7.5, 151.4		
Day 15				
n [b]	191	30		
Geometric Mean Titer [95% CI] [c]	1706.47 [1336.24, 2179.28]	8.38 [5.30, 13.25]	203.71 [131.45, 315.69]	<.0001
Median [d]	1886.90	7.50		
Min, Max [d]	7.5, 14584.7	7.5, 1267.5		
Day 183				
n [b]	192	32		
Geometric Mean Titer [95% CI] [c]	510.77 [417.13, 625.42]	7.51 [5.30, 10.66]	67.99 [48.41, 95.48]	<.0001
Median [d]	551.65	7.50		
Min, Max [d]	7.5, 4856.9	7.5, 7.5		

Table 37. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, subjects 18 to <46 years

Subgroup = Age 18 to <46

Study Day Statistic	Pooled PXVX0317 (N=1480) n (%)	Placebo (N=245) n (%)	Ratio or GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	1480	245		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	1440	242		
Geometric Mean Titer [95% CI] [c]	115.35 [105.14, 126.56]	7.78 [6.59, 9.18]	14.83 [12.60, 17.45]	<.0001
Median [d]	102.95	7.50		
Min, Max [d]	7.5, 48641.3	7.5, 9949.3		
Day 15				
n [b]	1406	228		
Geometric Mean Titer [95% CI] [c]	1368.99 [1258.90, 1488.71]	7.99 [6.87, 9.29]	171.33 [147.71, 198.73]	<.0001
Median [d]	1339.20	7.50		
Min, Max [d]	7.5, 52909.3	7.5, 1188.1		
Day 183				
n [b]	1292	229		
Geometric Mean Titer [95% CI] [c]	322.82 [297.70, 350.05]	8.34 [7.23, 9.63]	38.69 [33.60, 44.56]	<.0001
Median [d]	345.40	7.50		
Min, Max [d]	7.5, 8151.8	7.5, 583.5		

Table 38. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, subjects 46 to <65 years

Subgroup = Age 46 to <65

Study Day Statistic	Pooled PXVX0317 (N=878) n (%)	Placebo (N=146) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	878	146		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	871	144		
Geometric Mean Titer [95% CI] [c]	60.26 [52.75, 68.84]	7.04 [5.54, 8.96]	8.56 [6.72, 10.89]	<.0001
Median [d]	64.10	7.50		
Min, Max [d]	7.5, 31974.5	7.5, 63.0		
Day 15				
n [b]	837	137		
Geometric Mean Titer [95% CI] [c]	708.90 [632.84, 794.11]	6.88 [5.58, 8.48]	103.09 [83.62, 127.08]	<.0001
Median [d]	788.50	7.50		
Min, Max [d]	7.5, 42325.5	7.5, 7.5		
Day 183				
n [b]	817	140		
Geometric Mean Titer [95% CI] [c]	321.99 [288.50, 359.36]	7.97 [6.54, 9.70]	40.42 [33.15, 49.30]	<.0001
Median [d]	372.80	7.50		
Min, Max [d]	7.5, 9205.6	7.5, 376.3		

Figure 14 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 8 by Visit Immunogenicity Evaluable Population, All Ages Pooled

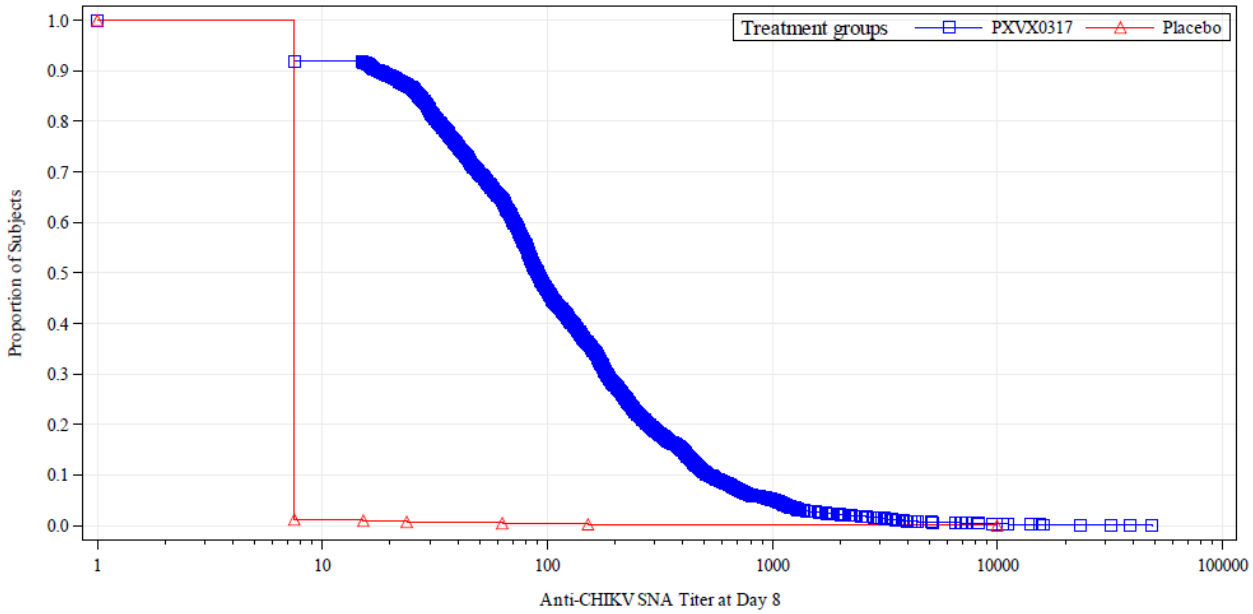


Figure 15 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 8 by Age Group – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

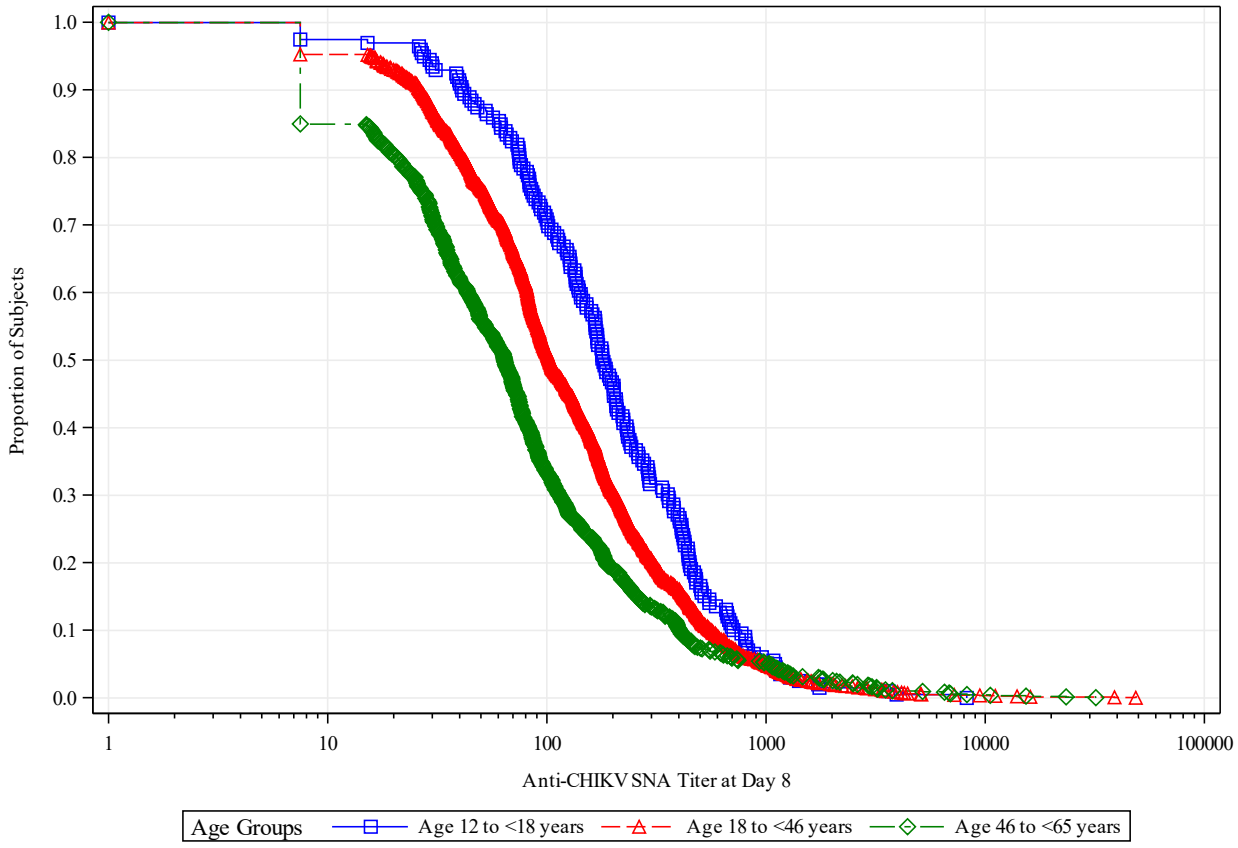


Figure 16 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Visit Immunogenicity Evaluable Population, All Ages Pooled

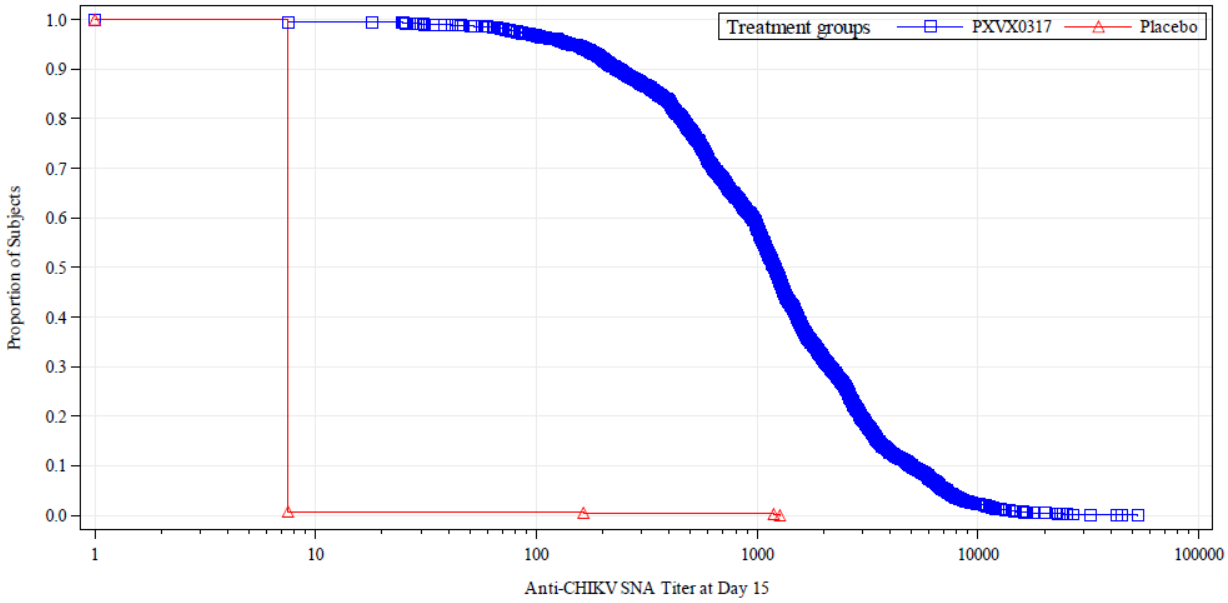


Figure 17 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Age Group – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

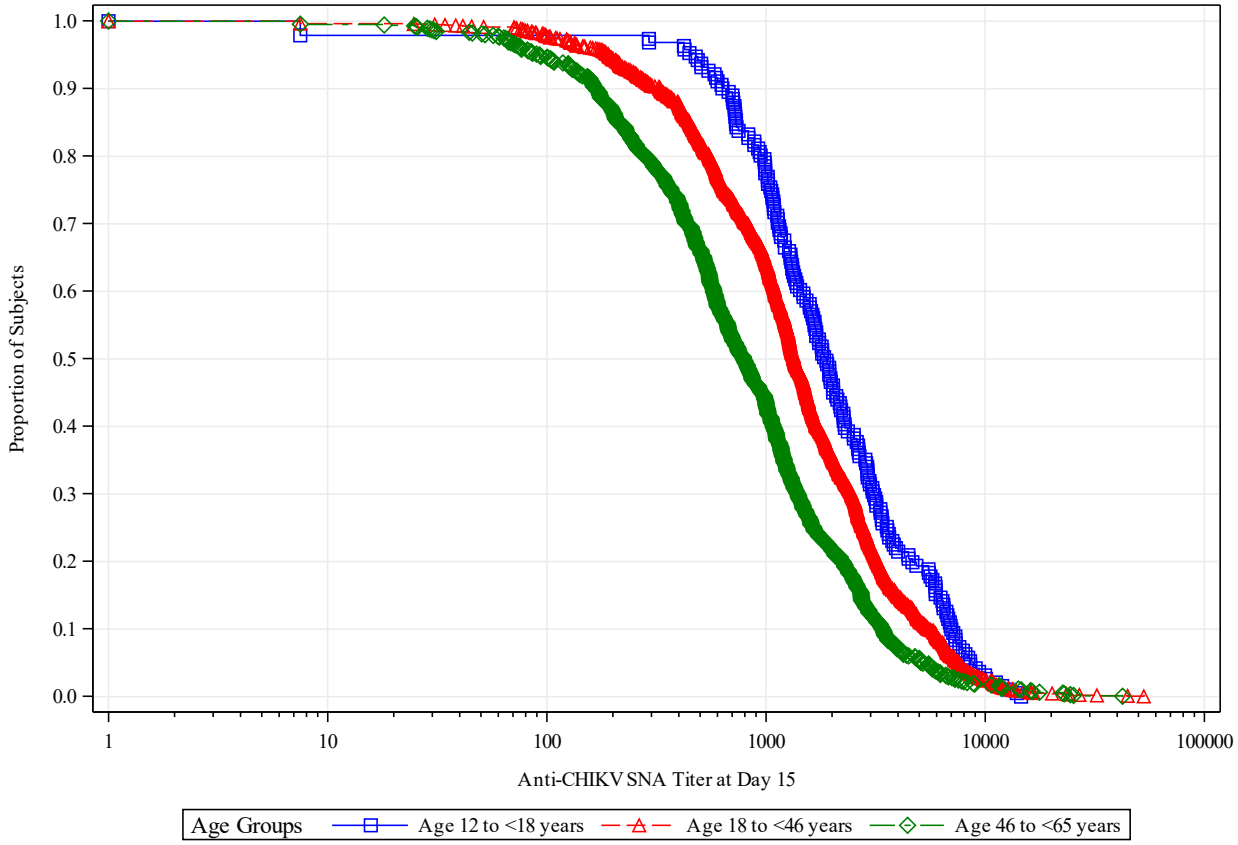


Figure 18. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Visit Immunogenicity Evaluable Population, All Ages Pooled

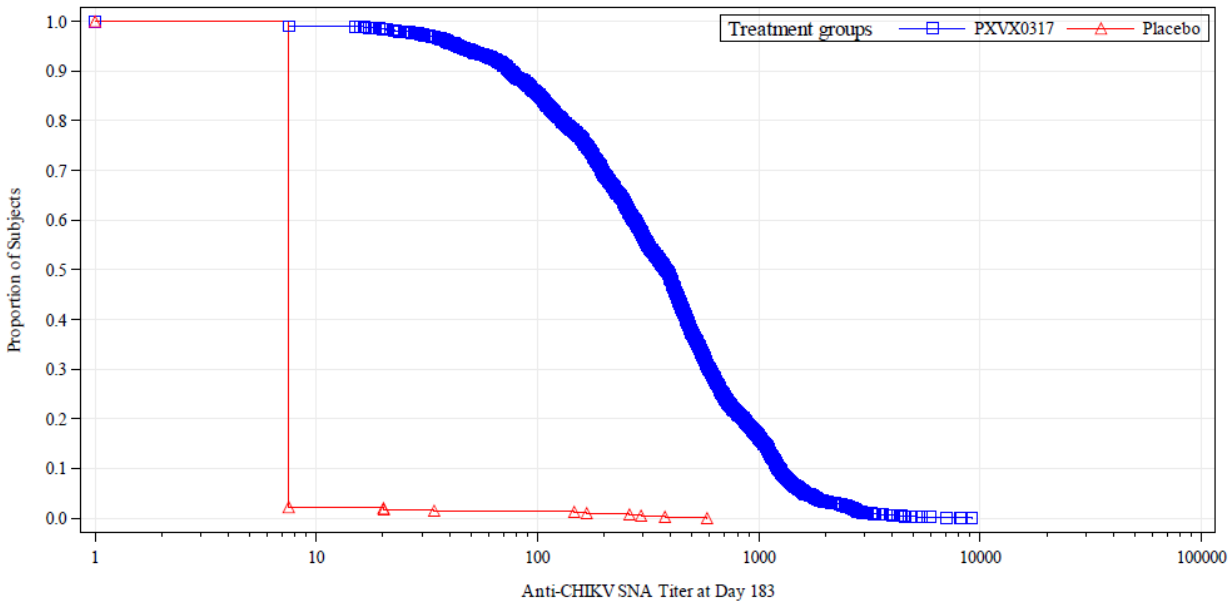


Figure 19. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Age Group – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

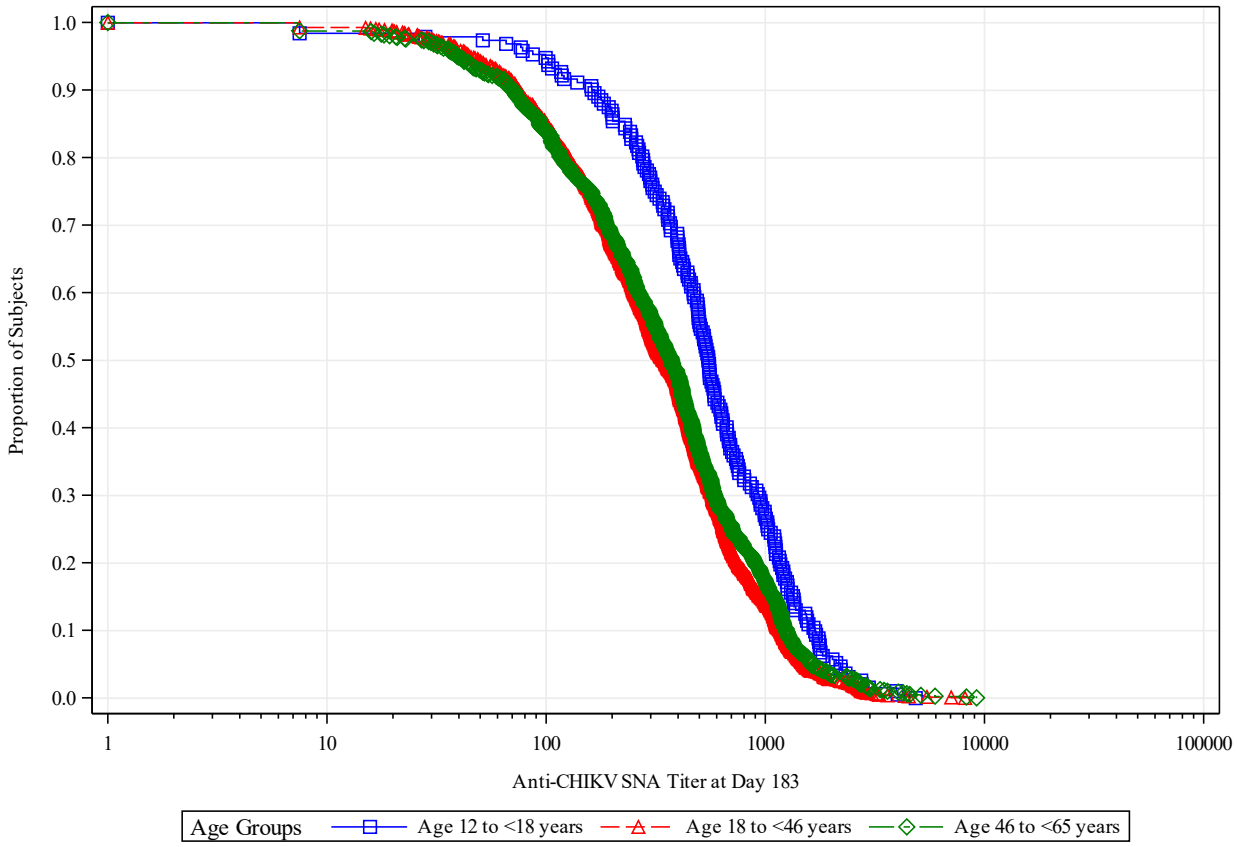


Table 39. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, male subjects

Subgroup = Male

Study Day Statistic	Pooled PXVX0317 (N=1241) n (%)	Placebo (N=210) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	1241	210		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	1216	207		
Geometric Mean Titer [95% CI] [c]	77.92 [70.65, 85.93]	7.19 [5.97, 8.65]	10.84 [8.98, 13.09]	<.0001
Median [d]	81.13	7.50		
Min, Max [d]	7.5, 31974.5	7.5, 151.4		
Day 15				
n [b]	1182	197		
Geometric Mean Titer [95% CI] [c]	827.58 [758.82, 902.58]	7.83 [6.63, 9.26]	105.64 [89.14, 125.21]	<.0001
Median [d]	948.60	7.50		
Min, Max [d]	7.5, 42325.5	7.5, 1267.5		
Day 183				
n [b]	1097	196		
Geometric Mean Titer [95% CI] [c]	241.92 [221.42, 264.32]	8.05 [6.87, 9.43]	30.05 [25.68, 35.17]	<.0001
Median [d]	256.30	7.50		
Min, Max [d]	7.5, 3338.6	7.5, 260.2		

Table 40. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, female subjects

Subgroup = Female

Study Day Statistic	Pooled PXVX0317 (N=1318) n (%)	Placebo (N=214) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	1318	214		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	1294	212		
Geometric Mean Titer [95% CI] [c]	112.86 [101.78, 125.15]	7.71 [6.37, 9.34]	14.63 [12.13, 17.65]	<.0001
Median [d]	102.25	7.50		
Min, Max [d]	7.5, 48641.3	7.5, 9949.3		
Day 15				
n [b]	1252	198		
Geometric Mean Titer [95% CI] [c]	1471.59 [1339.79, 1616.35]	7.79 [6.56, 9.23]	189.02 [160.17, 223.05]	<.0001
Median [d]	1505.20	7.50		
Min, Max [d]	7.5, 52909.3	7.5, 163.4		
Day 183				
n [b]	1204	205		
Geometric Mean Titer [95% CI] [c]	455.97 [420.11, 494.88]	8.29 [7.14, 9.63]	54.97 [47.49, 63.63]	<.0001
Median [d]	480.15	7.50		
Min, Max [d]	7.5, 9205.6	7.5, 583.5		

Figure 20. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 8 by Sex – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

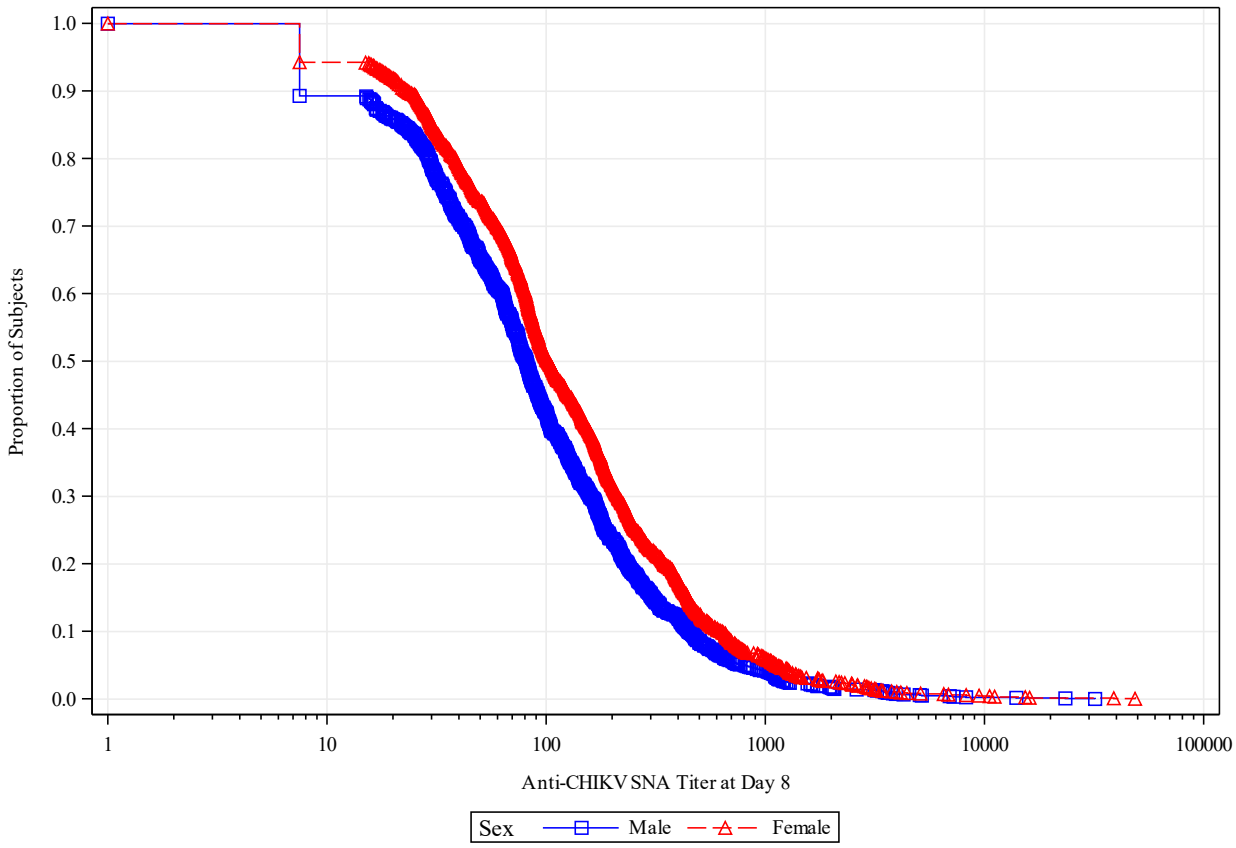


Figure 21. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Sex – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

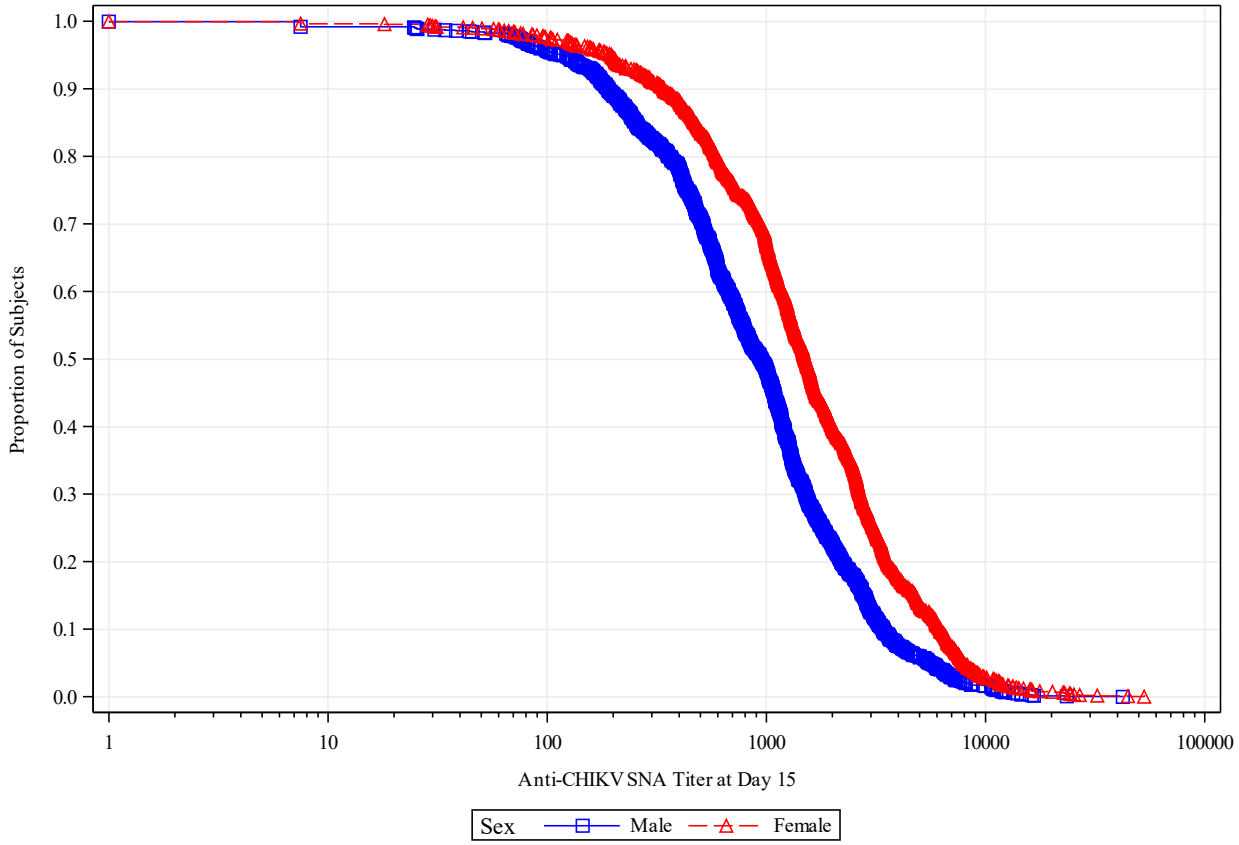


Figure 22. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Sex – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

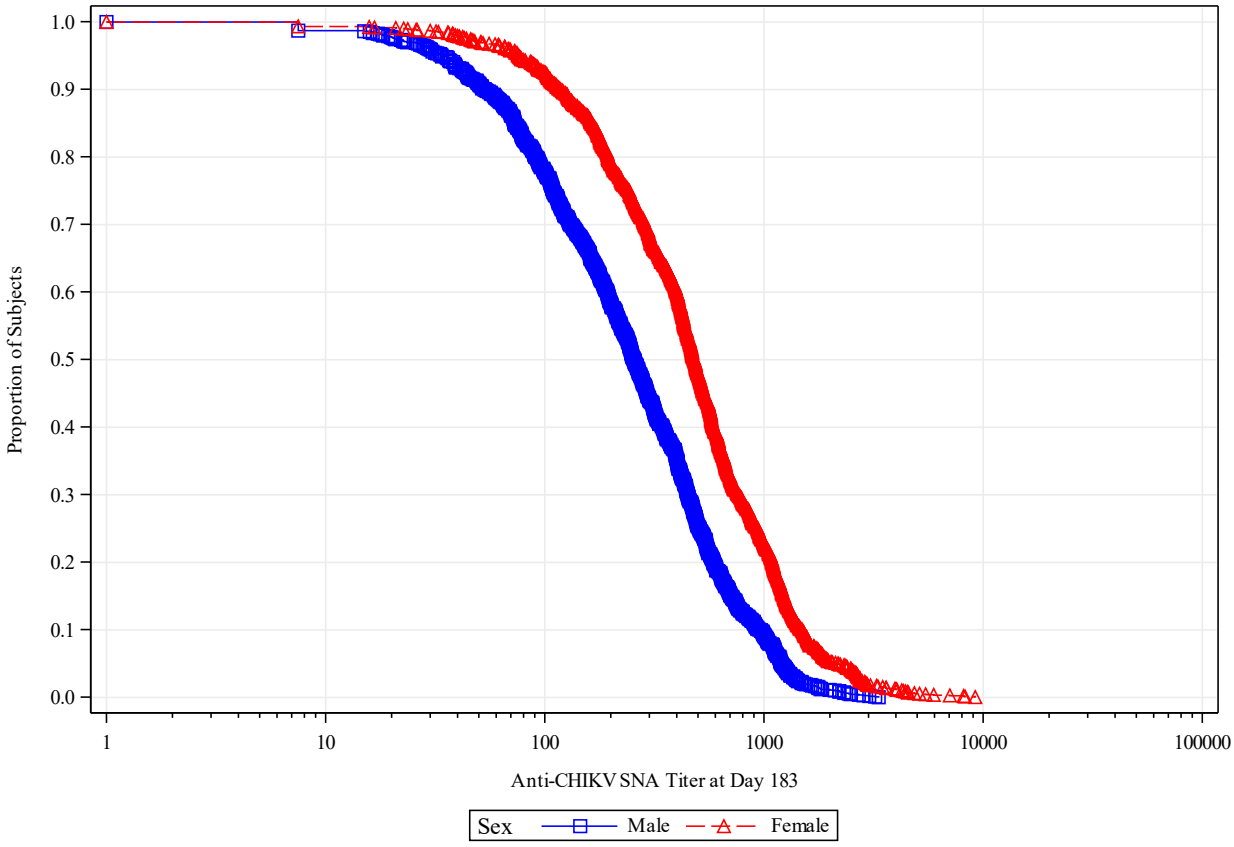


Table 41. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, subjects race white

Subgroup = White

Study Day Statistic	Pooled PXVX0317 (N=1887) n (%)	Placebo (N=315) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	1887	315		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	1853	313		
Geometric Mean Titer [95% CI] [c]	93.55 [85.35, 102.53]	7.44 [6.35, 8.73]	12.57 [10.78, 14.65]	<.0001
Median [d]	91.90	7.50		
Min, Max [d]	7.5, 48641.3	7.5, 9949.3		
Day 15				
n [b]	1799	291		
Geometric Mean Titer [95% CI] [c]	1061.88 [976.47, 1154.75]	7.48 [6.46, 8.67]	141.93 [123.22, 163.47]	<.0001
Median [d]	1169.70	7.50		
Min, Max [d]	7.5, 52909.3	7.5, 1267.5		
Day 183				
n [b]	1727	300		
Geometric Mean Titer [95% CI] [c]	369.12 [342.52, 397.77]	7.68 [6.75, 8.75]	48.04 [42.38, 54.45]	<.0001
Median [d]	423.40	7.50		
Min, Max [d]	7.5, 9205.6	7.5, 583.5		

Table 42.Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, subjects race non-white

Subgroup = Non-white

Study Day Statistic	Pooled PXVX0317 (N=652) n (%)	Placebo (N=105) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	652	105		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	638	102		
Geometric Mean Titer [95% CI] [c]	103.99 [88.37, 122.37]	8.34 [6.23, 11.17]	12.46 [9.43, 16.47]	<.0001
Median [d]	85.35	7.50		
Min, Max [d]	7.5, 31974.5	7.5, 7.5		
Day 15				
n [b]	615	100		
Geometric Mean Titer [95% CI] [c]	1209.08 [1050.92, 1391.03]	8.10 [6.30, 10.42]	149.21 [117.22, 189.93]	<.0001
Median [d]	1283.60	7.50		
Min, Max [d]	7.5, 42325.5	7.5, 7.5		
Day 183				
n [b]	556	97		
Geometric Mean Titer [95% CI] [c]	259.00 [227.76, 294.51]	9.75 [7.71, 12.33]	26.55 [21.11, 33.39]	<.0001
Median [d]	254.40	7.50		
Min, Max [d]	7.5, 4324.9	7.5, 376.3		

Figure 23. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 8 by Race – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

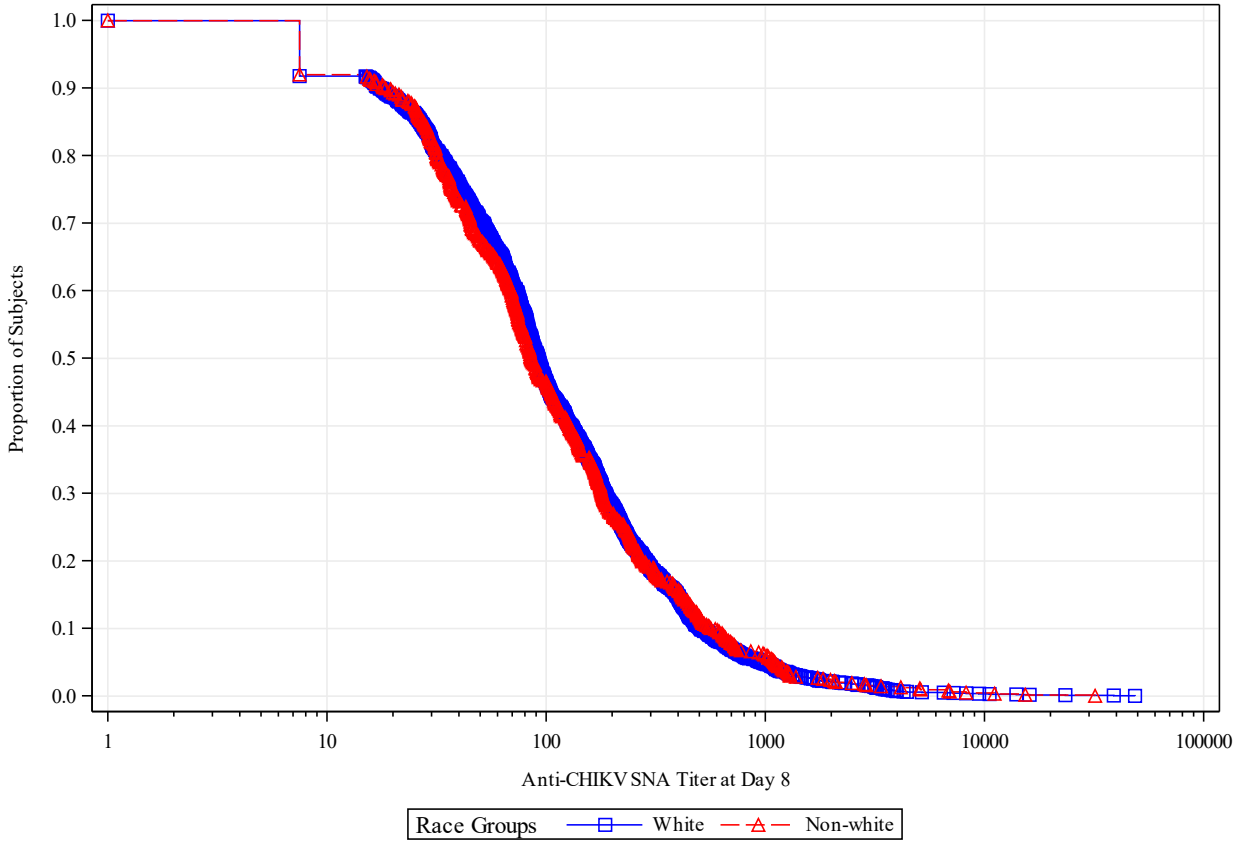


Figure 24. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Race – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

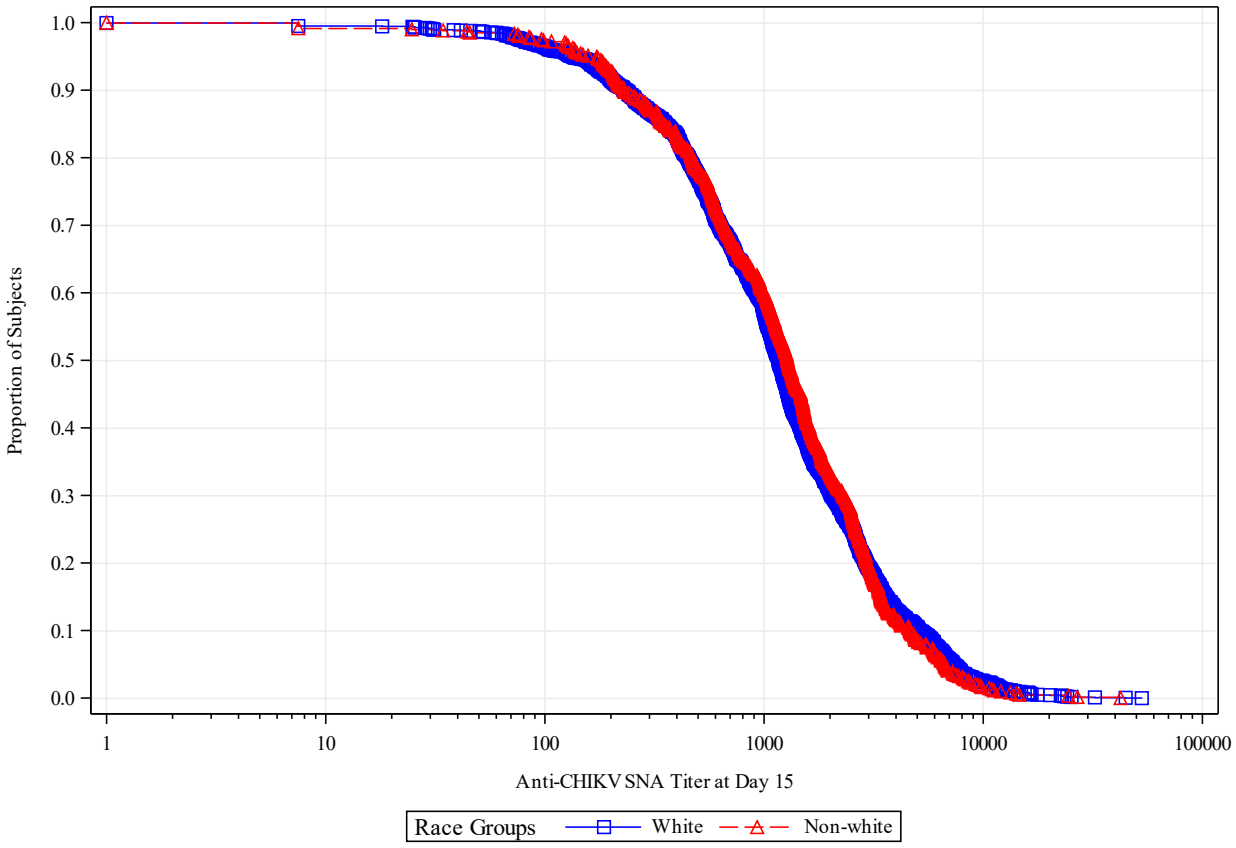


Figure 25 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Race – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

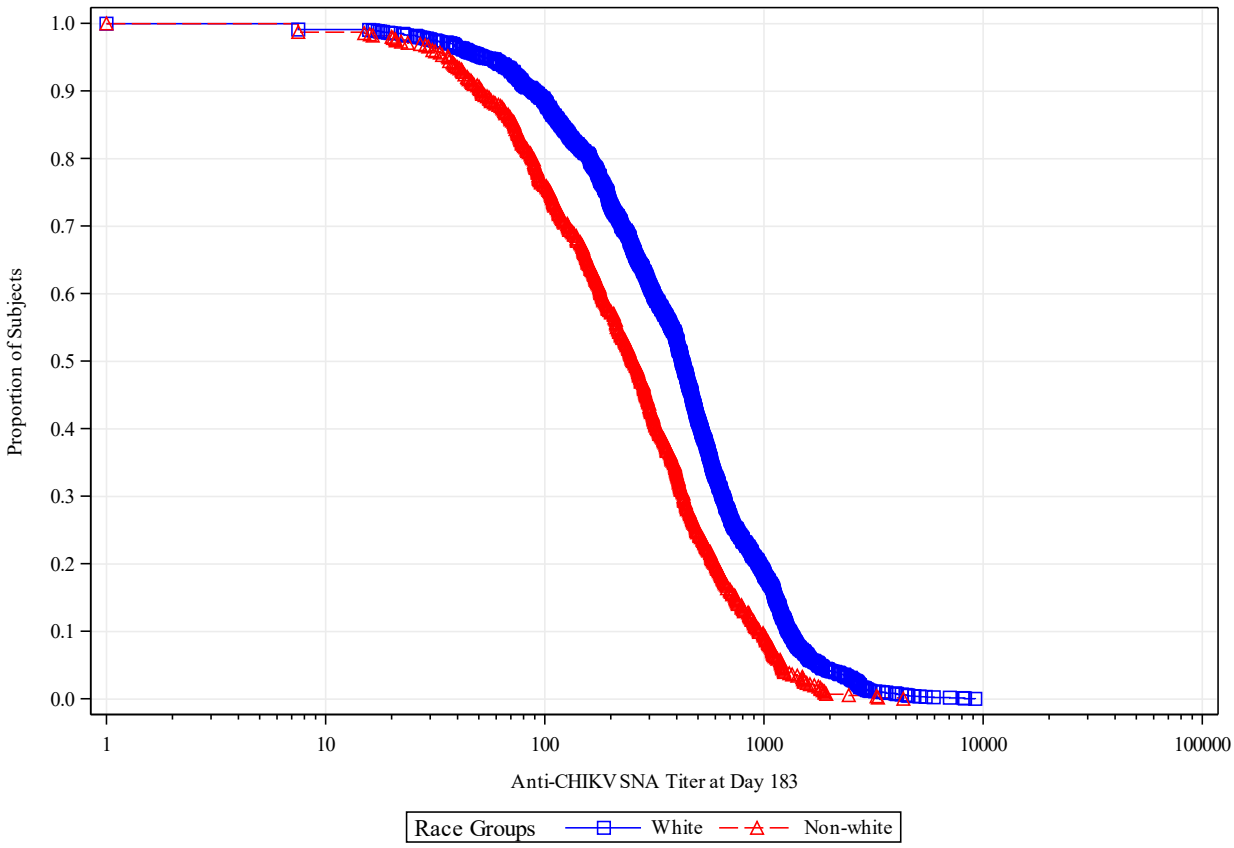


Table 43. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, Hispanic or Latino

Subgroup = Hispanic or Latino

Study Day Statistic	Pooled PXVX0317 (N=457) n (%)	Placebo (N=65) n (%)	Ratio or GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	457	65		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	444	64		
Geometric Mean Titer [95% CI] [c]	109.93 [89.16, 135.53]	9.06 [6.23, 13.19]	12.13 [8.51, 17.30]	<.0001
Median [d]	95.25	7.50		
Min, Max [d]	7.5, 13979.2	7.5, 9949.3		
Day 15				
n [b]	440	60		
Geometric Mean Titer [95% CI] [c]	1200.00 [989.66, 1455.04]	8.11 [5.69, 11.56]	147.99 [105.55, 207.48]	<.0001
Median [d]	1352.15	7.50		
Min, Max [d]	7.5, 25301.6	7.5, 1267.5		
Day 183				
n [b]	392	60		
Geometric Mean Titer [95% CI] [c]	317.68 [270.18, 373.53]	8.05 [5.99, 10.83]	39.44 [29.65, 52.47]	<.0001
Median [d]	362.05	7.50		
Min, Max [d]	7.5, 5950.0	7.5, 583.5		

Table 44. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, Not Hispanic or Latino

Subgroup = Not Hispanic or Latino

Study Day Statistic	Pooled PXVX0317 (N=2049) n (%)	Placebo (N=347) n (%)	Ratio or GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	2049	347		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 8				
n [b]	2013	343		
Geometric Mean Titer [95% CI] [c]	90.38 [83.11, 98.29]	7.13 [6.14, 8.29]	12.67 [10.94, 14.67]	<.0001
Median [d]	89.30	7.50		
Min, Max [d]	7.5, 48641.3	7.5, 151.4		
Day 15				
n [b]	1943	323		
Geometric Mean Titer [95% CI] [c]	1084.32 [998.50, 1177.52]	7.47 [6.50, 8.59]	145.12 [127.14, 165.64]	<.0001
Median [d]	1169.90	7.50		
Min, Max [d]	7.5, 52909.3	7.5, 1188.1		
Day 183				
n [b]	1857	329		
Geometric Mean Titer [95% CI] [c]	335.42 [312.31, 360.25]	8.01 [7.06, 9.08]	41.87 [37.02, 47.36]	<.0001
Median [d]	392.40	7.50		
Min, Max [d]	7.5, 9205.6	7.5, 376.3		

Figure 26. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 8 by Ethnicity – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

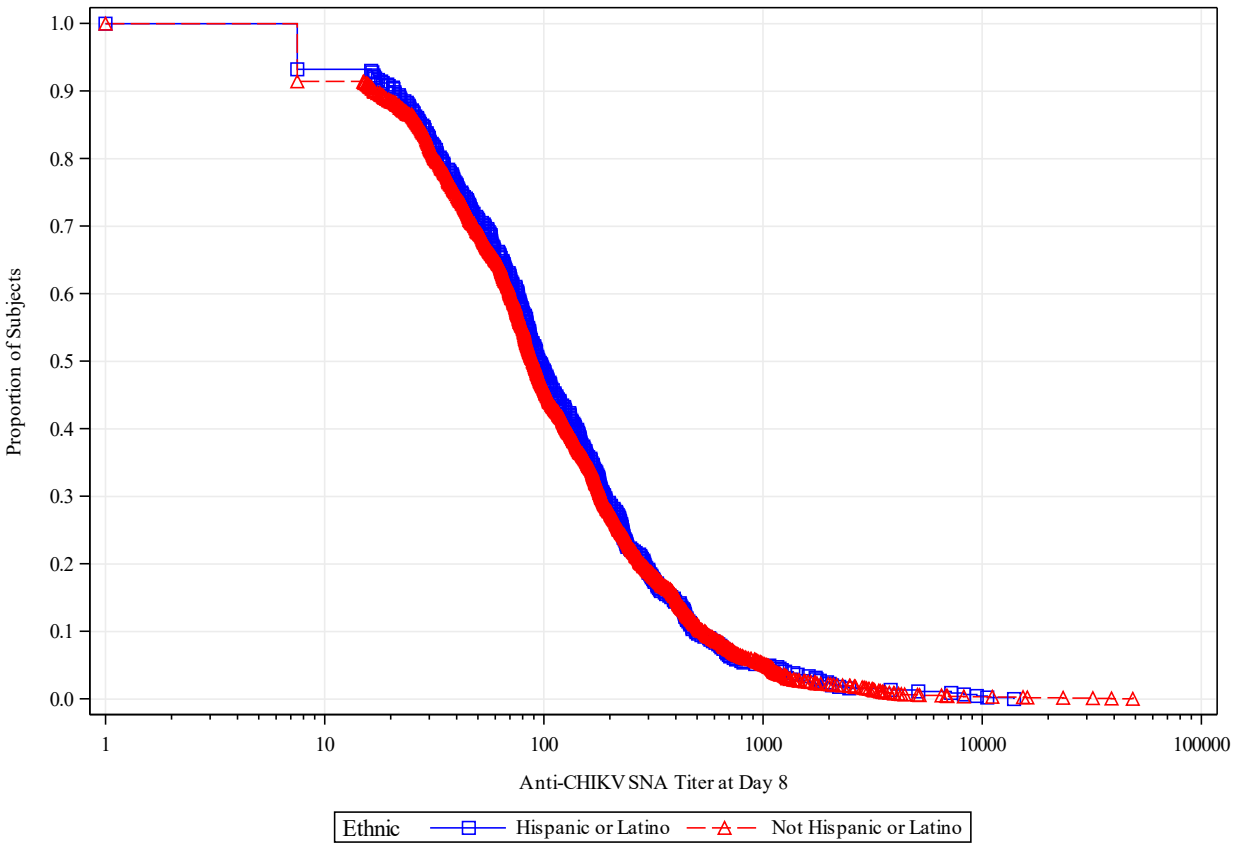


Figure 27. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Ethnicity – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

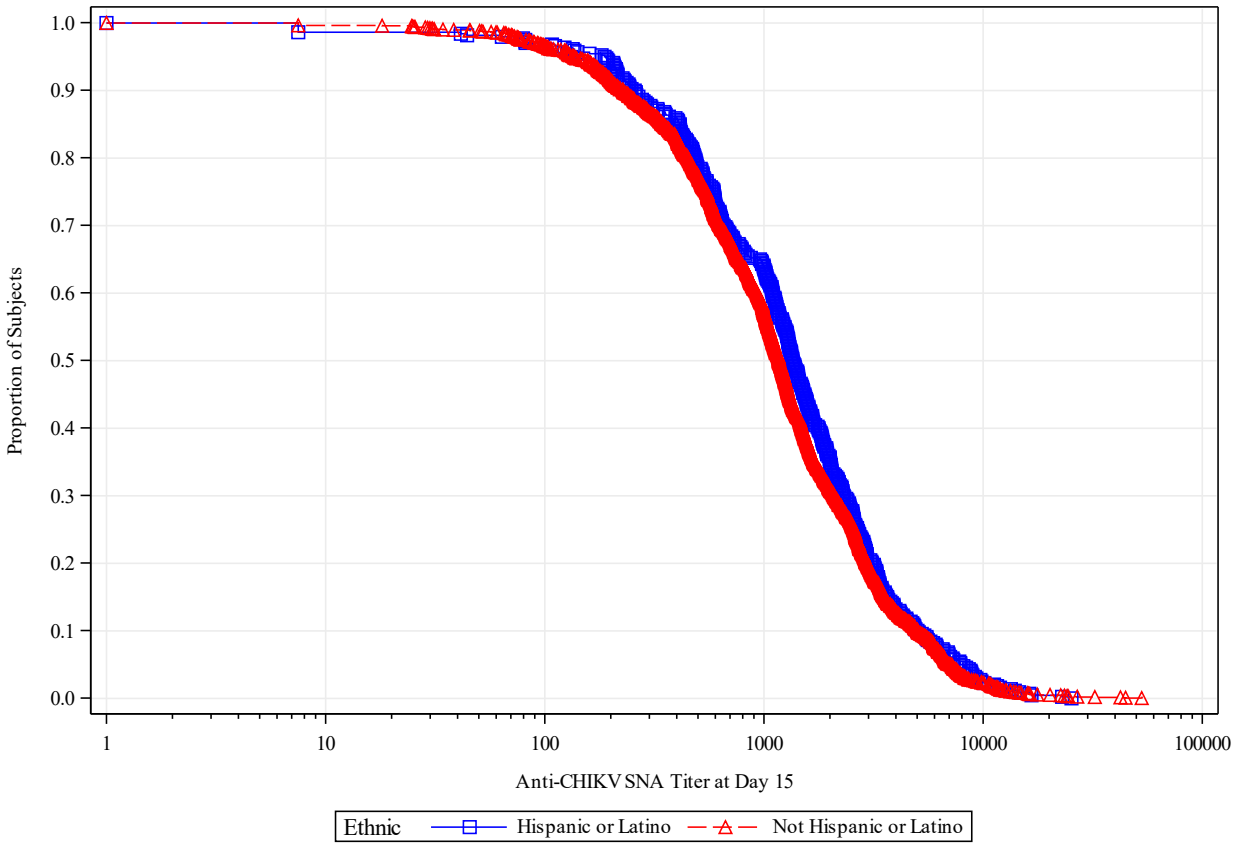


Figure 28. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Ethnicity – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

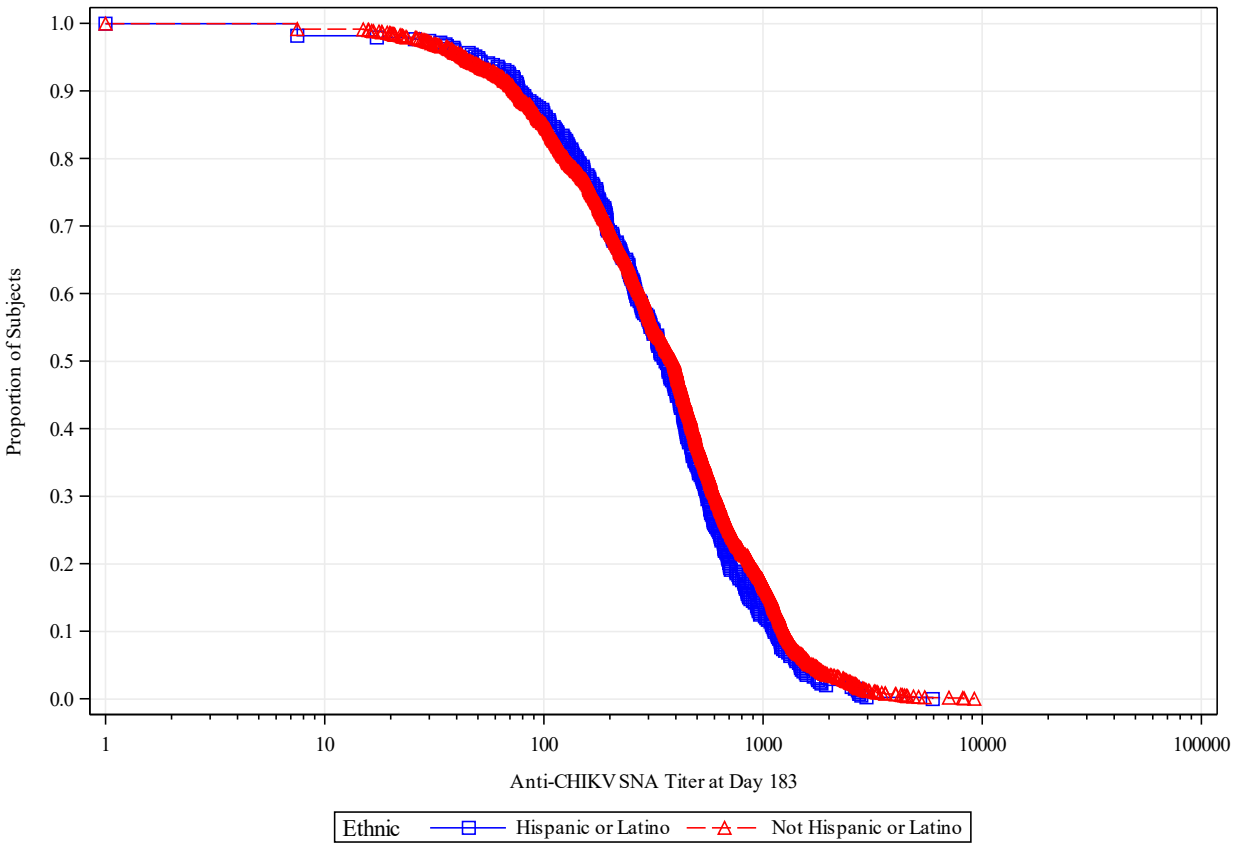
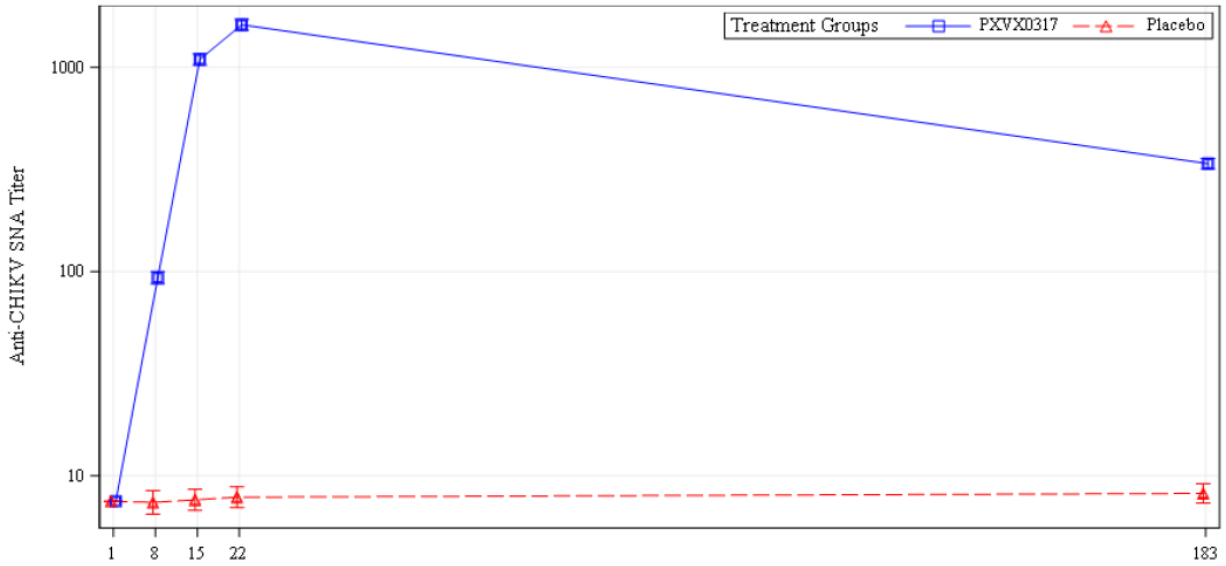


Figure 29. Geometric Mean Anti-CHIKV SNA Titers by Visit (Immunogenicity Evaluable Population, All Ages Pooled)



Note: Y-axis is represented on a $\log_{10}$ scale. Vertical bars denote the 95% confidence interval around the geometric mean, calculated based on the Newcombe hybrid score method. PXVX0317 = CHIKV VLP vaccine.

Secondary endpoint: Geometric mean fold increase (GMFI) from Day 1 to subsequent collection time points.

Figure 30. Geometric Mean Fold Increase in Anti-CHIKV SNA Titers by Visit (Immunogenicity Evaluable Population, All Ages Pooled)

Note: Y-axis is represented on a $\log_{10}$ scale. Geometric mean fold increase from Day 1 (baseline) is (Day x/Day 1). Vertical bars denote the 95% confidence interval around the geometric mean fold increase, calculated based on the t-statistics assuming a normal distribution of the log fold increase in titer. PXVX0317 = CHIKV VLP vaccine.

Secondary endpoint: Number and percentage of participants with an anti-CHIKV SNA titre ≥15 and 4-fold rise over baseline.

Table 45. Participants with Anti-CHIKV SNA titre at or above Selected Titers (≥15 and 4-fold rise over baseline) by Treatment Group and Visit (Immunogenicity Evaluable Population)

Study Day Statistic	Pooled PXVX0317 (N=2559) n (%)	Placebo (N=424) n (%)	Seroresponse Rate Difference [95% CI][a]	p-value[b]
Day 8				
n[c]	2510	419		
n (%) titer ≥15 [95% CI][d]	2306 (91.9%) [90.7%, 92.9%]	5 (1.2%) [0.5%, 2.8%]	90.7% [88.7%, 91.9%]	<0.0001
n (%) titer ≥ 4-fold rise [95% CI][d]	1644 (65.5%) [63.6%, 67.3 %]	3 (0.7%) [0.2%, 2.1%]	64.8% [62.5%, 66.7%]	<0.0001
Day 15				
n[c]	2434	395		
n (%) titer ≥15 [95% CI][d]	2421 (99.5%) [99.1%, 99.7%]	3 (0.8%) [0.3%, 2.2%]	98.7% [97.2%, 99.3%]	<0.0001
n (%) titer ≥ 4-fold Rise [95% CI][d]	2399 (98.6%) [98.0%, 99.0%]	3 (0.8%) [0.3%, 2.2%]	97.8% [96.3%, 98.4%]	<0.0001
Day 22				
n[c]	2559	424		
n (%) titer ≥15 [95% CI][d]	2539 (99.2%) [98.8%, 99.5%]	7 (1.7%) [0.8%, 3.4%]	97.6% [95.8%, 98.5%]	<0.0001
n (%) titer ≥ 4-fold Rise [95% CI][d]	2524 (98.6%) [98.1%, 99.0%]	6 (1.4%) [0.7%, 3.1%]	97.2% [95.5%, 98.1%]	<0.0001
Day 183				
n[c]	2301	401		
n (%) titer ≥15 [95% CI][d]	2279 (99.0%) [98.6%, 99.4%]	9 (2.2%) [1.2%, 4.2%]	96.8% [94.8%, 97.9%]	<0.0001
n (%) titer ≥ 4-fold Rise [95% CI][d]	2138 (92.9%) [91.8%, 93.9%]	6 (1.5%) [0.7%, 3.2%]	91.4% [89.4%, 92.7%]	<0.0001

Data Source: Table 14.2.5, Listing 16.2.5

CI = confidence interval; IEP = Immunogenicity Evaluable Population; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody.

Note: By definition, the IEP has no measurable anti-CHIKV SNA at Day 1, therefore Day 1 is omitted from this table.

[a] Seroresponse rate difference is (PXVX0317 minus placebo); 95% CIs are based on the Newcombe hybrid score method.

[b] p-value is from a two-sided chi-square test of equality of seroresponse percentages between groups.

[c] n is the number of participants with a sample result available at the indicated visit.

[d] 95% CIs of seroresponse rates are based on the Wilson method.

- ***Exploratory immunogenicity endpoint:***

Anti-CHIKV SNA seroresponse rate difference with associated 95% CIs for each pair of CHIKV VLP vaccine lots (104 minus 105, 105 minus 106, 104 minus 106) (adults 18 to <46 years of age) at Day 22.

Table 46. Day 22 Pairwise Lot Comparison of Anti-CHIKV SNA Seroresponse Rate (Immunogenicity Evaluable Population, Adults 18 to <46 Years)

Study Day 22 Statistic	Seroresponse PXVX0317 Lot 104 (N=488)	Seroresponse PXVX0317 Lot 105 (N=498)	Seroresponse PXVX0317 Lot 106 (N=494)
Participants with titer ≥100 (n/N, %) [95% CI][a]	479/488 (98.2%) [96.5%, 99.2%]	491/498 (98.6%) [97.1%, 99.4%]	485/494 (98.2%) [96.6%, 99.2%]
	Seroresponse Rate Difference		
Seroresponse Rate Difference Lot 104: Lot 105 [95% CI][b] p-value[c]	-0.4% [-2.2%, 1.3%] 0.6230	-	-
Seroresponse Rate Difference Lot 105: Lot 106 [95% CI][b] p-value[c]	0.4% [-1.3%, 2.2%] 0.6250	-	-
Seroresponse Rate Difference Lot 104: Lot 106 [95% CI][b] p-value[c]	-0.0% [-1.9%, 1.8%] 1.0000	-	-

Data Source: Listing 16.2.5, Table 14.2.6
CI = confidence interval; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody.
[a] 95% CIs of seroresponse rates are based on the Wilson method.
[b] 95% CIs of pairwise differences in seroresponse rates are based on the Newcombe hybrid score method.
[c] p-value is from a two-sided chi-square test of equality of seroresponse rates between groups.

Pre-defined and ad hoc important subgroup analyses

Immunogenicity in Baseline Seropositive Participants:

There were 69 baseline seropositive participants (defined as Day 1 pre-dose anti-CHIKV titre ≥15 [≥LLOQ]) in the EBSI-CV-317-004 study. Of these, 63 participants were in the CHIKV VLP group and 6 were in the placebo group. The discussion in this section pertains to the 63 baseline seropositive participants in the CHIKV VLP group.

An ad hoc analysis found that baseline seropositive CHIKV VLP vaccine group participants tended to have much higher immunogenicity than that for baseline seronegative CHIKV VLP vaccine group participants, as measured by SNA GMT. Seropositive participants had a Day 1 (baseline) GMT of 317, a Day 8 GMT of 1148, a Day 15 GMT of 3799, a Day 22 GMT of 3969, and a Day 183 GMT of 1140. Comparatively, seronegative CHIKV VLP vaccine group participants (the IEP) had a Day 1 GMT below the LLOQ, a Day 8 GMT of 93, a Day 15 GMT of 1096, a Day 22 GMT of 1618, and a Day 183 GMT of 338. Despite the small sample size among baseline seropositive CHIKV VLP vaccine recipients (n=63), this group appears to have had a notably higher immune response compared to that of baseline seronegative vaccine recipients at all subsequent timepoints.

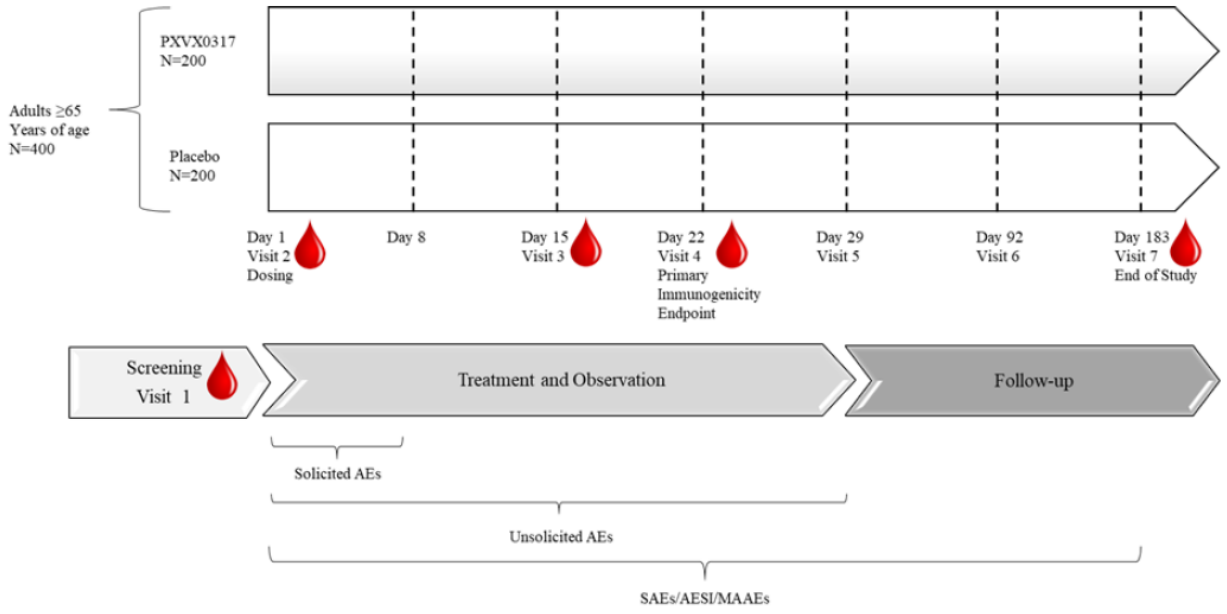
2.6.5.2.2. EBSI-CV-317-005

Methods

Study design

EBSI-CV-317-005 was a phase 3, randomized, double-blind, placebo-controlled, parallel-group study with two treatment groups. A total of 400 healthy participants ≥65 years of age were planned for enrolment and stratified in two age subgroups (≥65 to <75 and ≥75 years of age). Participants were randomized in a 1:1 ratio to receive either a single IM dose of CHIKV VLP vaccine or placebo at Day 1.

Figure 31. EBSI-CV-317-005: Study schema



Note: Days 29 and 92 were phone call follow-ups.

The per participant estimated total study duration was 212 days (including screening). The screening window was to be no greater than 30 days prior to Day 1. Day 1 included randomization and administration of investigational product (IP) (either CHIKV VLP vaccine or placebo). The immune response to CHIKV VLP vaccine was measured by anti-CHIKV SNA prior to vaccination on Day 1 and on Days 15, 22, and at the Day 183 End of Study (EOS) Visit.

- **Study Participants**

This multicentre study was conducted in the US, using 10 sites.

Inclusion criteria were standard as for all clinical trials and included generally healthy males and females 65 years of age or older.

Exclusion criteria were also standard and among others, included prior receipt of a CHIK vaccine or investigational vaccines and congenital or acquired immunodeficiency that could impact the response to vaccination or anticipated use of systemic immunomodulatory or immunosuppressive medications from six months prior to screening through Day 22.

- **Treatments**

Participants received either CHIKV VLP vaccine (lot number 2861-104A) as a single dose of 40 µg CHIKV VLP combined with 300 µg aluminium hydroxide or placebo or placebo.

- **Prior and Concomitant Therapy**

At the Screening Visit, the details of prior and concomitant medication usage were collected (through 30 days prior to screening, or 90 days for blood products, or six months prior to screening for systemic immunomodulatory or immunosuppressive medications). Concomitant medications were collected at each on site visit or by phone interview (or early discontinuation/withdrawal) for all groups. The details of all concomitant medications including those associated with solicited AEs and unsolicited AEs were collected though Day 29. Concomitant medications associated with SAEs, AESI, and MAAEs were collected through EOS.

- **Objectives and endpoints**

Primary objectives

- To compare the anti-CHIKV SNA response to CHIKV VLP vaccine and placebo at Day 22, as measured by geometric mean titre (GMT) and clinically relevant difference in seroresponse rate (CHIKV VLP vaccine minus placebo) in adults ≥65 years of age.

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA titre ≥100.

- To evaluate the safety of CHIKV VLP vaccine in adults ≥65 years of age.

Primary endpoints

Safety endpoints:

- Incidence of solicited adverse events (AEs) through Day 8 for CHIKV VLP vaccine and placebo.
- Incidence of unsolicited AEs through Day 29 for CHIKV VLP vaccine and placebo.
- Incidence of serious AEs (SAEs) medically attended AEs (MAAEs), and AEs of special interest (AESI) through Day 183 for CHIKV VLP vaccine and placebo.

Co-primary immunogenicity endpoints:

- Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) and associated 95% confidence interval (CI) at Day 22.
    - Success criterion: Statistical superiority to placebo and lower bound of the two-sided 95% CI on the difference in seroresponse rates between CHIKV VLP vaccine and placebo groups ≥70% (considered clinically significant).

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA titre ≥100.

- Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for CHIKV VLP vaccine and placebo.
    - Success criterion: Significant difference across all age groups combined using an analysis of variance (ANOVA) model with logarithmically transformed anti-CHIKV SNA titers (log10) as the dependent variable and treatment group and study site as the fixed effects with a two-sided significance level of 0.05.

Secondary objectives

- To compare the anti-CHIKV SNA response to CHIKV VLP vaccine and placebo at Day 15 and Day 183, as measured by GMT and seroresponse rate.

- To compare the anti-CHIKV SNA response to CHIKV VLP vaccine and placebo in participants ≥65 to <75 and ≥75 years of age as measured by GMT and seroresponse rate.

Secondary endpoints

- **Key secondary endpoints:** Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) with associated 95% CI at Day 15 and Day 183, in that order.
- Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at Day 15 and Day 183.
- Geometric mean fold increase (GMFI) from Day 1 to subsequent collection time points.
- Number and percentage of participants with an anti-CHIKV SNA titre ≥15 and 4-fold rise over baseline.

Exploratory objective

- To evaluate the ability of CHIKV VLP vaccine-induced CHIKV antibodies to neutralize various CHIKV genotypes.

Exploratory endpoint

- Geometric mean titers and associated two-sided 95% CIs for neutralizing antibodies against various CHIKV genotypes measured at Day 22 (and at baseline if necessary) for a subset of participants in the CHIKV VLP vaccine group.

- **Sample size**

Based on the data from the phase 2 study PXVX-CV-317-001, the seroresponse rate for CHIKV VLP vaccine was expected to be approximately 90% versus <5% for the placebo participants. With an assumed 10% rate of non-evaluable participants, the power to show superiority over placebo with 180 CHIKV VLP vaccine and 180 placebo evaluable participants was >99.9% for the combined age groups.

- **Randomisation and blinding (masking)**

Participants were randomized on Day 1 following confirmation of eligibility and immediately prior to IP administration. Participants were stratified by age subgroups (65 to <75 years and ≥75 years of age), with a target of 25% enrolment of participants ≥75 years of age. Participants were randomized to PXVX0317 or placebo in a 1:1 ratio.

Study participants, the investigator, and study site personnel remained blinded to all randomization assignments throughout the study. The sponsor's MM and personnel, who were in regular contact with the study site and/or involved with documentation associated with the study, remained blinded to all participant randomization assignments. Additional safeguards were employed to reduce the risk of inadvertent unblinding.

- **Statistical methods**

Planned analysis

The primary comparison was in the IEP across all age groups combined. Tests were repeated in the mITT population as a measure of robustness, along with tests for each population in the separate age strata. No multiplicity adjustment was made for the analysis of the separate age strata as the primary population was the combined age groups.

The superiority of the immune response to CHIKV VLP vaccine over that to placebo was demonstrated at Day 22 by comparing seroresponse rates (the proportion of participants with a human SNA assay ≥100; considered the presumptive seroprotection rate) between the two treatment groups. The difference in seroresponse rates between the CHIKV VLP vaccine and placebo groups were calculated, along with the 95% CI for the difference based on the Newcombe hybrid score method. The lower bound of the two-sided 95% CI on the difference in seroresponse rates between CHIKV VLP vaccine and placebo groups had to be ≥70% (clinical significance).

Day 22 GMTs were compared between CHIKV VLP vaccine and placebo treatment groups and were analysed via a linear model based on an alpha=0.05. The primary model was an ANOVA, with logarithmically transformed anti-CHIKV SNA titers (log10) as the dependent variable and treatment group and study site as the fixed effects in the model. The adjusted least square means and their 95% CIs calculated based on the ANOVA were back transformed and reported as the group GMT values.

Beyond the inclusion of site in the immunogenicity ANOVA models described in Section 6.1, no further adjustment for covariates is performed.

Participants were included in the analyses to the extent of their available data; missing immunogenicity data were not imputed.

Planned subgroup analysis

Immunogenicity analyses are conducted for both age strata pooled as well as by separate age strata.

Error probabilities, adjustment for multiplicity and interim analyses

The familywise error rate is fixed at a two-sided alpha of 0.05 by the requirement that both coprimary endpoints must be met for a successful outcome. Seroresponse key secondary immunogenicity endpoints are tested using a prespecified hierarchical strategy to preserve the type I error rate without the need for further multiplicity adjustment.

- **Study assessments**

Prior to study vaccine administration on Day 1 (baseline), eligibility, medical history and concomitant medications were reviewed, and a baseline anti-CHIKV SNA sample was drawn. Vital signs including blood pressure, heart rate, respiratory rate, and temperature were taken on Day 1 before and after IP administration. Directed physical examinations were performed if indicated at Days 1, 15, 22, and Day 183 EOS Visit. No safety laboratory samples were collected as part of the study.

The immune response to CHIKV VLP vaccine was measured by anti-CHIKV SNA prior to vaccination on Day 1 and on Days 15, 22, and at the Day 183 End of Study (EOS) Visit.

A preliminary analysis of safety and immunogenicity was performed after the last participant reached Day 29. This analysis was conducted by an unblinded vendor (Catalyst Clinical Research) and reported at the treatment group summary level only, without unblinding the sponsor study team to individual participant treatment assignments. A final analysis of data collected throughout the study from all participants was performed after the last participant completed the study and the immunogenicity and safety data had been locked and unblinded.

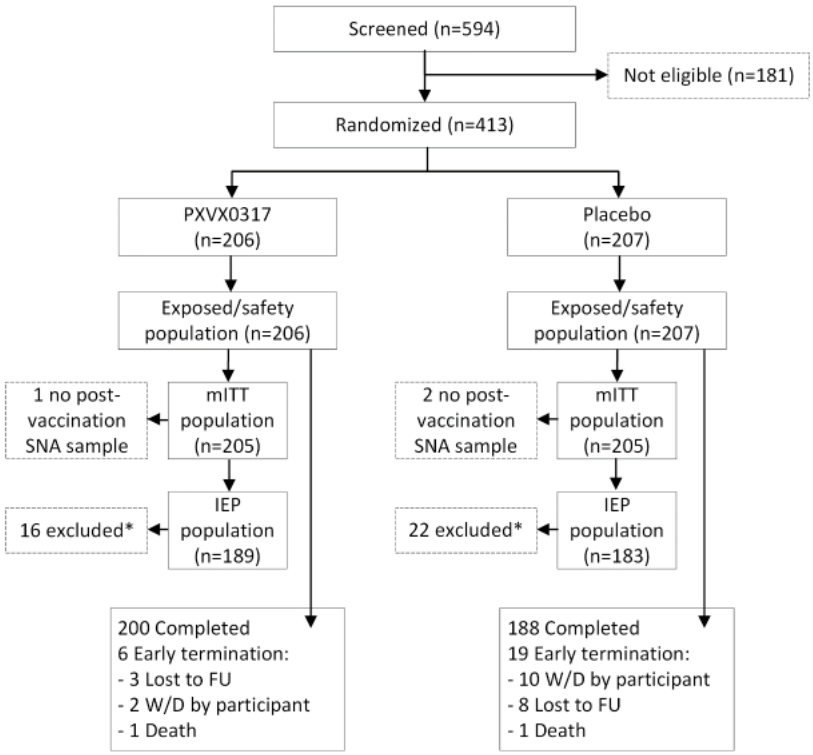
An independent Safety Monitoring Committee (SMC) provided safety oversight. The SMC reviewed aggregated, blinded safety data after the first 50 participants had completed 7 days of safety follow-up. The remaining participants were enrolled following the safety review by the SMC and based on the sponsor's consideration of the SMC's recommendation. A detailed scope of the safety review was provided in the SMC Charter and meeting outcomes were recorded in meeting minutes.

Results

- **Participant flow and numbers analysed**

The first participant was enrolled on 12-May-2022 and the last participant completed on 05-Jun-2023. A final analysis of data collected throughout the study from all participants was performed after the last participant completed the study and the immunogenicity and safety data had been locked and unblinded.

Figure 32. Disposition Flowchart of Participants for Study EBSI-CV-317-005



*Exclusions from IEP were due to: No SNA result in Day 22 window, measurable (baseline positive, ≥LLOQ) Day 1 SNA, prohibited vaccine/medication use, eligibility criteria not met, missing Day 1 SNA sample, and/or serum processing error.
FU = follow up; IEP = Immunogenicity Evaluable Population; LLOQ = lower limit of quantitation; mITT = modified intent-to-treat; PXVX0317 = CHIKV VLP vaccine; SNA = serum neutralizing antibody; W/D = withdrawn
Exposed Population = Randomized participants who received study vaccination.
Safety Population = Exposed participants who provided safety assessment data.
mITT Population = Exposed participants who had at least one post-injection CHIKV SNA result.
IEP = mITT population participants who had no measurable CHIKV SNA at Day 1, had an evaluable Day 22 serum sample result within window (Day 19 through Day 27, inclusive), and had no important exclusionary protocol deviation or other reason for exclusion as defined prior to unblinding.

Table 47. Summary of Participant Disposition (All Screened Participants)

Disposition	PXVX0317 (N=206) n (%)	Placebo (N=207) n (%)	Total (N=413) n (%)
All screened	-	-	594
Randomized	206 (100)	207 (100)	413 (100)
Treated/Exposed	206 (100)	207 (100)	413 (100)
Completed study	200 (97.1)	188 (90.8)	388 (93.9)
Primary reason for not completing study:			
Death	1 (0.5)	1 (0.5)	2 (0.5)
Lost to follow up	3 (1.5)	8 (3.9)	11 (2.7)
Withdrawn by participant	2 (1.0)	10 (4.8)	12 (2.9)
Study visit completion (based on database visit):			
Day 1	206 (100)	207 (100)	413 (100)
Day 15	197 (95.6)	198 (95.7)	395 (95.6)
Day 22	199 (96.6)	195 (94.2)	394 (95.4)
Day 29	203 (98.5)	203 (98.1)	406 (98.3)
Day 92	200 (97.1)	196 (94.7)	396 (95.9)
Day 183	200 (97.1)	188 (90.8)	388 (93.9)

Data Source: Listing 16.2.1.1, Listing 16.2.3, Table 14.1.2, Table 14.1.3
PXVX0317 = CHIKV VLP vaccine
Note: Percentages are based on the number of randomized participants in each treatment group. An individual participant was considered completed based on the Day 183 visit and completion of any required safety follow-up.

Table 48. Timing of Blood Sample Collection Randomized Population

Time Point Blood Sample Collection In Window or Out of Window	PXVX0317 (N=206) n (%)	Placebo (N=207) n (%)	Total (N=413) n (%)
Day 1 Blood Sample Collection			
Yes	206 (100)	206 (99.5)	412 (99.8)
Day 1 before Vaccination (or earlier)	206 (100)	206 (99.5)	412 (99.8)
Out of Window (after Vaccination)	0	0	0
No	0	1 (0.5)	1 (0.2)
Day 15 Blood Sample Collection			
Yes	197 (95.6)	200 (96.6)	397 (96.1)
Day 14 to Day 18	197 (95.6)	198 (95.7)	395 (95.6)
Out of Window	0	2 (1.0)	2 (0.5)
No	9 (4.4)	7 (3.4)	16 (3.9)
Day 22 Blood Sample Collection			
Yes	201 (97.6)	197 (95.2)	398 (96.4)
Day 19 to Day 27 (Analysis Window)	198 (96.1)	192 (92.8)	390 (94.4)
Out of Analysis Window	3 (1.5)	5 (2.4)	8 (1.9)
Day 21 to Day 25 (Protocol Window)	189 (91.7)	186 (89.9)	375 (90.8)
Out of Protocol Window	12 (5.8)	11 (5.3)	23 (5.6)
No	5 (2.4)	10 (4.8)	15 (3.6)

Table 49. Summary of Analysis Population Membership for All Enrolled Participants

Participant Population	PXVX0317 (N=206) n (%)	Placebo (N=207) n (%)	Total (N=413) n (%)
Randomized	206 (100)	207 (100)	413 (100)
Exposed Population[a]	206 (100)	207 (100)	413 (100)
Safety Population[b]	206 (100)	207 (100)	413 (100)
Modified Intent-to-treat (mITT) Population[c]	205 (99.5)	205 (99.0)	410 (99.3)
Immunogenicity Evaluable Population (IEP)[d]	189 (91.7)	183 (88.4)	372 (90.1)

Data Source: Listing 16.2.3, Table 14.1.2
N = number of participants per group; n = number of participants per parameter; PXVX0317 = CHIKV VLP vaccine
Note: Percentages are based on the number of randomized participants in each treatment group.
[a] Exposed Population = Randomized participants who received study vaccination.
[b] Safety Population = Exposed participants who provided safety assessment data.
[c] mITT Population = Exposed participants who had at least one post-injection anti-CHIKV SNA result.
[d] IEP = mITT population participants who had no measurable anti-CHIKV SNA at Day 1, had an evaluable Day 22 serum sample result within window (Day 19 through Day 27, inclusive), and had no important exclusionary protocol deviation or other reason for exclusion as defined prior to unblinding.

Reasons for exclusion from the IEP included 23 (5.6%) participants with no Day 22 SNA result in window, 5 (1.2%) with exclusionary PDs, 1 (0.2%) with no Day 1 SNA result, and 15 (3.6%) with measurable Day 1 SNA result. Note that participants could have more than one reason for exclusion. The IEP included 372 participants: 189 received CHIKV VLP vaccine and 183 received placebo.

- **Conduct of the study**

No participants received the wrong treatment or incorrect dose. There were 5 participants who received an excluded concomitant medication or vaccine.

Blood sample collection was performed in 412 (99.8%) participants on Day 1. Postbaseline blood sample collection tabulations used analysis visit windows and not database visit windows to match the IEP immunogenicity analysis. On Day 15, blood sample collection was performed in 395 (95.6%) participants with an additional 2 (0.5%) out of window and 16 (3.9%) missed blood collections; on Day 22 (primary endpoint) blood sample collection was performed in 390 (94.4%) participants with an additional 8 (1.9%) out of analysis window (Day 19 to Day 27) and 15 (3.6%) missed blood collections; on Day 183 blood collection was performed in 389 (94.2%) participants with 0 out of analysis window (Day 96 or higher) and 24 (5.8%) missed blood collections.

Overall, there were 95 (23.0%) participants with at least one iPD during the study. A total of 83 (20.1%) participants had study visit related iPDs including out of window or visits not done, 7 (1.7%) had laboratory assessment iPDs including missing results of sample collection date and/or time incomplete, 4 (1.0%) had noncompliance iPDs if they missed at least half of the required solicited AE entries in the eDiary or paper diary, 3 (0.7%) had eligibility criteria iPDs, and 3 (0.7%) had safety monitoring iPDs.

- **Baseline data**

Table 50.Summary of Study Population Demographics by Treatment Group (Randomized Population)

Parameter	PXVX0317 (N=206) n (%)	Placebo (N=207) n (%)	Total (N=413) n (%)
Age (years)			
Mean (SD)	71 (5.3)	71 (4.5)	71 (4.9)
Median	70	70	70
Min, Max	65, 95	65, 84	65, 95
Sex, n (%)			
Male	81 (39.3)	90 (43.5)	171 (41.4)
Female	125 (60.7)	117 (56.5)	242 (58.6)
Age, n (%)			
≥65 to <75 years	159 (77.2)	159 (76.8)	318 (77.0)
≥75 years	47 (22.8)	48 (23.2)	95 (23.0)
Race, n (%)			
White	176 (85.4)	168 (81.2)	344 (83.3)
American Indian or Alaska Native	1 (0.5)	1 (0.5)	2 (0.5)
Asian	4 (1.9)	1 (0.5)	5 (1.2)
Black or African American	20 (9.7)	29 (14.0)	49 (11.9)
Multiracial	4 (1.9)	5 (2.4)	9 (2.2)
Not reported	1 (0.5)	3 (1.4)	4 (1.0)
Ethnicity, n (%)			
Hispanic or Latino	93 (45.1)	90 (43.5)	183 (44.3)
Not Hispanic or Latino	112 (54.4)	116 (56.0)	228 (55.2)
Not reported	1 (0.5)	1 (0.5)	2 (0.5)
Height (cm)			
Mean (SD)	166.4 (9.42)	167.1 (9.84)	166.8 (9.63)
Median	165.1	167.6	166.6
Min, Max	144.8, 195.6	142.2, 190.5	142.2, 195.6
Weight (kg)			
Mean (SD)	75.8 (13.61)	77.2 (13.29)	76.5 (13.45)
Median	76.2	75.4	76.2
Min, Max	47.2, 110.2	44.9, 111.6	44.9, 111.6
BMI (kg/m^2)			
Mean (SD)	27.3 (3.98)	27.6 (3.90)	27.5 (3.94)
Median	27.5	27.5	27.5
Min, Max	17.5, 34.8	19.3, 34.9	17.5, 34.9
Baseline Anti-CHIKV SNA Serostatus, n (%)			
Negative (<LLOQ)	201 (97.6)	196 (94.7)	397 (96.1)
Positive (≥LLOQ)	5 (2.4)	10 (4.8)	15 (3.6)
Missing	0	1 (0.5)	1 (0.2)

Data Source: Listing 16.2.4.1; Table 14.1.4.1

BMI = body mass index; LLOQ = lower limit of quantitation; Min = minimum; Max = maximum; N = number of participants per Group; n = number of participants per parameter; PXVX0317 = CHIKV VLP vaccine; SD = standard deviation; SNA = Serum Neutralizing Antibody

Note: Percentages are based on the number of participants in each treatment group. Weight and body mass index were at screening visit.

Table 51. Summary of Study Population Demographics by Age Stratum for PXVX0317 and Placebo Groups (Randomized Population)

Parameter	≥65 to <75 years (N=318) n (%)	≥75 years (N=95) n (%)	Total (N=413) n (%)
Age			
Mean (SD)	69 (2.8)	78 (3.8)	71 (4.9)
Median	69	77	70
Min, Max	65, 74	75, 95	65, 95
Sex, n (%)			
Male	132 (41.5)	39 (41.1)	171 (41.4)
Female	186 (58.5)	56 (58.9)	242 (58.6)
Race, n (%)			
White	262 (82.4)	82 (86.3)	344 (83.3)
American Indian or Alaska Native	2 (0.6)	0	2 (0.5)
Asian	4 (1.3)	1 (1.1)	5 (1.2)
Black or African American	38 (11.9)	11 (11.6)	49 (11.9)
Multiracial	8 (2.5)	1 (1.1)	9 (2.2)
Not reported	4 (1.3)	0	4 (1.0)

Ethnicity, n (%)			
Hispanic or Latino	140 (44.0)	43 (45.3)	183 (44.3)
Not Hispanic or Latino	177 (55.7)	51 (53.7)	228 (55.2)
Not reported	1 (0.3)	1 (1.1)	2 (0.5)
Height (cm)			
Mean (SD)	167.1 (9.79)	165.5 (9.01)	166.8 (9.63)
Median	166.8	165.6	166.6
Min, Max	144.8, 195.6	142.2, 185.2	142.2, 195.6
Weight (kg)			
Mean (SD)	77.1 (13.12)	74.6 (14.41)	76.5 (13.45)
Median	76.4	74.8	76.2
Min, Max	44.9, 111.6	50.3, 110.2	44.9, 111.6
BMI (kg/m^2)			
Mean (SD)	27.6 (3.91)	27.1 (4.02)	27.5 (3.94)
Median	27.8	26.6	27.5
Min, Max	17.5, 34.9	18.4, 34.9	17.5, 34.9
Baseline Anti-CHIKV SNA Serostatus, n (%)			
Negative (<LLOQ)	307 (96.5)	90 (94.7)	397 (96.1)
Positive (≥LLOQ)	11 (3.5)	4 (4.2)	15 (3.6)
Missing	0	1 (1.1)	1 (0.2)

Data Source: Listing 16.2.4.1; Table 14.1.4.1

BMI = body mass index; LLOQ = lower limit of quantitation; Min = minimum; Max = maximum; N = number of participants per Group; n = number of participants per parameter; SD = standard deviation; PXVX0317 = CHIKV VLP vaccine

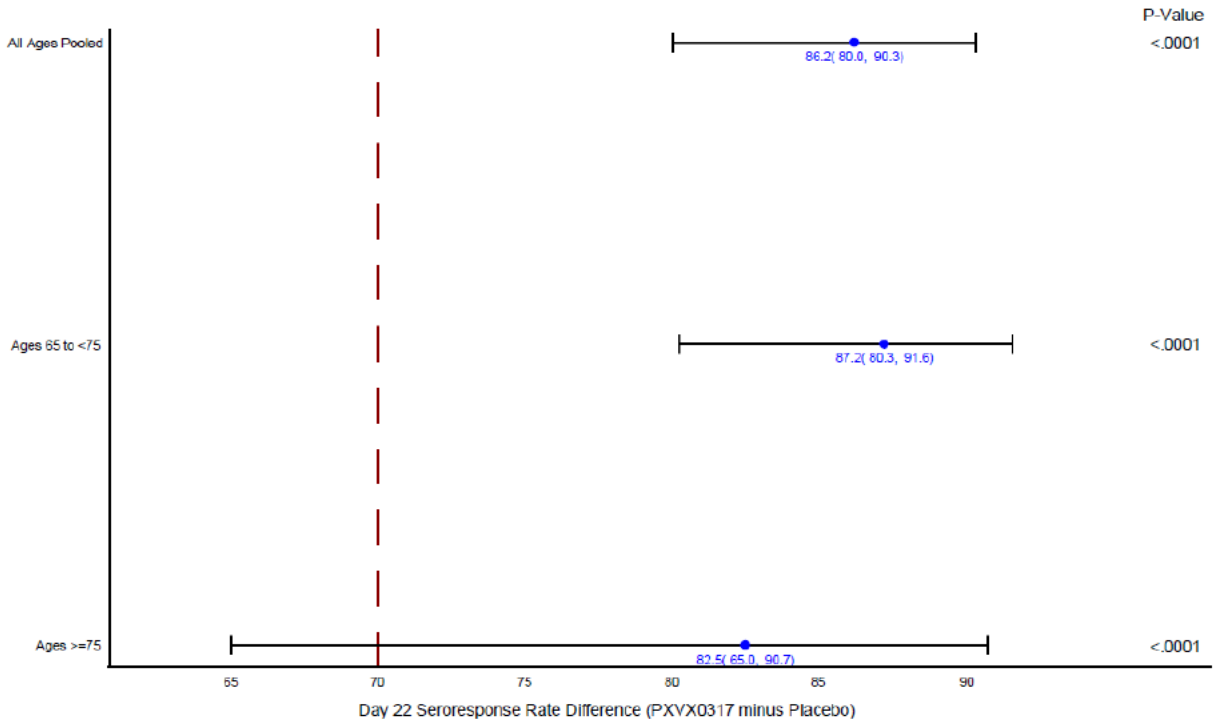
Note: Percentages are based on the number of participants in each treatment group. Weight and body mass index were at screening visit.

- **Outcomes and estimation**

Co-primary immunogenicity endpoints:

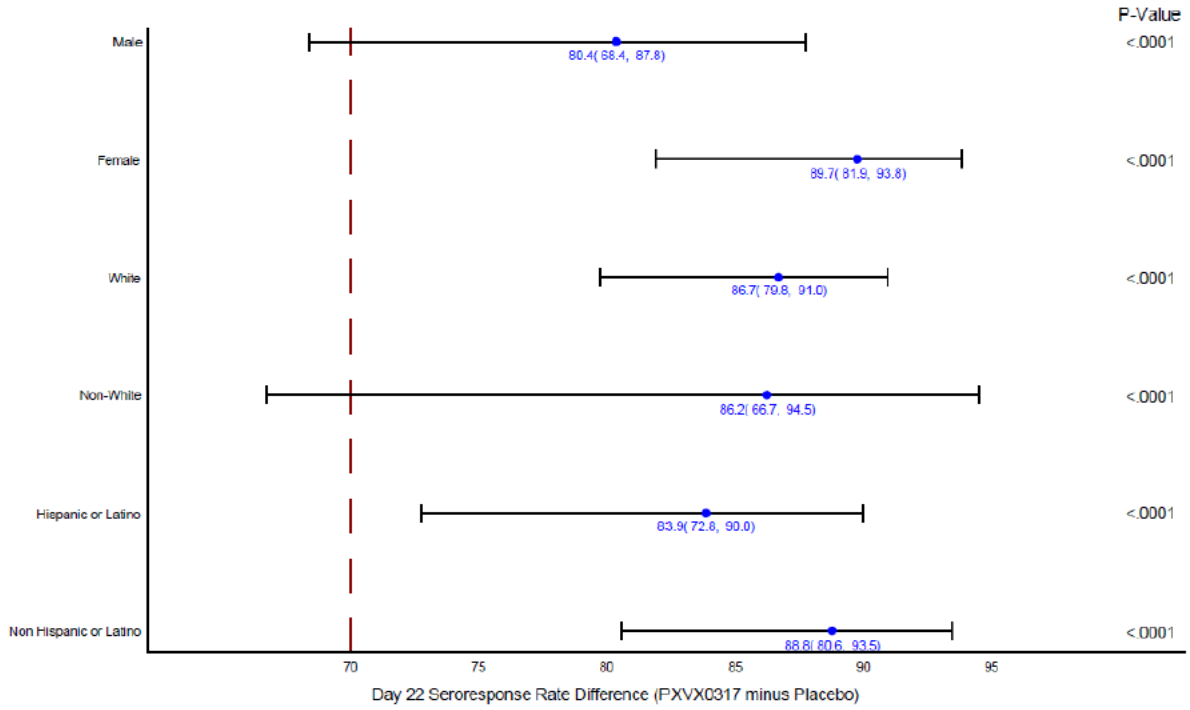
Co-primary endpoint: Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) and associated 95% confidence interval (CI) at Day 22.

Figure 33. Forest Plot of Seroresponse Rate Differences at Day 22 (Immunogenicity Evaluable Population, All Ages Pooled and by Age Group)



Reference Table: Table 14.2.1.1.1
PXVX0317 = CHIKV VLP vaccine
Note: Error bars are 95% confidence interval based on the Newcombe hybrid score method; p-values are from a two-sided chi-square test of equality of seroresponse percentages between groups.

Figure 34. Forest Plot of Seroresponse Rate Differences at Day 22 (Immunogenicity Evaluable Population, All Ages Pooled by Sex, Race, and Ethnicity Subgroups)



Reference Table: Table 14.2.1.1.2, Table 14.2.1.1.3, Table 14.2.1.1.4
PXVX0317 = CHIKV VLP vaccine
Note: Error bars are 95% confidence intervals based on the Newcombe hybrid score method; p-values are from a two-sided chi-square test of equality of seroresponse percentages between groups.

Table 52. Day 22 Difference in Anti-CHIKV SNA Seroresponse and Seroresponse Rate (Immunogenicity Evaluable Population)

Group	Subgroup	Seroresponse PXVX0317 n/N (%)[c] [95% CI][d]	Seroresponse Placebo n/N (%)[c] [95% CI][d]	Seroresponse Rate Difference [95% CI][a]	p-value[b]
All IEP	All IEP	165/189 (87.3%) [81.8, 91.3]	2/183 (1.1%) [0.3, 3.9]	86.2 [80.0, 90.3]	<0.0001
Sex	Male	58/71 (81.7%) [71.2, 89.0]	1/77 (1.3%) [0.2, 7.0]	80.4 [68.4, 87.8]	<0.0001
	Female	107/118 (90.7%) [84.1, 94.7]	1/106 (0.9%) [0.2, 5.2]	89.7 [81.9, 93.8]	<0.0001
Race	White	140/159 (88.1%) [82.1, 92.2]	2/145 (1.4%) [0.4, 4.9]	86.7 [79.8, 91.0]	<0.0001
	Non-White	25/29 (86.2%) [69.4, 94.5]	0/35 [0.0, 9.9]	86.2 [66.7, 94.5]	<0.0001
Ethnicity	Hispanic or Latino	70/81 (86.4%) [77.3, 92.2]	2/78 (2.6%) [0.7, 8.9]	83.9 [72.8, 90.0]	<0.0001
	Not Hispanic or Latino	95/107 (88.8%) [81.4, 93.5]	0/104 [0.0, 3.6]	88.8 [80.6, 93.5]	<0.0001
Age	≥65 to <75 years	131/149 (87.9%) [81.7, 92.2]	1/143 (0.7%) [0.1, 3.9]	87.2 [80.3, 91.6]	<0.0001
	≥75 years	34/40 (85.0%) [70.9, 92.9]	1/40 (2.5%) [0.4, 12.9]	82.5 [65.0, 90.7]	<0.0001

Data Source: Listing 16.2.5, Table 14.2.1.1.1, Table 14.2.1.1.2, Table 14.2.1.1.3, Table 14.2.1.1.4
CI = confidence interval; IEP = immune evaluable population; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody
[a] Seroresponse rate difference is (PXVX0317 minus placebo); 95% CIs are based on the Newcombe hybrid score method.
[b] p-value is from a two-sided chi-square test of equality of seroresponse percentages between groups.
[c] n is the number of participants with seroresponse ≥ titer 100. N is the total number of participants in the group. Seroresponse rate is n divided by N.
[d] 95% CIs of seroresponse rates are based on the Wilson method.

Table 53. Day 22 Difference in Anti-CHIKV SNA Seroresponse and Seroresponse Rate (Immunogenicity Evaluable Population), Hispanic or Latino

Hispanic or Latino

Study Day Statistic	PXVX0317 (N=81) n (%)	Placebo (N=78) n (%)	Seroresponse Rate Difference (%) [95% CI] [a]	p-value [b]
Day 22				
n [c]	81	78		
n (%) Participants with Titer >= 100 [95% CI] [d]	70 (86.4)[77.3, 92.2]	2 (2.6)[0.7, 8.9]	83.9 [72.8, 90.0]	<0.0001

Table 54. Day 22 Difference in Anti-CHIKV SNA Seroresponse and Seroresponse Rate (Immunogenicity Evaluable Population), Not Hispanic or Latino

Not Hispanic or Latino

Study Day Statistic	PXVX0317 (N=107) n (%)	Placebo (N=104) n (%)	Seroresponse Rate Difference (%) [95% CI] [a]	p-value [b]
Day 22				
n [c]	107	104		
n (%) Participants with Titer >= 100 [95% CI] [d]	95 (88.8)[81.4, 93.5]	0 (0.0)[0.0, 3.6]	88.8 [80.6, 93.5]	<0.0001

Co-primary endpoint: Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for CHIKV VLP vaccine and placebo.

Table 55. Day 22 Difference in Anti-CHIKV SNA GMT (Immunogenicity Evaluable Population)

Group	Subgroup	Statistic	PXVX0317 (N=189)	Placebo (N=183)	Ratio of GMTs [95% CI][a]	p-value[c]
All IEP	All IEP	n[b]	189	183	89.64 [68.62,117.10]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	723.93 [584.13, 897.20] 1009.60 7.5, 14518.9	8.08 [6.50, 10.04] 7.50 7.5, 1166.5		
Sex	Male	n[b]	71	77	52.56 [34.15, 80.88]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	424.67 [293.13, 615.23] 539.00 7.5, 14021.9	8.08 [5.75, 11.36] 7.50 7.5, 1166.5		
	Female	n[b]	118	106	124.62 [88.95,174.60]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	971.53 [746.70,1264.06] 1203.65 7.5, 14518.9	7.80 [5.91, 10.29] 7.50 7.5, 456.3		
Race	White	n[b]	159	145	88.08 [65.66,118.17]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	718.77 [565.89, 912.96] 963.50 7.5, 14518.9	8.16 [6.36, 10.47] 7.50 7.5, 1166.5		
	Non-White	n[b]	29	35	98.70 [48.36,201.46]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	870.55 [470.86,1609.50] 1176.30 7.5, 11907.5	8.82 [4.58, 16.97] 7.50 7.5, 7.5		
Age Group	≥65 to <75 years	n[b]	149	143	92.01 [68.69,123.27]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	726.42 [574.06, 919.20] 805.20 7.5, 14518.9	7.89 [6.22, 10.02] 7.50 7.5, 456.3		
	≥75 years	n[b]	40	40	83.59 [41.45,168.54]	<0.0001
		GMT [95% CI][c] Median[d] Min, Max[d]	716.25 [393.42,1304.01] 1127.10 7.5, 13147.9	8.57 [4.73, 15.51] 7.50 7.5, 1166.5		

Data Source: Table 14.2.2.1.1, Table 14.2.2.1.2, Table 14.2.2.1.3, Table 14.2.2.1.4, Listing 16.2.5

ANOVA = analysis of variance; CI = confidence interval; GMT = geometric mean titer; IEP = immunogenicity evaluable population; LLOQ = lower limit of quantitation; Max = maximum; Min = minimum; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody

Note: Values below lower limit of quantitation (LLOQ=15) are assigned the value LLOQ/2=7.5.

[a] Ratio of GMTs is (PXVX0317:placebo).

[b] n is the number of participants with a sample result available at the indicated visit.

[c] GMT estimates, together with their 95% CIs, are derived from an ANOVA model that includes site and treatment group as fixed effects, assuming normality of the log titers. Ratio of GMTs and 95% CIs are derived from the same model. P-value tests equivalence of group GMTs on the log scale (ie, ratio of GMTs equal to 1).

[d] Median and range are based on raw values with imputation per note.

Figure 35. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Age Group – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

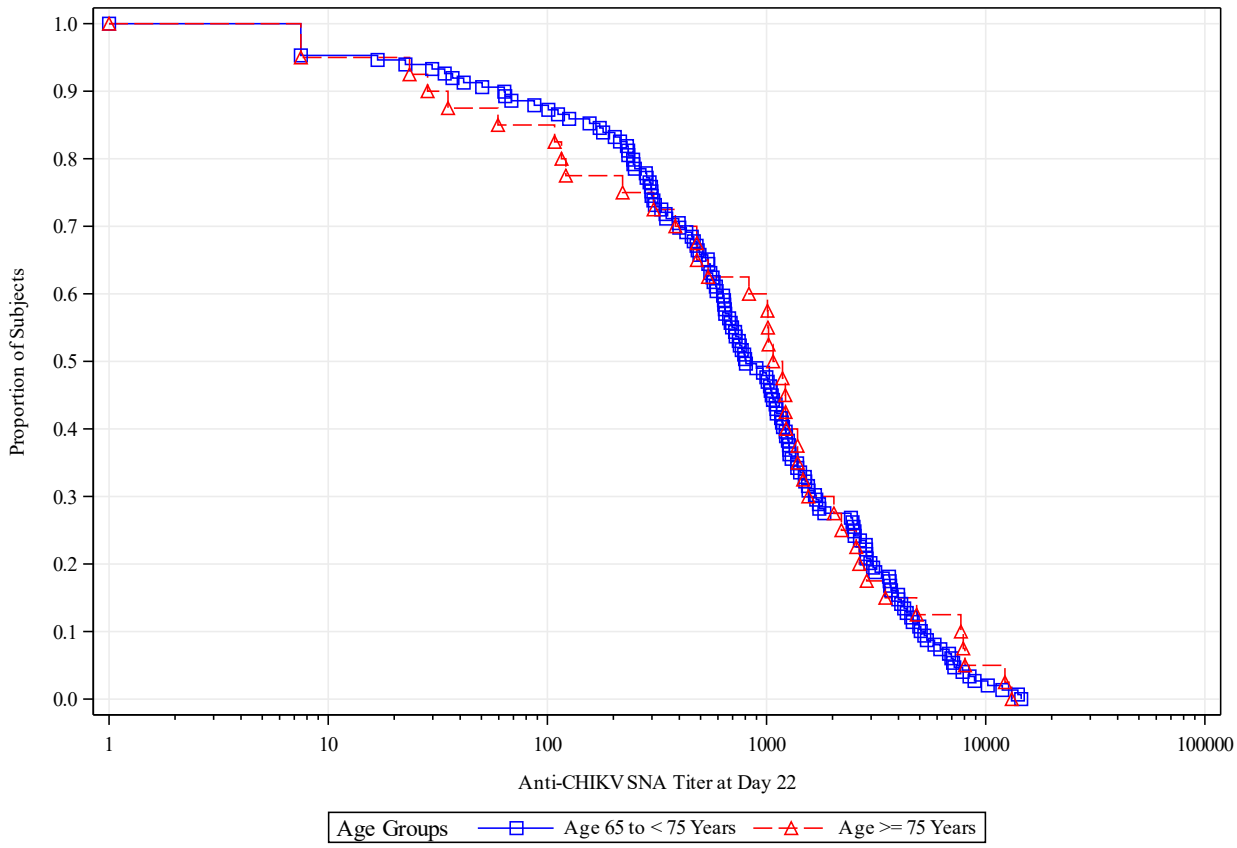


Figure 36. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Sex – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

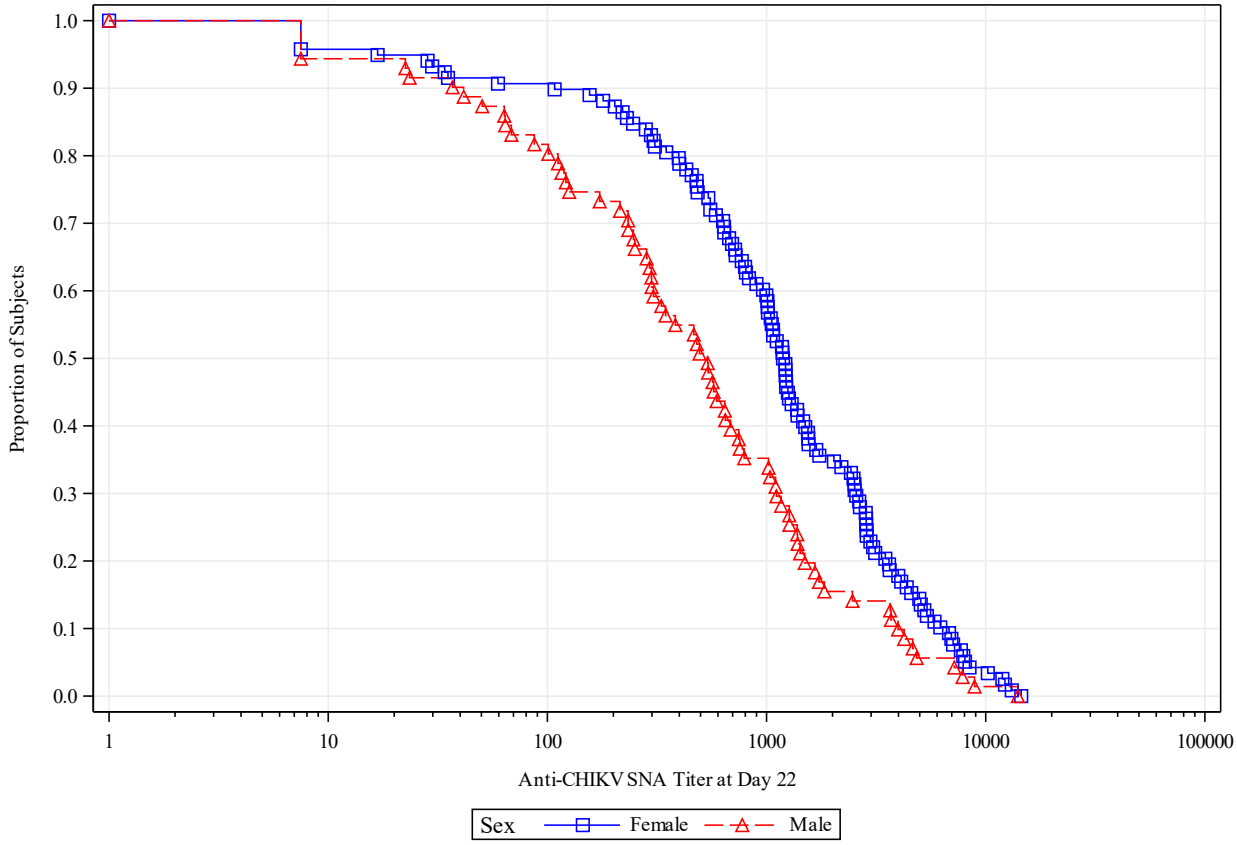


Figure 37. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Race – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

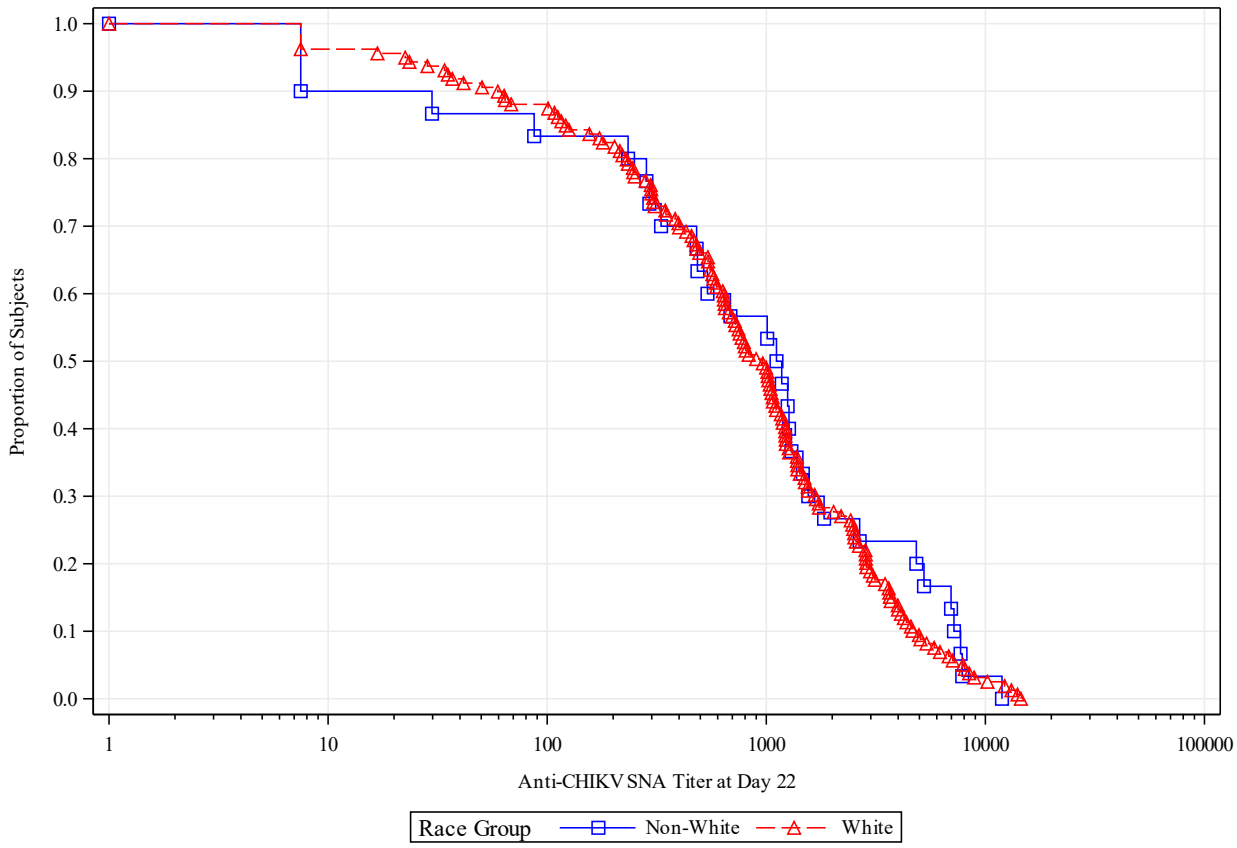


Table 56. Day 22 Difference in Anti-CHIKV SNA GMT (Immunogenicity Evaluable Population), Hispanic or Latino

Hispanic or Latino

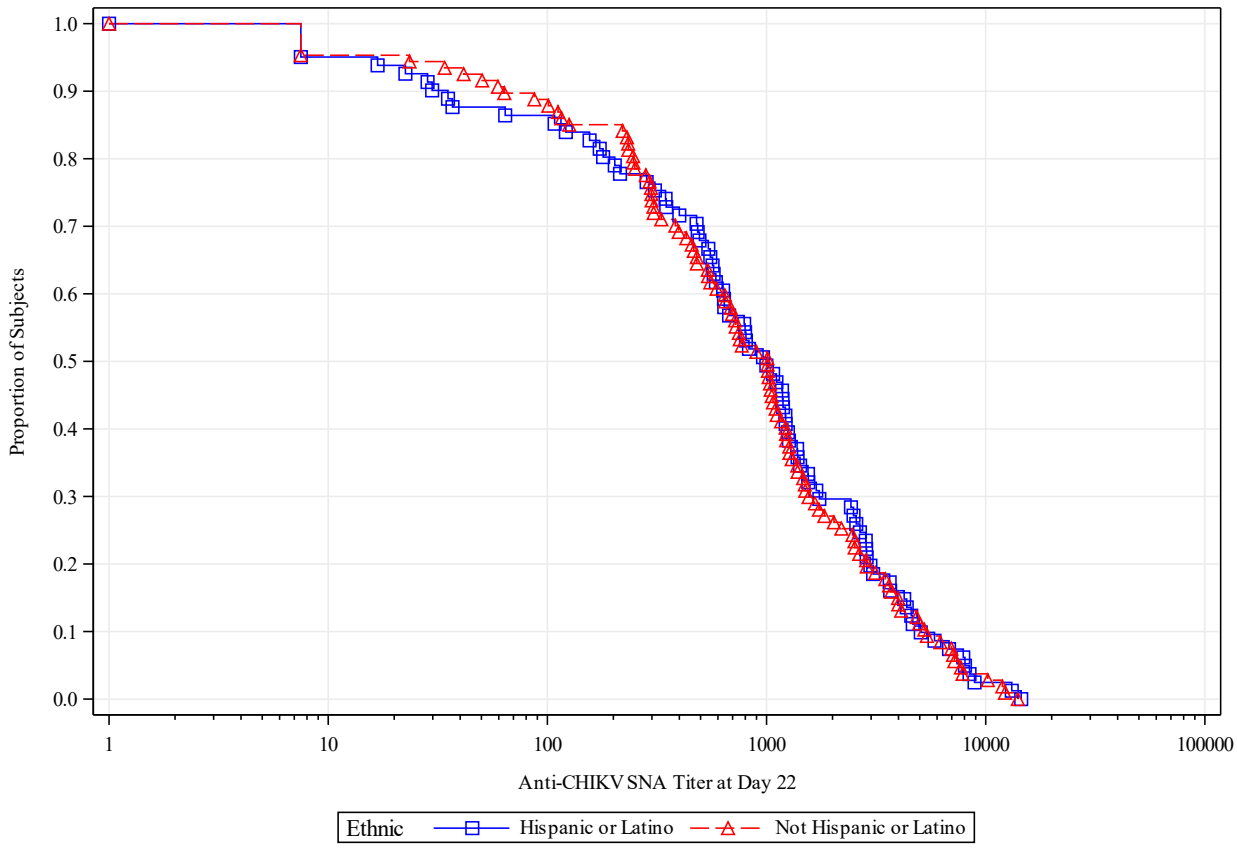
Study Day Statistic	PXVX0317 (N=81) n (%)	Placebo (N=78) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 22				
n [b]	81	78		
Geometric Mean Titer [95% CI] [c]	944.46 [495.80,1799.12]	11.80 [6.13, 22.74]	80.02 [51.22,125.00]	<0.0001
Median [d]	997.90	7.50		
Min, Max [d]	7.5, 14518.9	7.5, 1166.5		

Table 57 Day 22 Difference in Anti-CHIKV SNA GMT (Immunogenicity Evaluable Population), Not Hispanic or Latino

Not Hispanic or Latino

Study Day Statistic	PXVX0317 (N=107) n (%)	Placebo (N=104) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 22				
n [b]	107	104		
Geometric Mean Titer [95% CI] [c]	761.11 [525.21,1102.98]	7.94 [5.51, 11.43]	95.86 [68.50,134.16]	<0.0001
Median [d]	1011.90	7.50		
Min, Max [d]	7.5, 14021.9	7.5, 7.5		

Figure 38. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 22 by Ethnicity – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only



Key secondary immunogenicity endpoints:

Key secondary endpoints: Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) with associated 95% CI at Day 15 and Day 183, in that order.

Table 58. Anti-CHIKV SNA Seroresponse and Seroresponse Rate by Visit, Sex, Race, and Age (Immunogenicity Evaluable Population)

Day	Group	Subgroup	Seroresponse PXVX0317 (N=189) n/N (%)[c] [95% CI][d]	Seroresponse Placebo (N=183) n/N (%)[c] [95% CI][d]	Seroresponse Rate Difference [95% CI][a,b]
Day 15	All IEP	All IEP	149/181 (82.3) [76.1, 87.2]	5/176 (2.8) [1.2, 6.5]	79.5 [72.3, 84.6]*
	Sex	Male	51/70 (72.9) [61.5, 81.9]	3/75 (4.0) [1.4, 11.1]	68.9 [55.4, 78.3]*
		Female	98/111 (88.3) [81.0, 93.0]	2/101 (2.0) [0.5, 6.9]	86.3 [77.5, 91.3]*
	Race	White	123/151 (81.5) [74.5, 86.8]	3/139 (2.2) [0.7, 6.2]	79.3 [71.3, 84.9]*
		Non-White	26/29 (89.7) [73.6, 96.4]	2/34 (5.9) [1.6, 19.1]	83.8 [63.0, 91.8]*
	Age	≥65 to <75 years	121/144 (84.0) [77.2, 89.1]	4/138 (2.9) [1.1, 7.2]	81.1 [73.0, 86.5]*
		≥75 years	28/37 (75.7) [59.9, 86.6]	1/38 (2.6) [0.5, 13.5]	73.0 [53.9, 84.2]*
Day 183	All IEP	All IEP	139/184 (75.5) [68.9, 81.2]	2/173 (1.2) [0.3, 4.1]	74.4 [67.1, 80.1]*
	Sex	Male	43/70 (61.4) [49.7, 72.0]	1/74 (1.4) [0.2, 7.3]	60.1 [47.0, 70.7]*
		Female	96/114 (84.2) [76.4, 89.8]	1/99 (1.0) [0.2, 5.5]	83.2 [74.2, 88.8]*
	Race	White	119/155 (76.8) [69.5, 82.7]	1/135 (0.7) [0.1, 4.1]	76.0 [68.1, 82.0]*
		Non-White	20/28 (71.4) [52.9, 84.7]	1/35 (2.9) [0.5, 14.5]	68.6 [46.7, 82.1]*
	Age	≥65 to <75 years	112/147 (76.2) [68.7, 82.4]	2/135 (1.5) [0.4, 5.2]	74.7 [66.3, 81.0]*
		≥75 years	27/37 (73.0) [57.0, 84.6]	0/38 [0.0, 9.2]	73.0 [54.6, 84.6]*

Data Source: Table 14.2.1.1.1, Table 14.2.1.1.2, Table 14.2.1.1.3, Listing 16.2.5
CI = confidence interval; IEP = immunogenicity evaluable population; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody
*p-value <0.0001
[a] Seroresponse rate difference is (PXVX0317 minus placebo); 95% CIs are based on the Newcombe hybrid score method.
[b] p-value is from a two-sided chi-square test of equality of seroresponse percentages between groups.
[c] n is the number of participants with seroresponse ≥ titer 100. N is the total number of participants in the group. Seroresponse rate is n divided by N.
[d] 95% Cis of seroresponse rates are based on the Wilson method.

Table 59. Anti-CHIKV SNA Seroresponse and Seroresponse Rate by Treatment Group and Visit Immunogenicity Evaluable Population, Hispanic or Latino

Hispanic or Latino

Study Day Statistic	PXVX0317 (N=81) n (%)	Placebo (N=78) n (%)	Seroresponse Rate Difference (%) [95% CI] [a]	p-value [b]
Day 1				
n [c]	81	78		
n (%) Participants with Titer >= 100 [95% CI] [d]	0 (0.0)[0.0, 4.5]	0 (0.0)[0.0, 4.7]		
Day 15				
n [c]	77	74		
n (%) Participants with Titer >= 100 [95% CI] [d]	63 (81.8)[71.8, 88.8]	1 (1.4)[0.2, 7.3]	80.5 [68.8, 87.6]	<0.0001
Day 183				
n [c]	77	73		
n (%) Participants with Titer >= 100 [95% CI] [d]	60 (77.9)[67.5, 85.7]	0 (0.0)[0.0, 5.0]	77.9 [66.3, 85.7]	<0.0001

Table 60. Anti-CHIKV SNA Seroresponse and Seroresponse Rate by Treatment Group and Visit Immunogenicity Evaluable Population, Not Hispanic or Latino

Not Hispanic or Latino

Study Day Statistic	PXVX0317 (N=107) n (%)	Placebo (N=104) n (%)	Seroresponse Rate Difference (%) [95% CI] [a]	p-value [b]
Day 1				
n [c]	107	104		
n (%) Participants with Titer >= 100 [95% CI] [d]	0 (0.0)[0.0, 3.5]	0 (0.0)[0.0, 3.6]		
Day 15				
n [c]	103	101		
n (%) Participants with Titer >= 100 [95% CI] [d]	86 (83.5)[75.1, 89.4]	4 (4.0)[1.6, 9.7]	79.5 [69.4, 85.9]	<0.0001
Day 183				
n [c]	106	99		
n (%) Participants with Titer >= 100 [95% CI] [d]	78 (73.6)[64.5, 81.0]	2 (2.0)[0.6, 7.1]	71.6 [61.1, 79.2]	<0.0001

Secondary immunogenicity endpoints:

Secondary endpoints: Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at Day 15 and Day 183.

Table 61. Anti-CHIKV SNA Geometric Mean Titre and Geometric Mean Fold Increase from Baseline by Visit (Immunogenicity Evaluable Population)

Study Day Statistic	PXVX0317 (N=189)	Placebo (N=183)	Ratio of GMTs [95% CI][a]	p-value [c,e]
Day 1				
n[b]	189	183		
GMT[c] [95% CI] Median[d] Min, Max[d]	7.50 [7.50, 7.50] 7.50 7.5, 7.5	7.50 [7.50, 7.50] 7.50 7.5, 7.5	-	-
Day 15				
n[b]	181	176		
GMT[c] [95% CI] Median[d] Min, Max[d]	378.42 [301.04, 475.69] 499.80 7.5, 17062.5	8.96 [7.09, 11.32] 7.50 7.5, 9907.9	42.23 [32.13, 55.51]	<0.0001
GMFI[e] [95% CI] Median[d] Min, Max[d]	48.52 [40.12, 58.69] 66.64 1.0, 2275.0	1.16 [0.95, 1.40] 1.00 1.0, 1321.1	-	<0.0001
Day 22				
n[b]	189	183		
GMT[c] [95% CI] Median[d] Min, Max[d]	723.93 [584.13, 897.20] 1009.60 7.5, 14518.9	8.08 [6.50, 10.04] 7.50 7.5, 1166.5	89.64 [68.62, 117.10]	<0.0001
GMFI[e] [95% CI] Median[d] Min, Max[d]	93.94 [78.03, 113.11] 134.61 1.0, 1935.9	1.05 [0.87, 1.27] 1.00 1.0, 155.5	-	<0.0001
Day 183				
n[b]	184	173		
GMT[c] [95% CI] Median[d] Min, Max[d]	233.02 [194.05, 279.82] 284.25 7.5, 4091.2	8.29 [6.87, 10.02] 7.50 7.5, 193.9	28.09 [22.31, 35.38]	<0.0001
GMFI[e] [95% CI] Median[d] Min, Max[d]	29.32 [25.01, 34.37] 37.90 1.0, 545.5	1.04 [0.88, 1.22] 1.00 1.0, 25.9	-	<0.0001

Data Source: Table 14.2.2.1.1, Table 14.2.3, Listing 16.2.5

ANOVA = analysis of variance; CI = confidence interval; GMT = geometric mean titer; GMFI = geometric mean fold increase; LLOQ = lower limit of quantitation; Max = maximum; Min = minimum; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody

Note: Values below lower limit of quantitation (LLOQ=15) were assigned the value LLOQ/2=7.5.

[a] Ratio of GMTs is (PXVX0317:placebo).

[b] n is the number of participants with a sample result available at the indicated visit.

[c] Geometric mean titer estimates and Ratios of GMTs, together with their 95% CIs, are derived from an ANOVA model that includes site and treatment group as fixed effects, assuming normality of the log titers. p-value tests equivalence of group GMTs on the log scale.

[d] Median and range are based on raw values with imputation per Note.

[e] Geometric mean fold increase from Day 1 (baseline) is (Day x/Day 1). GMFI estimates and 95% CIs are derived from an ANOVA model that includes site and treatment group as fixed effects, assuming normality of the log fold increase in titer. p-value tests equality of log fold increase in titer between groups.

Table 62. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, subjects ≥65 to <75 years

Age 65 to <75

Study Day Statistic	PXVX0317 (N=149) n (%)	Placebo (N=143) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	149	143		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	144	138		
Geometric Mean Titer [95% CI] [c]	406.53 [317.61, 520.34]	8.97 [6.94, 11.59]	45.34 [33.77, 60.87]	<0.0001
Median [d]	491.90	7.50		
Min, Max [d]	7.5, 9258.3	7.5, 9907.9		
Day 183				
n [b]	147	135		
Geometric Mean Titer [95% CI] [c]	222.27 [181.60, 272.05]	8.28 [6.71, 10.22]	26.83 [20.79, 34.63]	<0.0001
Median [d]	268.50	7.50		
Min, Max [d]	7.5, 4091.2	7.5, 193.9		

Table 63. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, subjects ≥75 years

Age >=75

Study Day Statistic	PXVX0317 (N=40) n (%)	Placebo (N=40) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	40	40		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	37	38		
Geometric Mean Titer [95% CI] [c]	299.20 [156.16, 573.25]	8.97 [4.81, 16.72]	33.36 [15.76, 70.58]	<0.0001
Median [d]	511.50	7.50		
Min, Max [d]	7.5, 17062.5	7.5, 3783.1		
Day 22				
n [b]	40	40		
Geometric Mean Titer [95% CI] [c]	716.25 [393.42,1304.01]	8.57 [4.73, 15.51]	83.59 [41.45,168.54]	<0.0001
Median [d]	1127.10	7.50		
Min, Max [d]	7.5, 13147.9	7.5, 1166.5		
Day 183				
n [b]	37	38		
Geometric Mean Titer [95% CI] [c]	280.69 [172.33, 457.19]	8.87 [5.51, 14.27]	31.65 [17.77, 56.38]	<0.0001
Median [d]	419.20	7.50		
Min, Max [d]	7.5, 3247.3	7.5, 7.5		

Figure 39. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Age Group – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

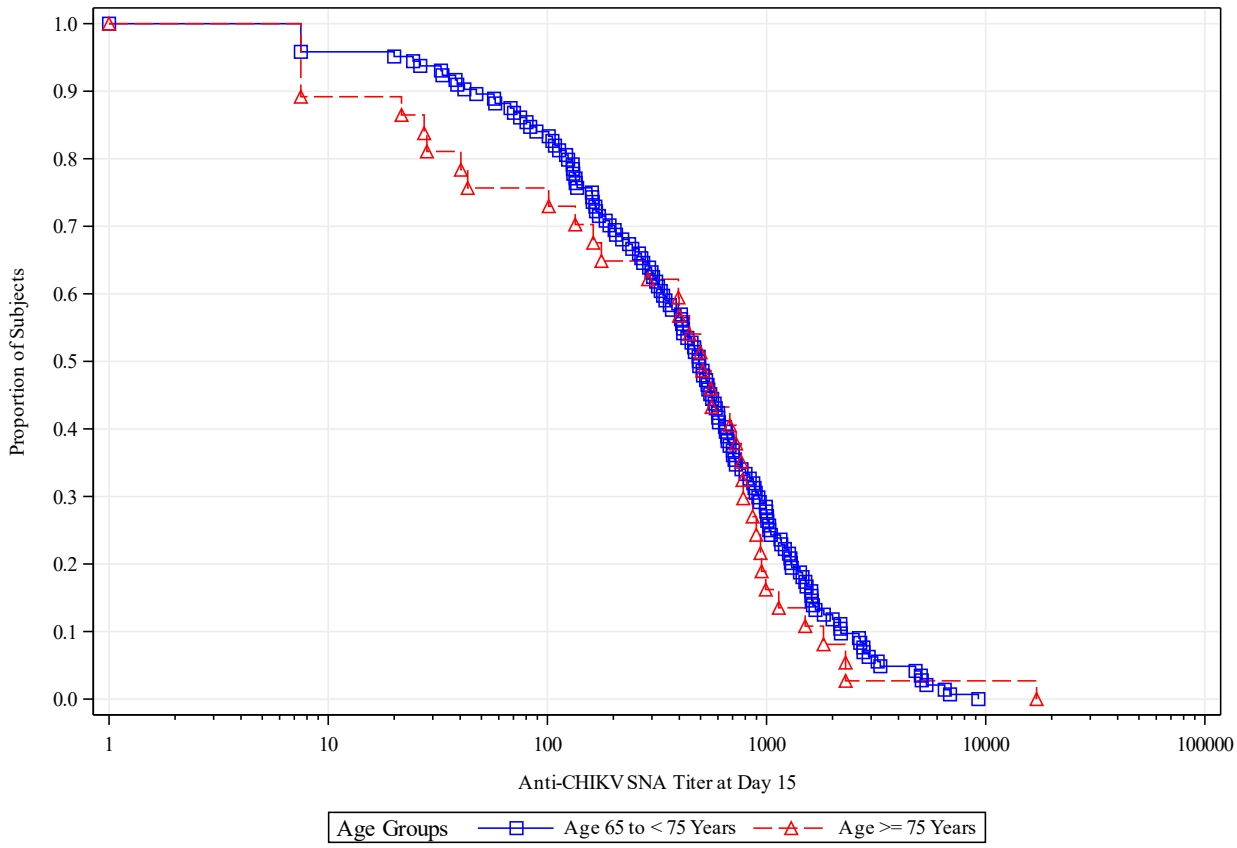


Figure 40 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Age Group – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

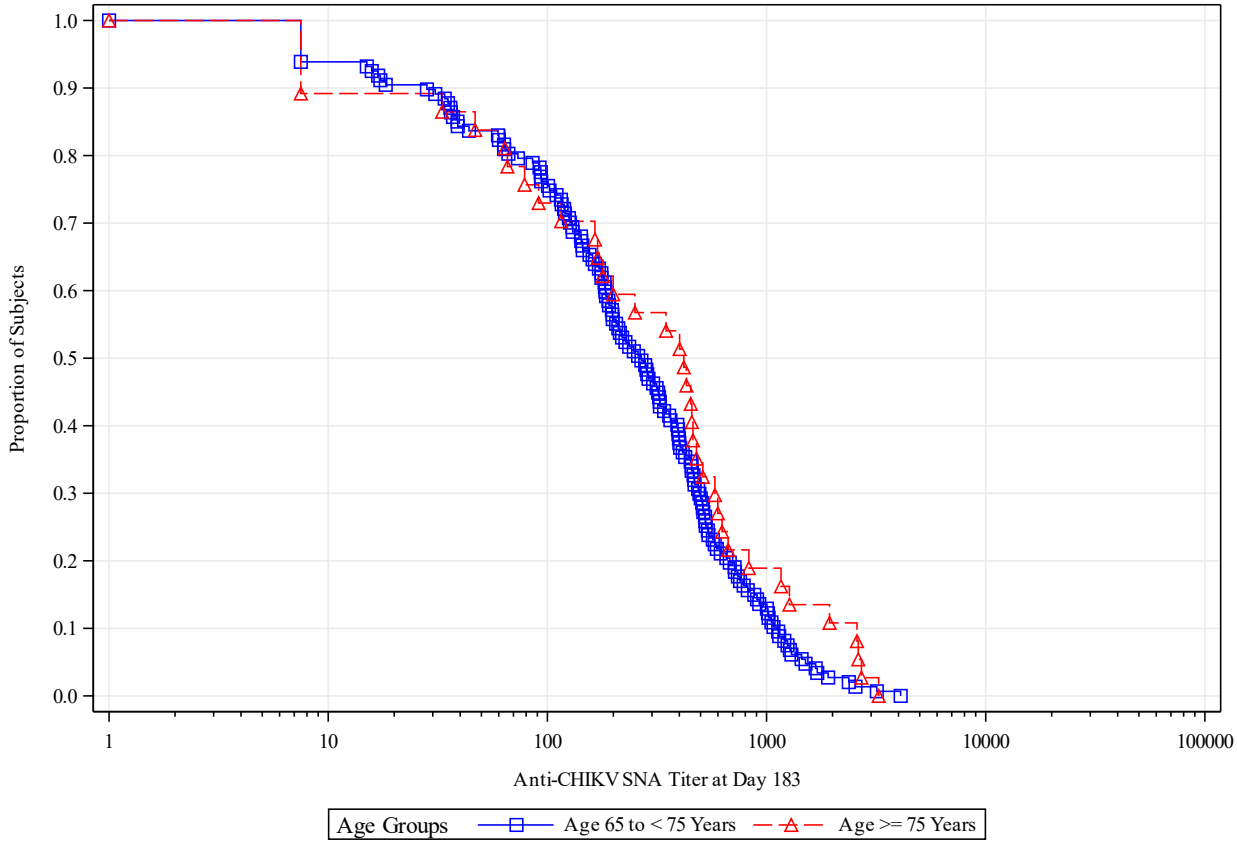


Table 64. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, male subjects

Males

Study Day Statistic	PXVX0317 (N=71) n (%)	Placebo (N=77) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	71	77		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	70	75		
Geometric Mean Titer [95% CI] [c]	247.50 [161.41, 379.51]	9.93 [6.65, 14.82]	24.92 [15.35, 40.45]	<0.0001
Median [d]	252.90	7.50		
Min, Max [d]	7.5, 5364.2	7.5, 9907.9		
Day 183				
n [b]	70	74		
Geometric Mean Titer [95% CI] [c]	139.59 [102.11, 190.83]	8.30 [6.21, 11.09]	16.82 [11.68, 24.22]	<0.0001
Median [d]	143.60	7.50		
Min, Max [d]	7.5, 3185.4	7.5, 193.9		

Table 65. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, female subjects

Females

Study Day Statistic	PXVX0317 (N=118) n (%)	Placebo (N=106) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	118	106		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	111	101		
Geometric Mean Titer [95% CI] [c]	508.25 [390.97, 660.70]	8.28 [6.27, 10.92]	61.40 [44.57, 84.60]	<0.0001
Median [d]	664.20	7.50		
Min, Max [d]	7.5, 17062.5	7.5, 384.6		
Day 183				
n [b]	114	99		
Geometric Mean Titer [95% CI] [c]	313.84 [251.27, 391.98]	8.10 [6.37, 10.30]	38.73 [29.00, 51.73]	<0.0001
Median [d]	409.05	7.50		
Min, Max [d]	7.5, 4091.2	7.5, 161.4		

Figure 41. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Sex – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

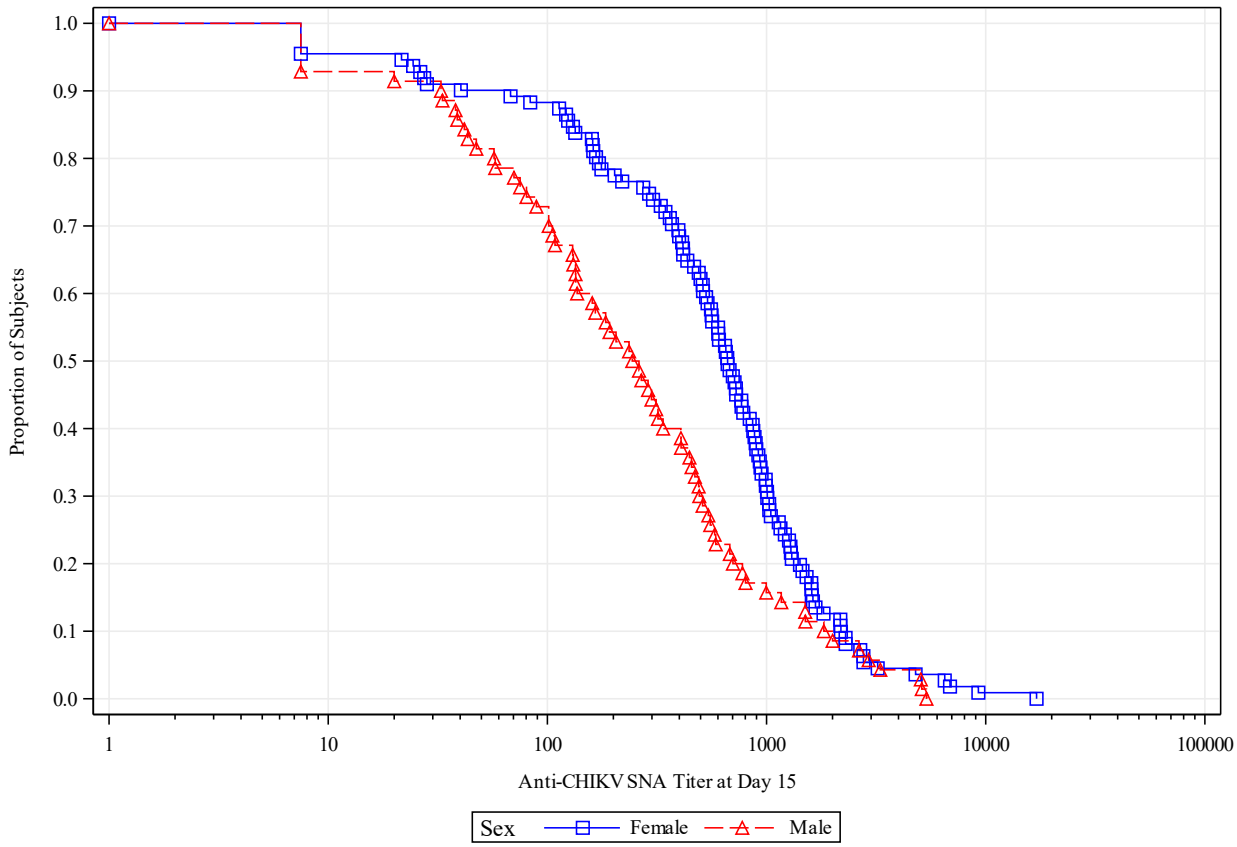


Figure 42. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Sex – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

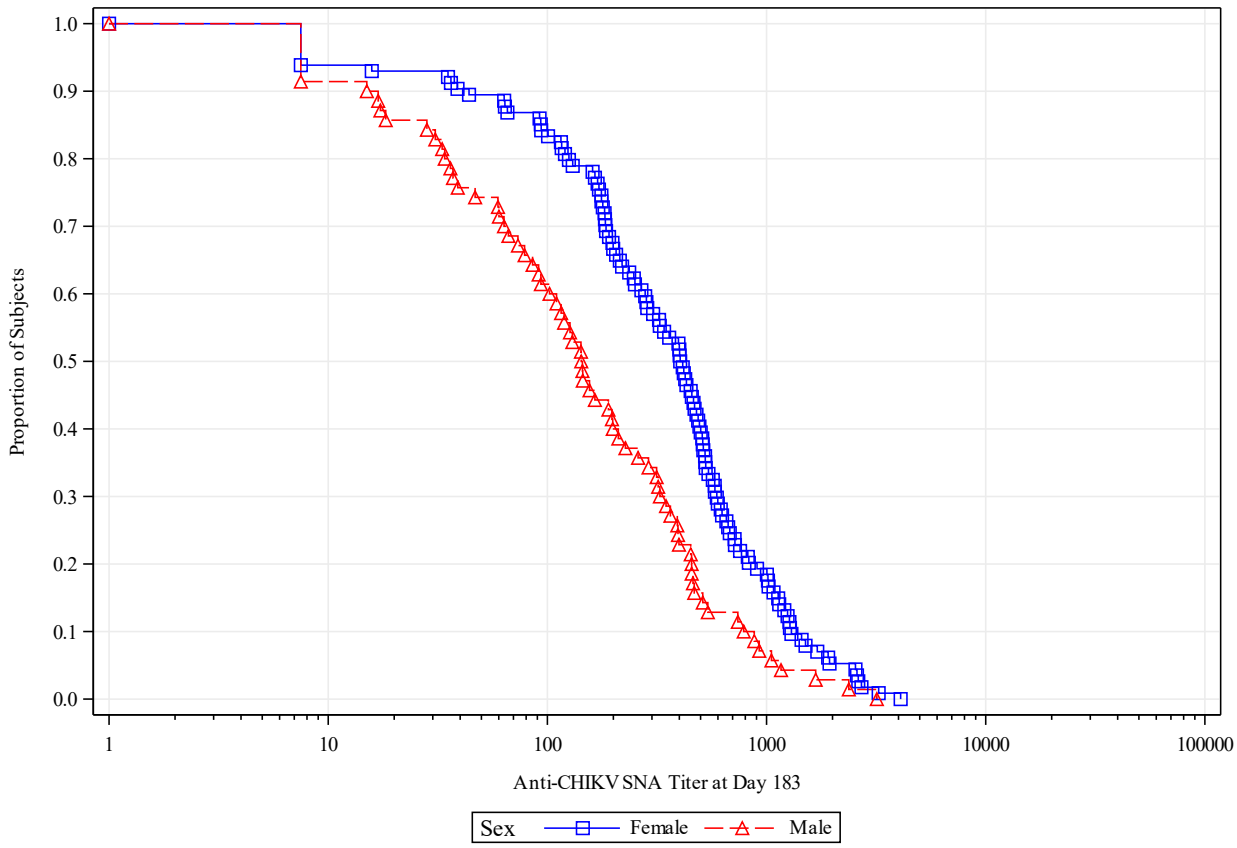


Table 66. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, white subjects

White

Study Day Statistic	PXVX0317 (N=159) n (%)	Placebo (N=145) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	159	145		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	151	139		
Geometric Mean Titer [95% CI] [c]	379.27 [292.06, 492.51]	9.04 [6.88, 11.88]	41.96 [31.03, 56.74]	<0.0001
Median [d]	493.30	7.50		
Min, Max [d]	7.5, 17062.5	7.5, 9907.9		
Day 183				
n [b]	155	135		
Geometric Mean Titer [95% CI] [c]	254.93 [208.10, 312.29]	8.06 [6.49, 10.00]	31.65 [24.56, 40.77]	<0.0001
Median [d]	325.70	7.50		
Min, Max [d]	7.5, 4091.2	7.5, 193.9		

Table 67. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, non-white subjects

Non-White

Study Day Statistic	PXVX0317 (N=29) n (%)	Placebo (N=35) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	29	35		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	29	34		
Geometric Mean Titer [95% CI] [c]	400.25 [210.33, 761.64]	8.63 [4.29, 17.35]	46.37 [22.01, 97.70]	<0.0001
Median [d]	542.40	7.50		
Min, Max [d]	7.5, 6502.8	7.5, 384.6		
Day 183				
n [b]	28	35		
Geometric Mean Titer [95% CI] [c]	153.37 [90.27, 260.57]	10.20 [5.81, 17.91]	15.04 [8.09, 27.93]	<0.0001
Median [d]	198.30	7.50		
Min, Max [d]	7.5, 2545.2	7.5, 161.4		

Figure 43 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Race – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

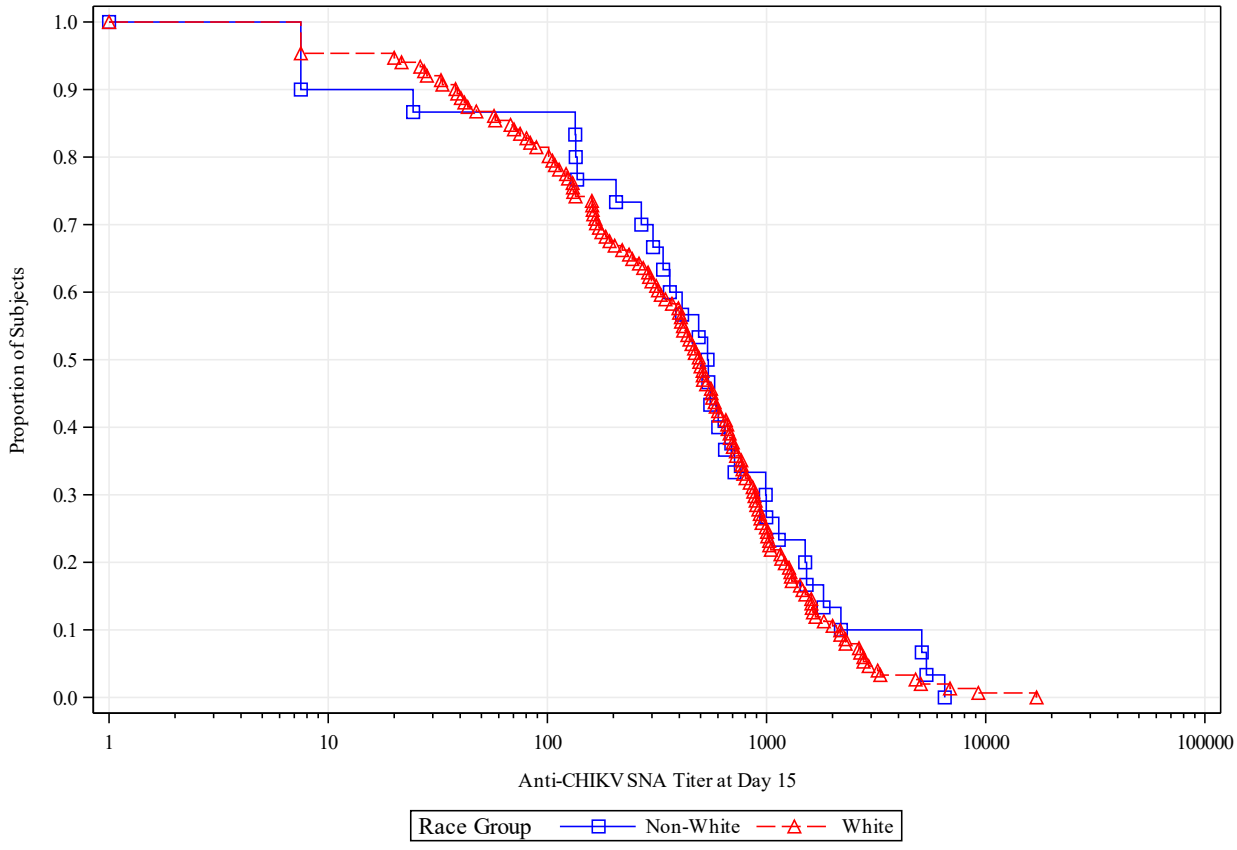


Figure 44 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Race – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

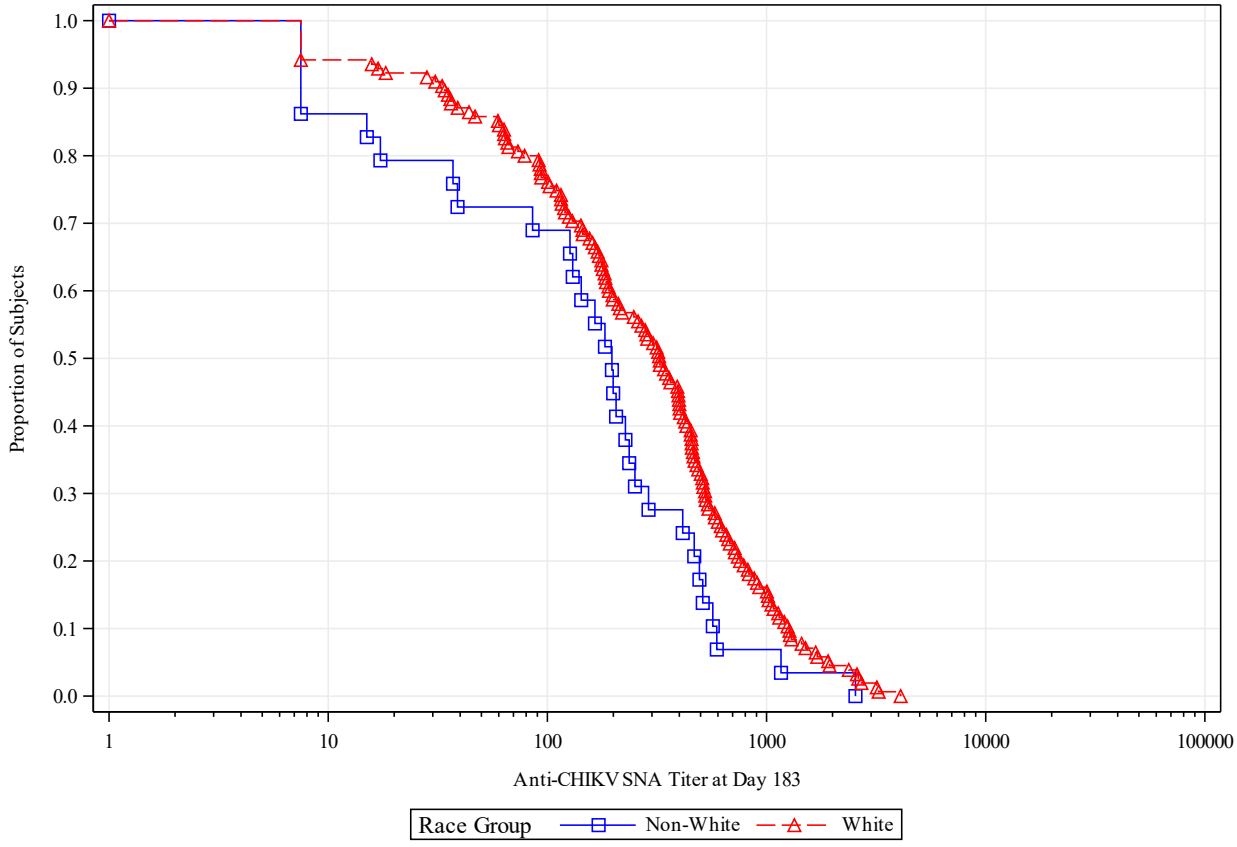


Table 68. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, Hispanic or Latino

Hispanic or Latino

Study Day Statistic	PXVX0317 (N=81) n (%)	Placebo (N=78) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	81	78		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	77	74		
Geometric Mean Titer [95% CI] [c]	429.83 [232.82, 793.55]	9.95 [5.33, 18.57]	43.21 [28.00, 66.70]	<0.0001
Median [d]	579.70	7.50		
Min, Max [d]	7.5, 17062.5	7.5, 3783.1		
Day 183				
n [b]	77	73		
Geometric Mean Titer [95% CI] [c]	229.73 [142.27, 370.97]	7.55 [4.63, 12.31]	30.43 [21.65, 42.77]	<0.0001
Median [d]	283.00	7.50		
Min, Max [d]	7.5, 2710.5	7.5, 7.5		

Table 69. Anti-CHIKV SNA GMT by Treatment Group and Visit Immunogenicity Evaluable Population, Not Hispanic or Latino

Not Hispanic or Latino

Study Day Statistic	PXVX0317 (N=107) n (%)	Placebo (N=104) n (%)	Ratio of GMTs [95% CI] [a]	p-value [c]
Day 1				
n [b]	107	104		
Geometric Mean Titer [95% CI] [c]	7.50 [7.50, 7.50]	7.50 [7.50, 7.50]		
Median [d]	7.50	7.50		
Min, Max [d]	7.5, 7.5	7.5, 7.5		
Day 15				
n [b]	103	101		
Geometric Mean Titer [95% CI] [c]	362.37 [241.58, 543.54]	8.78 [5.89, 13.11]	41.25 [28.59, 59.52]	<0.0001
Median [d]	490.00	7.50		
Min, Max [d]	7.5, 6872.2	7.5, 9907.9		
Day 183				
n [b]	106	99		
Geometric Mean Titer [95% CI] [c]	229.42 [174.01, 302.49]	8.65 [6.46, 11.59]	26.51 [19.17, 36.65]	<0.0001
Median [d]	287.45	7.50		
Min, Max [d]	7.5, 4091.2	7.5, 193.9		

Figure 45 Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 15 by Ethnicity – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

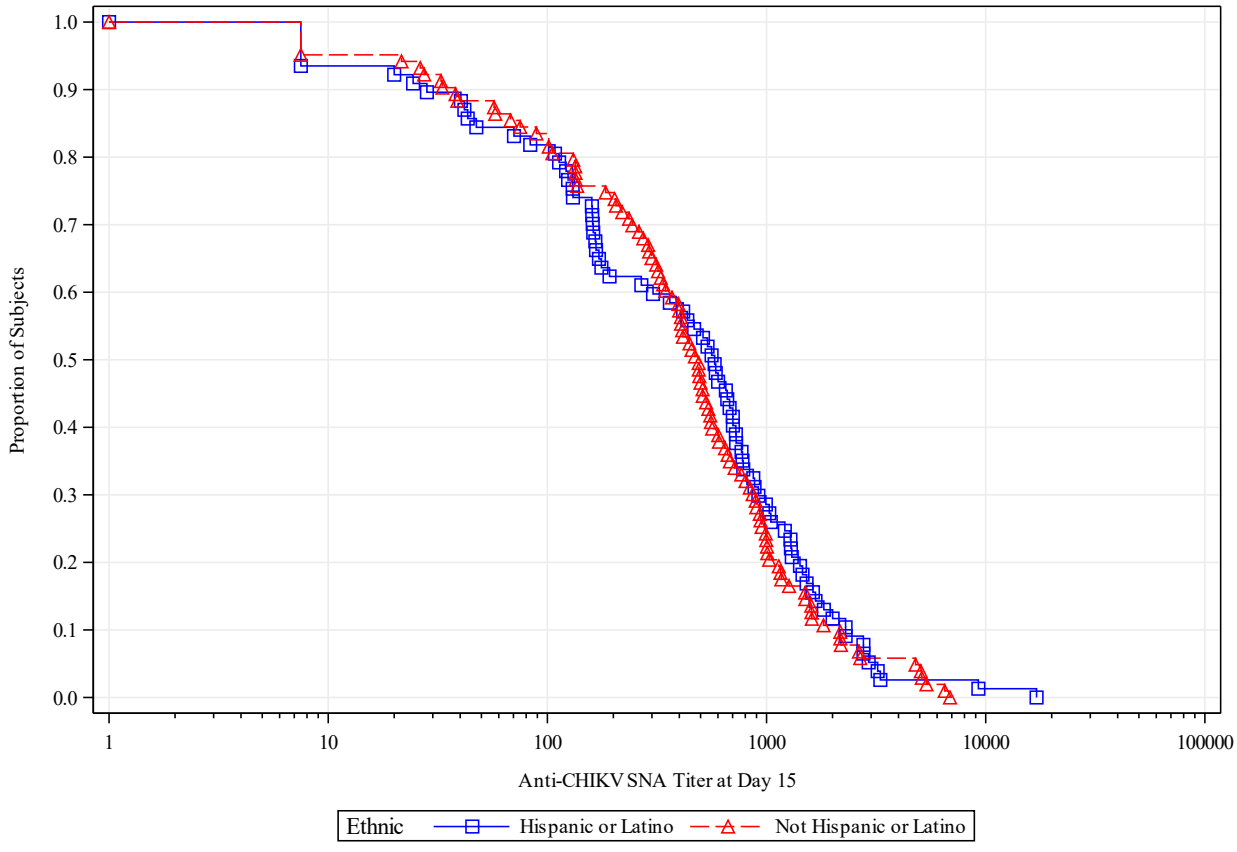


Figure 46. Reverse Cumulative Distribution of Anti-CHIKV SNA Titers on Day 183 by Ethnicity – Immunogenicity Evaluable Population, CHIKV VLP Single Dose 40/300 µg Group only

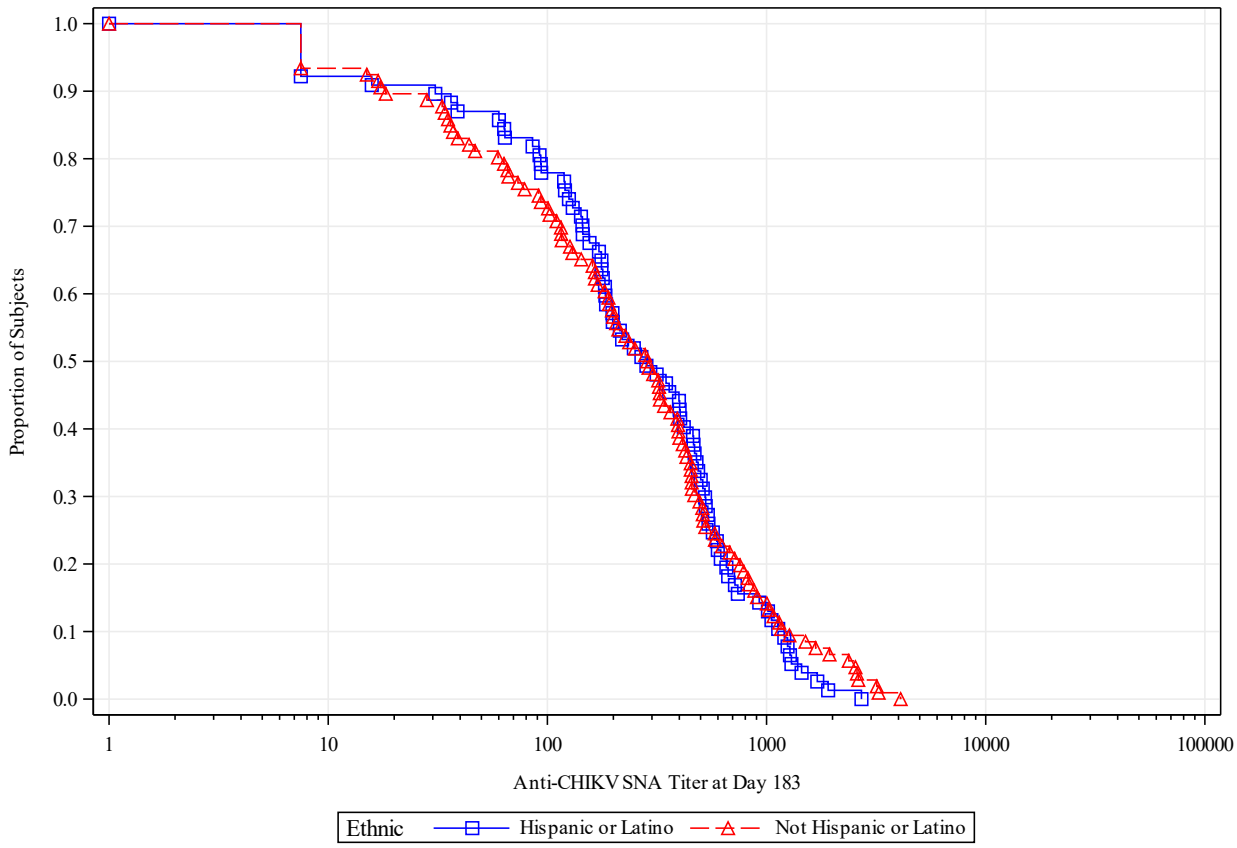
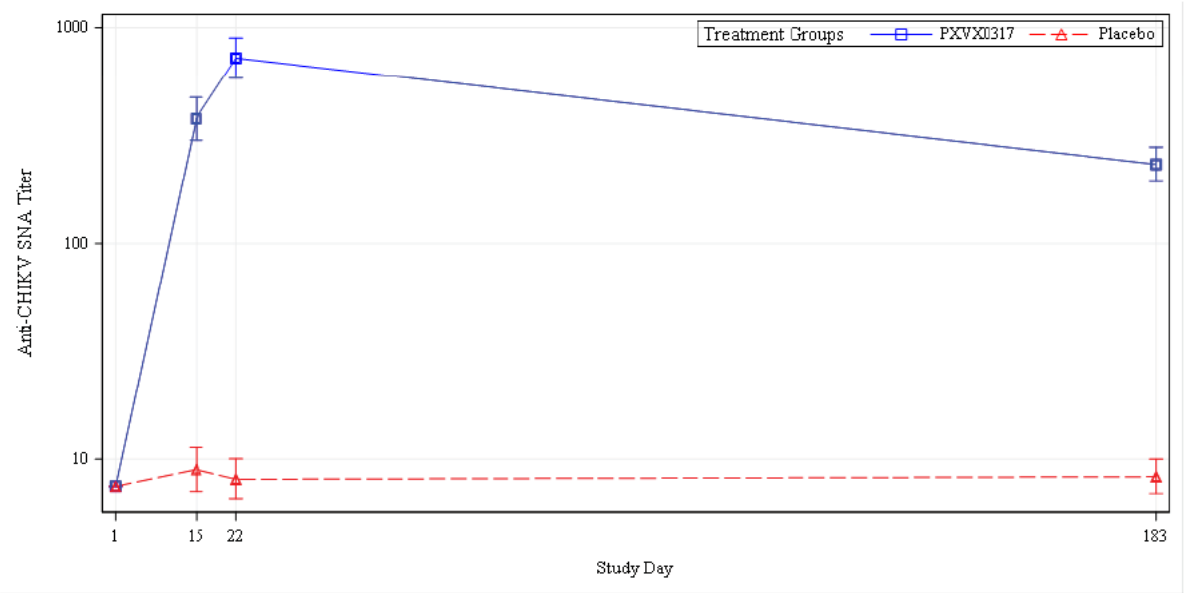


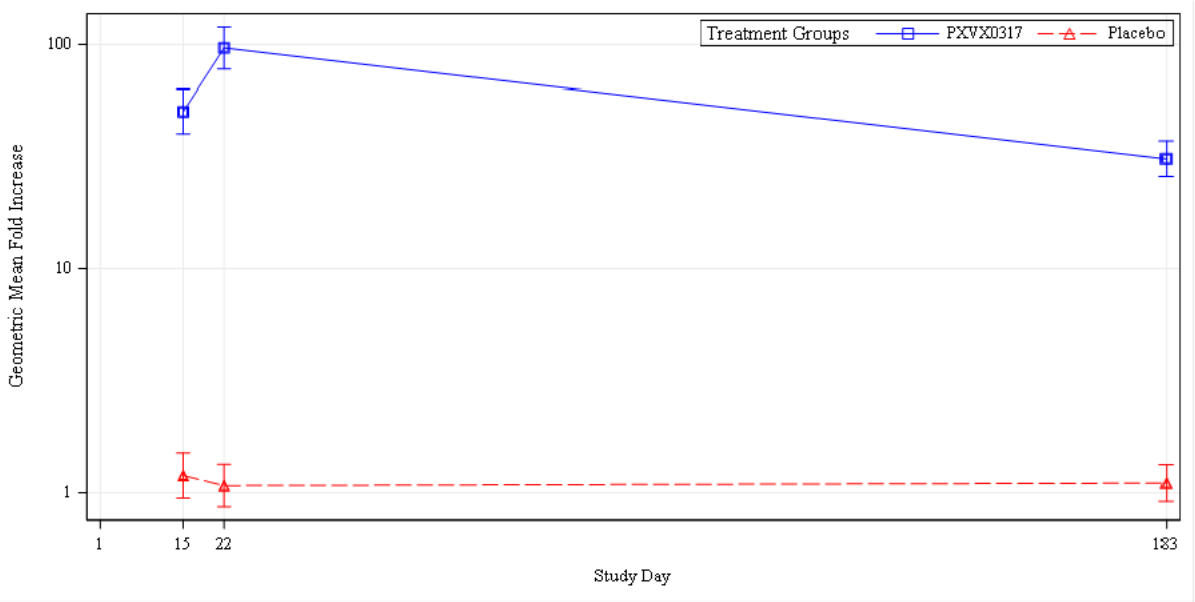
Figure 47. Geometric Mean Anti-CHIKV SNA Titers by Visit (Immunogenicity Evaluable Population, All Ages Pooled)



Data Source: Table 14.2.2.1.1
LLOQ = lower limit of quantitation; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody
Note: Y-axis is represented on a $\log_{10}$ scale. Vertical bars denote the 95% confidence interval around the geometric mean, calculated based on the Newcombe hybrid score method. Values below lower limit of quantitation (LLOQ=15) were assigned the value LLOQ/2=7.5.

Secondary endpoint: Geometric mean fold increase (GMFI) from Day 1 to subsequent collection time points.

Figure 48. Geometric Mean Fold Increase in Anti-CHIKV SNA Titers by Visit (Immunogenicity Evaluable Population, All Ages Pooled)



Data Source: Table 14.2.3
ANOVA = analysis of variance; CI = confidence interval; GMFI = geometric mean fold increase; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody
Note: Y-axis is represented on a $\log_{10}$ scale. Geometric mean fold increase from Day 1 (baseline) is (Day x/Day 1). Vertical bars denote the 95% confidence interval around the geometric mean fold increase. GMFI estimates and 95% CIs are derived from an ANOVA model that includes site and treatment group as fixed effects, assuming normality of the log fold increase in titer.

Secondary endpoint: Number and percentage of participants with an anti-CHIKV SNA titre ≥15 and 4-fold rise over baseline.

Table 70. Participants with Anti-CHIKV SNA Titre at or above Selected Titers (≥15 and 4-fold rise over baseline) by Treatment Group and Visit (Immunogenicity Evaluable Population)

Study Day Statistic	PXVX0317 (N=189) n (%)	Placebo (N=183) n (%)	Seroresponse Rate Difference [95% CI][a]	p-value[b]
Day 15				
n[c]	181	176		
n (%) titer ≥15 [95% CI][d]	171 (94.5) [90.1, 97.0]	5 (2.8) [1.2, 6.5]	91.6 [86.0, 94.6]	<0.0001
n (%) titer ≥ 4-fold Rise [95% CI][d]	155 (85.6) [79.8, 90.0]	5 (2.8) [1.2, 6.5]	82.8 [75.9, 87.5]	<0.0001
Day 22				
n[c]	189	183		
n (%) titer ≥15 [95% CI][d]	180 (95.2) [91.2, 97.5]	2 (1.1) [0.3, 3.9]	94.1 [89.2, 96.5]	<0.0001
n (%) titer ≥ 4-fold Rise [95% CI][d]	169 (89.4) [84.2, 93.0]	2 (1.1) [0.3, 3.9]	88.3 [82.4, 92.0]	<0.0001
Day 183				
n[c]	184	173		
n (%) titer ≥15 [95% CI][d]	171 (92.9) [88.3, 95.8]	2 (1.2) [0.3, 4.1]	91.8 [86.3, 94.8]	<0.0001
n (%) titer ≥ 4-fold Rise [95% CI][d]	153 (83.2) [77.1, 87.9]	2 (1.2) [0.3, 4.1]	82.0 [75.2, 86.8]	<0.0001

Data Source: Table 14.2.4, Listing 16.2.5
CI = confidence interval; IEP = Immunogenicity Evaluable Population; PXVX0317 = CHIKV VLP vaccine; SNA = Serum Neutralizing Antibody
Note: By definition, the IEP has no measurable anti-CHIKV SNA at Day 1, therefore Day 1 is omitted from this table.
[a] Seroresponse rate difference is (PXVX0317 minus placebo); 95% Cis are based on the Newcombe hybrid score method.
[b] p-value is from a two-sided chi-square test of equality of seroresponse percentages between groups.
[c] n is the number of participants with a sample result available at the indicated visit.
[d] 95% Cis of seroresponse rates are based on the Wilson method.

Exploratory immunogenicity endpoint:

- **Geometric mean titers and associated two-sided 95% CIs for neutralizing antibodies against various CHIKV genotypes measured at Day 22 (and at baseline if necessary) for a subset of participants in the CHIKV VLP vaccine group.**

The exploratory endpoint was to examine CHIKV VLP vaccine immunogenicity against various genotypes of CHIKV in the CHIKV genotype subset population for both age strata combined. This report does not include the analysis of exploratory objectives; this will be included in a future addendum when data are available.

- **Pre-defined and ad hoc important subgroup analyses**

Immunogenicity in Baseline Seropositive Participants:

There were 15 baseline seropositive participants (defined as Day 1 pre-dose anti-CHIKV titre ≥15 [≥LLOQ]) in the EBSI-CV-317-005 study. Of these, 5 participants were in the CHIKV VLP vaccine group and 10 were in the placebo group. The discussion in this section pertains to the 5 baseline seropositive participants in the CHIKV VLP vaccine group.

An ad hoc analysis found that baseline seropositive CHIKV VLP vaccine group participants tended to have much higher immunogenicity than that for baseline seronegative CHIKV VLP vaccine group participants, as measured by SNA GMT. Seropositive participants had a Day 1 (baseline) GMT of 209, a Day 15 GMT of 4082, a Day 22 GMT of 5072, and a Day 183 GMT of 1257. Comparatively, seronegative participants (the IEP) had a Day 1 GMT below the LLOQ, a Day 15 GMT of 378, a Day 22 GMT of 724, and a Day 183 GMT of 233. Despite the small sample size among baseline seropositive CHIKV VLP vaccine recipients (n=5), this group appears to have had a notably higher immune response compared to that for baseline seronegative vaccine recipients at all subsequent timepoints. It is unclear why participants located in the US were baseline seropositive.

- **Ancillary analyses**

Comparison of Preliminary Versus Final Immunogenicity Results:

The final analysis included unblinded results through Day 183 EOS. Estimates for the coprimary immunogenicity endpoints and key secondary immunogenicity endpoints at Day 22 changed slightly because of three additional participants being excluded from the IEP at final analysis whose exclusion was overlooked at preliminary analysis. Two participants should have been screen failed as they were found to be taking an anticoagulant (Eliquis, a factor Xa inhibitor anticoagulant) which in the opinion of the investigators met exclusion criterion #8, and one participant missed the Day 22 SNA sample. Changes to the preliminary coprimary immunogenicity endpoints and key secondary immunogenicity endpoints at Day 22 were summarized together with new data for the Day 183 immunogenicity time point. Briefly, between the preliminary and the final analysis, the Day 22 CHIKV VLP vaccine group seroresponse rate changed at the tenths place from 167/191 (87.4%) to 165/189 (87.3%), and the seroresponse rate difference between treatment groups at Day 22 vaccine group changed at the tenths place from 86.3% to 86.2%. Geometric mean titers changed by a larger magnitude from 730 at preliminary analysis to 724 at final analysis for Day 22 CHIKV VLP vaccine group, but the GMT ratio between treatment groups at Day 22 changed only at the tenths place from 90.0% to 89.6%. In summary, changes from the preliminary to final immunogenicity analyses were minor and did not affect integer-rounded percentages.

2.6.5.2.3. Summary of main efficacy results

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

Table 71. Summary of efficacy for study EBSI-CV-317-004

Title: A Phase 3 Safety, Immunogenicity, and Lot-consistency Trial of the VLP-based Chikungunya Vaccine PXVX0317 in Healthy Adults and Adolescents		
Study identifier	EBSI-CV-317-004, EudraCT Number: 2023-001124-42, NCT05072080	
Design	Phase 3 multicentre, randomized, placebo-controlled, double-blind, parallel-group study.	
	Duration of main phase:	182 days
	Duration of run-in phase:	Not applicable
	Duration of extension phase:	Not applicable
Hypothesis	Superiority	

Treatments groups	CHIKV VLP vaccine (PXVX0317)		CHIKV VLP vaccine 40 µg with aluminium hydroxide adjuvant (corresponding to approximately 300 µg of aluminium), single dose at Day 1, N=2790 randomized
	Placebo		Placebo (formulation buffer), single dose at Day 1, N=464 randomized
Endpoints and definitions	Co-Primary endpoint	Anti-CHIKV SNA seroresponse rate at Day 22	Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) and associated 95% CI at Day 22.
	Co-Primary endpoint	Anti-CHIKV SNA GMT at Day 22	Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for CHIKV VLP vaccine and placebo.
	Key secondary endpoint	Anti-CHIKV SNA seroresponse rate at Day 15, 183, and 8	Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) with associated 95% CI at Day 15, Day 183, and Day 8, in that order.
	Secondary endpoint	Anti-CHIKV SNA GMTs at Day 8, 15, and 183	Anti-CHIKV SNA GMTs by study arm with associated 95% CI at Day 8, Day 15, and Day 183.
	Secondary endpoint	Anti-CHIKV SNA GMFIs at Day 8, 15, 22, and 183	Anti-CHIKV SNA GMFIs by study arm from Day 1 to Day 8, Day 15, Day 22, and Day 183.
	Secondary endpoint	Seroconversion at other titers	Number and percentage of participants with an anti-CHIKV SNA titre ≥15 and 4-fold rise over baseline at Day 8, Day 15, Day 22, and Day 183
Database lock	30 June 2023		

Results and Analysis

Analysis description	**Co-Primary Analysis** **Anti-CHIKV SNA seroresponse rate at Day 22**		
Analysis population and time point description	**Population:** IEP, defined as all participants in the mITT population (all randomized and vaccinated participants who had at least one postvaccination anti-CHIKV human SNA assay result) who provided an evaluable Day 22 anti-CHIKV human SNA assay sample within the analysis window of Day 19 through Day 27, had no measurable (defined as ≥LLOQ) anti-CHIKV human SNA assay titre at Day 1 (i.e., seronegative at baseline; participants missing a Day 1 titre were excluded from IEP), and had no important protocol deviation deemed exclusionary or other reason to be excluded as defined prior to unblinding. **Timepoint:** Day 22 (21 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	2559	424
	Seroresponse rate n/N (%) [95% CI]	 2503/2559 (97.8%) [97.2%, 98.3%]	 5/424 (1.2%) [0.5%, 2.7%]
Effect estimate per comparison	Anti-CHIKV SNA seroresponse rate at Day 22	Comparison groups	CHIKV VLP vaccine / Placebo
		Difference in seroresponse rate [95% CI]	96.6% [95.0%, 97.5%]
		P-value (two-sided chi-square test)	<0.0001

Analysis description	**Co-Primary Analysis** **Anti-CHIKV SNA GMT at Day 22**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Day 22 (21 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	2559	424
	n subjects with results	2559	424
	GMT	1618.05	7.85
	95% CI	[1522.11, 1720.04]	[6.98, 8.83]
Effect estimate per comparison	Anti-CHIKV SNA GMT at Day 22	Comparison groups	CHIKV VLP vaccine / Placebo
		Ratio of GMT [95% CI]	206.12 [183.17, 231.95]
		P-value (equivalence of group GMTs on the log scale)	<0.0001

Analysis description	**Key Secondary analysis** **Anti-CHIKV SNA seroresponse rate at Day 15, 183, and 8**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 15, 183, 8 (14, 182, 7 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	2559	424
	Seroresponse rate		
	n/N (%) (Day 15)	2355/2434 (96.8%)	3/395 (0.8%)
	[95% CI]	[96.0%, 97.4%]	[0.3%, 2.2%]
	n/N (%) (Day 183)	1967/2301 (85.5%)	6/401 (1.5%)
	[95% CI]	[84.0%, 86.9%]	[0.7%, 3.2%]
	n/N (%) (Day 8)	1169/2510 (46.6%)	2/419 (0.5%)
	[95% CI]	[44.6%, 48.5%]	[0.1%, 1.7%]
Effect estimate per comparison	Anti-CHIKV SNA seroresponse rate at Day 15, Day 183, and Day 8	Comparison groups	CHIKV VLP vaccine / Placebo
		Difference in seroresponse rate [95% CI]:	
		Day 15	96.0% [94.3%, 96.8%]
		Day 183	84.0% [81.7%, 85.6%]
		Day 8	46.1% [43.8%, 48.1%]
		P-value (two-sided chi-square test):	
		Day 15	<0.0001
		Day 183	<0.0001
		Day 8	<0.0001

Analysis description	**Secondary analysis** **Anti-CHIKV SNA GMTs at Day 8, 15, and 183**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 8, 15, 183 (7, 14, 182 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	2559	424
	Day 8		
	n subjects with results	2510	419
	GMT	93.36	7.40
	[95% CI]	[87.17, 99.99]	[6.49, 8.44]
	Day 15		
	n subjects with results	2434	395
	GMT	1095.83	7.62
	[95% CI]	[1029.28, 1166.69]	[6.76, 8.59]
	Day 183		
	n subjects with results	2301	401
	GMT	337.73	8.19
	[95% CI]	[318.27, 358.37]	[7.33, 9.14]
Effect estimate per comparison	Anti-CHIKV SNA GMT at Day 8, Day 15, and Day 183	Comparison groups	CHIKV VLP vaccine / Placebo
		Ratio of GMT [95% CI]:	
		Day 8	12.62 [11.06, 14.41]
		Day 15	143.88 [127.60, 162.24]
		Day 183	41.26 [36.95, 46.07]
		P-value (equivalence of group GMTs on the log scale):	
		Day 8	<0.0001
		Day 15	<0.0001
		Day 183	<0.0001
Analysis description	**Secondary analysis** **Anti-CHIKV SNA GMFIs at Day 8, 15, 22, and 183**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 8, 15, 22, 183 (7, 14, 21, 182 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	2559	424
	Day 8	2510	419
	n subjects with results	12.45	0.99
	GMFI	[11.62, 13.33]	[0.86, 1.13]
	[95% CI]		
	Day 15	2434	395
	n subjects with results	146.11	1.02

	GMFI	[137.24, 155.56]	[0.90, 1.14]
	[95% CI]		
	Day 22	2559	424
	n subjects with results	215.74	1.05
	GMFI	[202.95, 229.34]	[0.93, 1.18]
	[95% CI]		
	Day 183	2301	401
	n subjects with results	45.03	1.09
	GMFI	[42.44, 47.78]	[0.98, 1.22]
	[95% CI]		
Effect estimate per comparison	Secondary endpoint: Anti-CHIKV SNA GMFI at Day 8, Day 15, Day 22, and Day 183	Comparison groups	CHIKV VLP vaccine / Placebo
		P-value (equality of log fold increase):	
		Day 8	<0.0001
		Day 15	<0.0001
		Day 22	<0.0001
		Day 183	<0.0001
Analysis description	**Secondary analysis** **Seroconversion at other titers**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 8, 15, 22, 183 (7, 14, 21, 182 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	2559	424
	Seroconversion at titre ≥15:		
	n/N (%) (Day 8)	2306/2510 (91.9%)	5/419 (1.2%)
	[95% CI]	[90.7%, 92.9%]	[0.5%, 2.8%]
	n/N (%) (Day 15)	2421/2434 (99.5%)	3/395 (0.8%)
	[95% CI]	[99.1%, 99.7%]	[0.3%, 2.2%]
	n/N (%) (Day 22)	2539/2559 (99.2%)	7/424 (1.7%)
	[95% CI]	[98.8%, 99.5%]	[0.8%, 3.4%]
	n/N (%) (Day 183)	2279/2301 (99.0%)	9/401 (2.2%)
	[95% CI]	[98.6%, 99.4%]	[1.2%, 4.2%]

	Titre ≥4-fold increase:		
	n/N (%) (Day 8)	1644/2510 (65.5%)	3/419 (0.7%)
	[95% CI]	[63.6%, 67.3 %]	[0.2%, 2.1%]
	n/N (%) (Day 15)	2399/2434 (98.6%)	3/395 (0.8%)
	[95% CI]	[98.0%, 99.0%]	[0.3%, 2.2%]
	n/N (%) (Day 22)	2524/2559 (98.6%)	6/424 (1.4%)
	[95% CI]	[98.1%, 99.0%]	[0.7%, 3.1%]
	n/N (%) (Day 183)	2138/2301 (92.9%)	6/401 (1.5%)
	[95% CI]	[91.8%, 93.9%]	[0.7%, 3.2%]
Effect estimate per comparison	Seroconversion at other titers	Comparison groups	CHIKV VLP vaccine / Placebo
		Titre ≥15	
		Difference in seroresponse rate [95% CI]:	
		Day 8	90.7% [88.7%, 91.9%]
		Day 15	98.7% [97.2%, 99.3%]
		Day 22	97.6% [95.8%, 98.5%]
		Day 183	96.8% [94.8%, 97.9%]
		P-value (two-sided chi-square test):	
		Day 8	<0.0001
		Day 15	<0.0001
		Day 22	<0.0001
		Day 183	<0.0001
		Titre ≥4-fold rise	
		Difference in seroresponse rate [95% CI]:	
		Day 8	64.8% [62.5%, 66.7%]
		Day 15	97.8% [96.3%, 98.4%]
		Day 22	97.2% [95.5%, 98.1%]
		Day 183	91.4% [89.4%, 92.7%]
		P-value (two-sided chi-square test):	
		Day 8	<0.0001
		Day 15	<0.0001
		Day 22	<0.0001
		Day 183	<0.0001
Notes	Study EBSI-CV-317-004 also included a third coprimary endpoint, anti-CHIKV SNA GMT ratio between all three pairs of CHIKV VLP vaccine lots (104:105, 105:106, 104:106) in adults 18 to <46 years of age at Day 22. As the efficacy assessment of CHIKV VLP vaccine is based on an integrated summary of efficacy with a dedicated statistical analysis plan which did not include this coprimary endpoint, it has been omitted from this summary of main efficacy		

	results. For details and results on the third coprimary endpoint, refer to the Clinical Study Report. For GMT results, values below the LLOQ (15) were assigned the value LLOQ/2=7.5.

CHIKV = chikungunya virus; CI = confidence interval; GMFI = geometric mean titre fold increase; GMT = geometric mean titre; IEP = Immunogenicity Evaluable Population; IP = investigational product; LLOQ = lower limit of quantitation; mITT = modified Intent-To-Treat; PXVX0317 = previous designation of CHIKV VLP vaccine; SNA = serum neutralizing antibody; VLP = virus-like particle.

Table 72. ***Summary of efficacy for study EBSI-CV-317-005***

Title: A Phase 3 Safety and Immunogenicity Trial of the VLP-based Chikungunya Virus Vaccine PXVX0317 in Adults ≥65 Years of Age			
Study identifier	EBSI-CV-317-005, NCT05349617		
Design	Phase 3 multicentre, randomized, placebo-controlled, double-blind, parallel-group study.		
	Duration of main phase:		182 days
	Duration of Run-in phase:		Not applicable
	Duration of Extension phase:		Not applicable
Hypothesis	Superiority		
Treatments groups	CHIKV VLP vaccine (PXVX0317)		CHIKV VLP vaccine 40 µg with aluminium hydroxide adjuvant (corresponding to approximately 300 µg of aluminium), single dose at Day 1, N=206 randomized
	Placebo		Placebo (formulation buffer), single dose at Day 1, N=207 randomized
Endpoints and definitions	Co-Primary endpoint	Anti-CHIKV SNA seroresponse rate at Day 22	Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) and associated 95% CI at Day 22.
	Co-Primary endpoint	Anti-CHIKV SNA GMT at Day 22	Anti-CHIKV SNA GMT and associated 95% CIs at Day 22 for CHIKV VLP vaccine and placebo.
	Key secondary endpoint	Anti-CHIKV SNA seroresponse rate at Day 15 and Day 183	Difference in anti-CHIKV SNA seroresponse rate (CHIKV VLP vaccine minus placebo) with associated 95% CI at Day 15 and Day 183, in that order.
	Secondary endpoint	Anti-CHIKV SNA GMTs at Day 15 and Day 183	Anti-CHIKV SNA GMTs by study arm with associated 95% CIs at Day 15 and Day 183.
	Secondary endpoint	Anti-CHIKV SNA GMFIs at Day 15, Day 22 and Day 183	Anti-CHIKV SNA GMFIs by study arm from Day 1 to Day 15, Day 22, and Day 183.
	Secondary endpoint	Seroconversion at other titers	Number and percentage of participants with an anti-CHIKV SNA titre ≥15 and 4-fold rise over baseline at Day 15, Day 22, and Day 183.
Database lock	08 August 2023		
Results and Analysis			
Analysis description	**Co-Primary Analysis** **Anti-CHIKV SNA seroresponse rate at Day 22**		

Analysis population and time point description	**Population:** IEP, defined as all participants in the mITT population (all randomized and vaccinated participants who had at least one postvaccination anti-CHIKV human SNA assay result) who provided an evaluable Day 22 anti-CHIKV human SNA assay sample within the analysis window of Day 19 through Day 27, had no measurable (defined as ≥LLOQ) anti-CHIKV human SNA assay titre at Day 1 (i.e., seronegative at baseline; participants missing a Day 1 titre were excluded from IEP), and had no important protocol deviation deemed exclusionary or other reason to be excluded as defined prior to unblinding. **Timepoint:** Day 22 (21 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	189	183
	Seroresponse rate n/N (%) [95% CI]	 165/189 (87.3%) [81.8%, 91.3%]	 2/183 (1.1%) [0.3%, 3.9%]
Effect estimate per comparison	Anti-CHIKV SNA seroresponse rate at Day 22	Comparison groups	CHIKV VLP vaccine / Placebo
		Difference in seroresponse rate [95% CI]	86.2% [80.0%, 90.3%]
		P-value (two-sided chi-square test)	<0.0001
Analysis description	**Co-Primary Analysis** **Anti-CHIKV SNA GMT at Day 22**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Day 22 (21 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	189	183
	n subjects with results GMT [95% CI]	 189 723.93 [584.13, 897.20]	 183 8.08 [6.50, 10.04]
Effect estimate per comparison	Anti-CHIKV SNA GMT at Day 22	Comparison groups	CHIKV VLP vaccine / Placebo
		Ratio of GMT [95% CI]	89.64 [68.62,117.10]
		P-value (equivalence of group GMTs on the log scale)	<0.0001
Analysis description	**Key Secondary analysis** **Anti-CHIKV SNA seroresponse rate at Day 15 and 183**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 15 and 183 (14 and 182 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	189	183
	Seroresponse rate n/N (%) (Day 15)	149/181 (82.3%) [76.1%, 87.2%]	5/176 (2.8%) [1.2%, 6.5%]

	[95% CI] n/N (%) (Day 183) [95% CI]	139/184 (75.5%) [68.9%, 81.2%]	2/173 (1.2%) [0.3%, 4.1%]
Effect estimate per comparison	Anti-CHIKV SNA seroresponse rate at Day 15 and Day 183	Comparison groups	CHIKV VLP vaccine / Placebo
		Difference in seroresponse rate [95% CI]: Day 15 Day 183	 79.5% [72.3%, 84.6%] 74.4% [67.1%, 80.1%]
		P-value (two-sided chi-square test): Day 15 Day 183	 <0.0001 <0.0001
Analysis description	**Secondary analysis** **Anti-CHIKV SNA GMTs at Day 15 and 183**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 15 and 183 (14 and 182 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	189	183
	<u>Day 15</u> n subjects with results GMT 95% CI <u>Day 183</u> n subjects with results GMT 95% CI	 181 378.42 [301.04, 475.69] 184 233.02 [194.05, 279.82]	 176 8.96 [7.09, 11.32] 173 8.29 [6.87, 10.02]
Effect estimate per comparison	Anti-CHIKV SNA GMT at Day 15 and Day 183	Comparison groups	CHIKV VLP vaccine / Placebo
		Ratio of GMT [95% CI]: Day 15 Day 183	 42.23 [32.13, 55.51] 28.09 [22.31, 35.38]
		P-value (equivalence of group GMTs on the log scale): Day 15 Day 183	 <0.0001 <0.0001

Analysis description	**Secondary analysis** **Anti-CHIKV SNA GMFIs at Day 15, 22, and 183**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 15, 22, 183 (14, 21, 182 days after IP administration).		
Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	189	183
	<u>Day 15</u> n subjects with results GMFI [95% CI] <u>Day 22</u> n subjects with results GMFI [95% CI] <u>Day 183</u> n subjects with results GMFI [95% CI]	 181 48.52 [40.12, 58.69] 189 93.94 [78.03, 113.11] 184 29.32 [25.01, 34.37]	 176 1.16 [0.95, 1.40] 183 1.05 [0.87, 1.27] 173 1.04 [0.88, 1.22]
Effect estimate per comparison	Anti-CHIKV SNA GMFIs at Day 15, Day 22, and Day 183	Comparison groups	CHIKV VLP vaccine / Placebo
		P-value (equality of log fold increase): Day 15 Day 22 Day 183	 <0.0001 <0.0001 <0.0001
Analysis description	**Secondary analysis** **Seroconversion at other titers**		
Analysis population and time point description	**Population:** IEP. **Timepoint:** Days 15, 22, 183 (14, 21, 182 days after IP administration).		

Descriptive statistics and estimate variability	**Treatment group**	**CHIKV VLP vaccine**	**Placebo**
	Number of subjects	189	183
	Seroconversion at titre ≥15:		
	n/N (%) (Day 15)	171/181 (94.5%)	5/176 (2.8%)
	[95% CI]	[90.1%, 97.0%]	[1.2%, 6.5%]
	n/N (%) (Day 22)	180/189 (95.2%)	2/183 (1.1%)
	[95% CI]	[91.2%, 97.5%]	[0.3%, 3.9%]
	n/N (%) (Day 183)	171/184 (92.9%)	2/173 (1.2%)
	[95% CI]	[88.3%, 95.8%]	[0.3%, 4.1%]
	Titre ≥4-fold increase:		
	n/N (%) (Day 15)	155/181 (85.6%)	5/176 (2.8%)
	[95% CI]	[79.8%, 90.0%]	[1.2%, 6.5%]
	n/N (%) (Day 22)	169/189 (89.4%)	2/183 (1.1%)
	[95% CI]	[84.2%, 93.0%]	[0.3%, 3.9%]
	n/N (%) (Day 183)	153/184 (83.2%)	2/173 (1.2%)
	[95% CI]	[77.1%, 87.9%]	[0.3%, 4.1%]
Effect estimate per comparison	Seroconversion at other titers	Comparison groups	CHIKV VLP vaccine / Placebo
		Titre ≥15	
		Difference in seroresponse rate [95% CI]:	
		Day 15	91.6% [86.0%, 94.6%]
		Day 22	94.1% [89.2%, 96.5%]
		Day 183	91.8% [86.3%, 94.8%]
		P-value (two-sided chi-square test):	
		Day 15	<0.0001
		Day 22	<0.0001
		Day 183	<0.0001
		Titre ≥4-fold rise	
		Difference in seroresponse rate [95% CI]:	
		Day 15	82.8% [75.9%, 87.5%]
		Day 22	88.3% [82.4%, 92.0%]
		Day 183	82.0% [75.2%, 86.8%]
		P-value (two-sided chi-square test):	
		Day 15	<0.0001
		Day 22	<0.0001
		Day 183	<0.0001

Notes	For GMT results, values below the LLOQ (15) were assigned the value LLOQ/2=7.5.

CHIKV = chikungunya virus; CI = confidence interval; GMFI = geometric mean titre fold increase; GMT = geometric mean titre; IEP = Immunogenicity Evaluable Population; IP = investigational product; LLOQ = lower limit of quantitation; mITT = modified Intent-To-Treat; PXVX0317 = previous designation of CHIKV VLP vaccine; SNA = serum neutralizing antibody; VLP = virus-like particle.

2.6.5.3. Clinical studies in special populations

Immune responses in adolescents and elderly subjects were investigated in the pivotal studies EBSI-CV-317-004 and EBSI-CV-317-005, respectively.

The efficacy and safety of CHIKV VLP vaccine has not been assessed in individuals with altered immunocompetence. It is not known whether individuals with impaired immune responsiveness, including individuals receiving immunosuppressive therapy, will elicit the same response to CHIKV VLP vaccine as immunocompetent individuals.

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

2.6.5.4.1. Cross-neutralization with other lineages and strains

The CHIKV VLP vaccine is based on the chikungunya virus (CHIKV) Senegal strain 37997 from the West African (WA) lineage.

During a scientific advice procedure, the MAH had initially been asked to evaluate neutralization across strains representing the main CHIKV lineages at least in a subset of study EBSI-CV-317-005 individuals (≥65 yoa). It was later agreed at the PRIME submission readiness meeting that cross-protection data from study EBSI-CV-317-002 (<65 yoa) are sufficient for MAA provided that these data are generated with the clinically relevant formulation of the CHIKV VLP vaccine and cross-protection data from study EBSI-CV-317-005 may be submitted post authorization.

The data of EBSI-CV-317-002 were provided with the current application (see section supportive studies): The 3 major lineages Asian, WA, and ECSA were tested for CHIKV VLP vaccine cross-neutralization using the following 4 strains: 15561 (Asian – Thailand 1962), 181/25 (Asian – attenuated strain of 15561), LR (ECSA/IOL – La Reunion 2006), and PM2951 (WA – Senegal 1966). At Day 22, GMTs for strains 181/25, 15561, LR, and PM2951 were 584, 551, 905, and 2584, respectively, and seroconversion (titre ≥20) was seen in 100% of participants. At Day 182, GMTs for strains 181/25, 15561, LR, and PM2951 were 204, 184, 148, and 686, respectively, and seroconversion remained high (100% for strains 181/25 and PM2951 and 98% and 95% for strains 15561 and LR, respectively).

Additional (exploratory) data on cross-neutralization of other arthritogenic alphaviruses were provided in study EBSI-CV-317-002 (see section 2.6.5.5. supportive studies).

2.6.5.5. Supportive studies

The following studies are considered as supportive:

- Phase 1 dose escalation VRC 311 (publication only, Chang et al 2014, The Lancet)
- Phase 2 VRC 704
- Phase 2 EBSI-CV-317-002
- Phase 2 EBSI-CV-317-010

Studies **VRC 311** and **VRC 704** were conducted early in development and evaluated safety and immunogenicity of unadjuvanted CHIKV VLP formulations (VRC 311: sequential dose level groups 10 µg, 20 µg, or 40 µg given at weeks 0, 4, and 20; VRC 704: 20 µg given at weeks 0 and 4).

Studies **EBSI-CV-317-002** and **EBSI-CV-317-010** evaluated the clinically relevant single-dose adjuvanted CHIKV VLP formulation (40/300 µg).

2.6.5.5.1. Phase 1 Study VRC 311

VRC 311 was a phase 1 open-label, dose escalation clinical trial in healthy adults 18-50 years of age where participants were assigned to sequential dose level groups to receive IM injections of 10 µg, 20 µg, or 40 µg (unadjuvanted) on weeks 0, 4, and 20, with follow-up for 44 weeks after enrolment.

Primary endpoint was safety and tolerability. Secondary endpoints were chikungunya virus specific immune responses assessed by ELISA and neutralising antibody assays. Twenty-five healthy adult participants 18 to 50 years of age were assigned sequentially to receive IM injections of 10 µg (n=5), 20 µg (n=10), or 40 µg (n=10) CHKVLP059 at Weeks 0, 4, and 20, with follow-up for a total of 44 weeks after the first injection.

In summary, unadjuvanted CHIKV VLP vaccine (CHKVLP059) was immunogenic at all three dose levels in healthy volunteers at Weeks 0, 4, and 20, with neutralizing antibody IC_{50} GMTs at 4 weeks after the second dose (i.e., Week 8) of 2688 in the 10 µg group (n=5), 1775 in the 20 µg group (n=10), and 7246 in the 40 µg group (n=10)(Chang, 2014).

The sera from 12 VRC 311 participants neutralized all tested strains at Week 44 with no significant differences between the 50% neutralization titre (NT50), except for the Nigeria 1965 strain (WA lineage) which was neutralized with an NT50 approximately 3-times higher than that for OPY1 strain (Indian Ocean Lineage [IOL]) (Goo, 2016).

The sera from 12 VRC 311 participants neutralized all tested strains at Week 44 with no significant differences between the 50% neutralization titre (NT_{50}), except for the Nigeria 1965 strain (WA lineage) which was neutralized with an NT_{50} approximately 3-times higher than that for OPY1 strain (Indian Ocean Lineage [IOL]). No safety concern was identified.

2.6.5.5.2. Phase 2 Study VRC 704

This was a phase II, multicentre, randomized, placebo-controlled, double-blind study to evaluate the safety and immunogenicity of a 2-injection vaccine regimen with the Chikungunya virus (CHIKV) virus-like particle vaccine (CHIKV VLP, VRC-CHKVLP059-00-VP) in healthy adults.

A total of 400 participants 18 to 60 years of age who resided in CHIKV-endemic regions of the Caribbean were assigned in a 1:1 ratio to receive 2 IM injections of either 20 µg CHKVLP059 (n=201) or phosphate buffered saline placebo (n=199) at 0 and 4 weeks. Participants were followed for a total duration of 72 weeks. Immune responses to CHIKV were assessed using NIH VRC's focus reduction neutralization test, reported as half- maximal effective concentration (EC50) values, and the human SNA assay, reported as NT80 values.

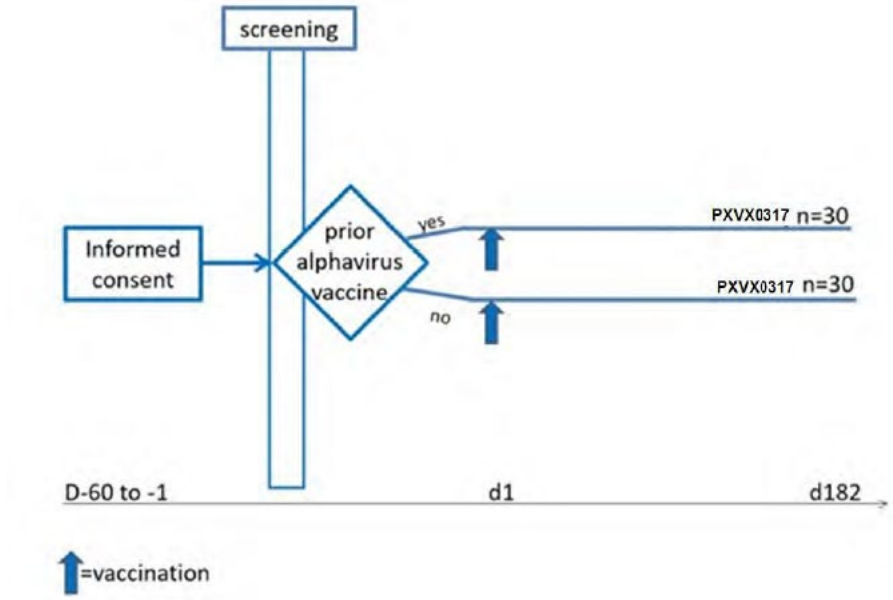
The EC50 GMT at Week 8 (4 weeks after second dose at Week 4) was 43 for placebo recipients (n=192) versus 2005 for vaccinees (n=192). Use of a responder analysis with threshold of ≥30 showed that 20.8% of placebo recipients responded versus a 99.5% response rate for vaccinees; there were high baseline GMTs in some participants because the study was conducted in CHIKV-endemic countries. No clinically consistent CHIKV cases were diagnosed in either treatment group in this study, so vaccine efficacy could not be assessed.

2.6.5.5.3. Phase 2 Study EBSI-CV-317-002

This was a Phase 2, parallel-group, age- and gender-matched, open label study in healthy adults 18–65 years of age to assess safety and immunogenicity of an Alum-Adjuvanted Chikungunya Virus-Like Particle Vaccine (PXVX0317) in Prior Recipients of Other Alphavirus Vaccines Versus Alphavirus Naïve Controls

A total of 60 subjects were planned to be enrolled with up to 30 at each of two sites. Up to 60 healthy adults (with replacements allowed for those who dropped out prior to Day 29), including 30 prior recipients of investigational alphavirus vaccines, recruited from USAMRIID (United States Army Medical Research Institute of Infectious Disease), and an equal number of gender and age-matched controls recruited from WRAIR (Walter Reed Army Institute of Research).

Figure 49. ESVI-CV-317-002 Study schema



d = days, n = sample size

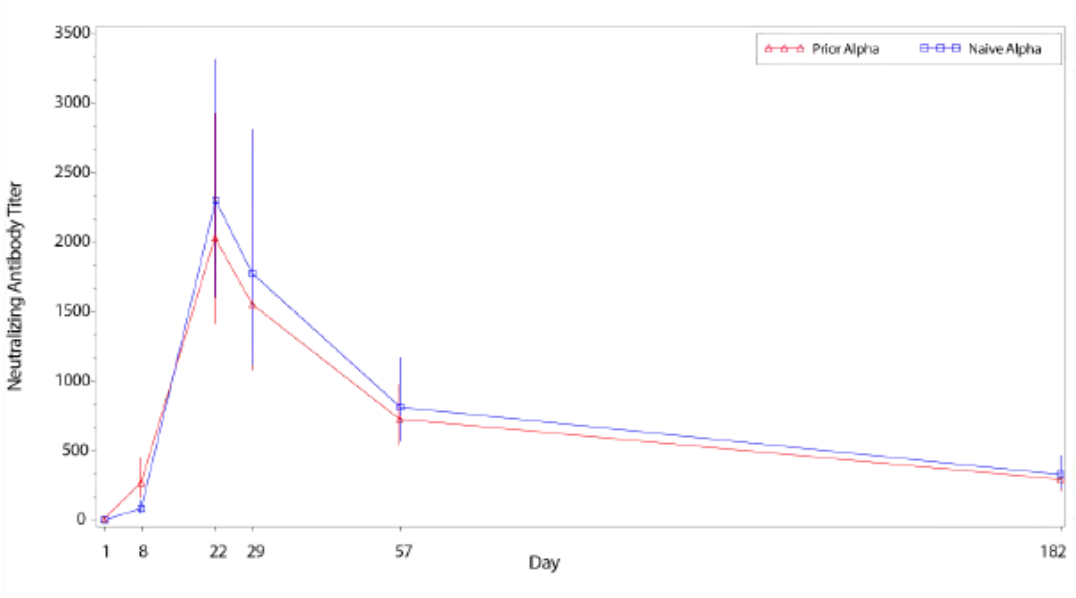
Immunogenicity Results

The primary immunogenicity objective (percentage of participants with a 4-fold rise or greater in anti-CHIKV SNA at Day 22) was met. The comparison yielded no difference as all participants in both groups (prior alphavirus vaccine recipients and alphavirus-naïve controls) developed a 4-fold or greater rise between baseline (Day 1, vaccination day), and Day 22 in SNA.

Vaccination with CHIKV VLP vaccine resulted in a more rapid immune response in the prior alphavirus vaccine recipients versus the alphavirus- naïve controls. There was a statistically significant difference in GMTs between the prior alpha and naïve alpha group at Day 8. The SNA GMT of the prior alphavirus vaccine recipients was 3.11 (95% CI: 1.55, 6.23) times higher than the SNA GMT of the alphavirus-naïve controls. However, the GMTs of both groups peaked at Day 22 and were similar on Day 22 and all subsequent visits. Also, at every visit after the Day 22 primary endpoint, all participants continued to exhibit a 4-fold rise in neutralizing antibody (except on Day 182 where 86.7% of participants in the prior alpha virus recipient group showed a ≥4-fold rise versus 100% of participant in the alphavirus-naïve control group). However, this difference is not statistically significant (p=0.1124). The study also summarized SNA categorically as the percentage of participants with titers of at least 15, 40, 100, 160, and 640 at each visit. In the prior alphavirus vaccine recipients, a significantly higher proportion of

participants had a titre ≥40, 100, and 160 compared to the naïve alpha group on Day 8 but no significant difference between groups was observed at Day 22 and later visits.

Figure 50. Geometric Mean SNA Titre Over Time



Note: Vertical bars denote extent of 95% confidence interval on the geometric mean.

Cross-neutralization other CHIKV genotypes

The 3 major CHIKV lineages include Asian, WA, and ECSA lineages, with a newer sub-lineage, the Indian Ocean Lineage (IOL), which emerged from the ECSA lineage (Goo, 2016; Sanchez-Vargas, 2019; Bartholomeeusen, 2023). The 3 major lineages were tested for CHIKV VLP vaccine cross-neutralization using the following strains: 15561 (Asian – Thailand 1962), 181/25 (Asian – attenuated strain of 15561), LR (ECSA/IOL – La Reunion 2006), and PM2951 (WA – Senegal 1966).

The CHIKV VLP vaccine is based on the chikungunya virus (CHIKV) Senegal strain 37997 from the West African lineage. Antibodies from participants vaccinated with CHIKV VLP vaccine were shown, by PRNT assay, to neutralize all 4 CHIKV strains tested. At Day 22, geometric mean titers (GMTs) for strains 181/25, 15561, LR, and PM2951 were 584, 551, 905, and 2584, respectively, and seroconversion (titre ≥20) was seen in 100% of participants.

At Day 182, PRNT50 GMTs for strains 181/25, 15561, LR, and PM2951 were 204, 184, 148, and 686, respectively, and seroconversion remained high (100% for strains 181/25 and PM2951 and 98% and 95% for strains 15561 and LR, respectively).

The 181/25 strain was used as the comparator for the other strains in calculating raw and geometric fold increase titers. The 15561 strain was similar in cross-neutralization. The more contemporary LR strain was neutralized at a greater potency of 3.91× at Day 22 and 1.77× at Day 182 using raw mean fold increase titers (GMFI titers were 1.55× at Day 22 and 0.72× at Day 182). The PM2951 strain (WA, Senegal 1966) was neutralized with the greatest potency, 10.81× at Day 22 and 5.69× at Day 182 using raw mean fold increase titers (GMFI titers were 4.43× at Day 22 and 3.36× at Day 182). For all strains, at both Day 22 and Day 182, naïve participants had a higher GMT than the prior alpha vaccine recipients, though in some instances, the difference was small.

Table 73. $PRNT_{50}$ Geometric Mean Titre, seroconversion and fold increase for other CHIKV strains

Strain	Day	Group	N	GMT [95% CI][a]	Seroconversion n (%)	Raw Mean Fold Titer Increase Relative to 181/25 [95% CI][b]	Geometric Mean Fold Titer Increase Relative to 181/25 [95% CI][c]
181/25	22	Naïve	30	655.0 [353.3, 1214.3]	30 (100%)	-	-
		Prior alpha	30	519.8 [367.2, 735.9]	30 (100%)	-	-
		Total	60	583.5 [413.3, 823.8]	60 (100%)	-	-
	182	Naïve	30	221.1 [177.0, 276.3]	30 (100%)	-	-
		Prior alpha	30	188.1 [125.3, 282.3]	30 (100%)	-	-
		Total	60	203.9 [162.7, 255.5]	60 (100%)	-	-
15561	22	Naïve	30	702.0 [430.5, 1144.6]	30 (100%)	2.89 [0.66, 5.12]	1.07 [0.64, 1.79]
		Prior alpha	30	432.1 [259.0, 720.9]	30 (100%)	1.79 [0.62, 2.96]	0.83 [0.54, 1.29]
		Total	60	550.8 [388.5, 780.8]	60 (100%)	2.34 [1.11, 3.57]	0.94 [0.68, 1.31]
	182	Naïve	30	188.1 [104.1, 339.8]	29 (96.7%)	2.43 [0.91, 3.96]	0.85 [0.48, 1.51]
		Prior alpha	30	179.6 [117.4, 274.7]	30 (100%)	1.26 [0.89, 1.62]	0.95 [0.72, 1.27]
		Total	60	183.8 [129.1, 261.7]	59 (98.3%)	1.85 [1.07, 2.62]	0.90 [0.66, 1.23]
LR	22	Naïve	30	1436.8 [1015.4, 2033.0]	30 (100%)	5.89 [1.17, 10.61]	2.19 [1.37, 3.51]
		Prior alpha	30	570.2 [350.8, 926.8]	30 (100%)	1.92 [1.05, 2.79]	1.10 [0.74, 1.63]
		Total	60	905.1 [661.4, 1238.5]	60 (100%)	3.91 [1.52, 6.29]	1.55 [1.14, 2.12]
	182	Naïve	30	376.2 [246.5, 574.1]	30 (100%)	3.06 [1.56, 4.56]	1.70 [1.14, 2.54]
		Prior alpha	30	57.9 [39.0, 85.9]	27 (90.0%)	0.49 [0.22, 0.75]	0.31 [0.22, 0.43]
		Total	60	147.6 [101.8, 214.0]	57 (95.0%)	1.77 [0.96, 2.58]	0.72 [0.52, 1.01]
PM2951	22	Naïve	30	3300.8 [2227.4, 4891.4]	30 (100%)	12.43 [6.12, 18.74]	5.04 [2.84, 8.95]
		Prior alpha	30	2022.9 [1195.5, 3422.7]	30 (100%)	9.20 [3.12, 15.28]	3.89 [2.47, 6.13]
		Total	60	2584.0 [1867.3, 3575.8]	60 (100%)	10.81 [6.54, 15.09]	4.43 [3.10, 6.33]
	182	Naïve	30	970.1 [635.9, 1479.8]	30 (100%)	6.87 [4.36, 9.37]	4.39 [2.97, 6.47]
		Prior alpha	30	485.0 [325.2, 723.5]	30 (100%)	4.52 [2.32, 6.71]	2.58 [1.69, 3.93]
		Total	60	685.9 [510.1, 922.4]	60 (100%)	5.69 [4.05, 7.34]	3.36 [2.53, 4.48]

Data Source: Table 14.2.6.1; Table 14.2.6.2; Table 14.2.6.3; Table 14.2.6.4; Listing 16.2.6.4
CI = confidence interval; GMT = geometric mean titer; N = number of participants with a sample result; n = number of participants with a seroresponse titer ≥20; PRNT = plaque-reduction neutralization test
Note: CHIKV strain 181/25 was the least sensitive (most conservative titer) so was used as the baseline titer for fold increase comparison with other strains.
[a] The GMTs were calculated by taking the mean and standard deviation of the titers on a $\log_{10}$ scale and then back-transforming to produce the GMT and associated 95% CI.
[b] Titer fold increase relative to strain 181/25 was computed, for each participant, by dividing the non-181/25 strain titer by the 181/25 titer and then calculating the mean and 95% CI for the group.
[c] The geometric mean fold increase was similarly calculated but using a $\log_{10}$ scale transformation on the titer, taking the difference between the non-181/25 strain and the other strains, calculating the mean and SD on the log scale and then back-transforming to produce the GMFI and associated 95% CI.

Cross-neutralization alphaviruses:

Table 74. Summary of PRNT results against arthritogenic alphaviruses

Virus	Cohort	Subjects with no neutralizing Ab[1]	Subjects with neutralizing Ab[1]	GMT PRNT50[2]	PRNT50 Range[2]	GMT PRNT80[2]	PRNT80 Range[2]
CHIKV-PM2951	WRAIR	0	30	3536	160-20480	996	20-5120
	USAMRIID	0	30	2829	80-20480	794	20-10240
CHIKV-15561	WRAIR	0	30	1009	40-5120	139	20-1280
	USAMRIID	0	30	667	20-10240	112	20-1280
CHIKV-181/25	WRAIR	0	30	1045	80-10240	114	20-1280
	USAMRIID	0	30	529	80-2560	69	20-320
CHIKV-LR	WRAIR	0	30	1317	40-5120	107	20-640
	USAMRIID	0	30	732	20-10240	69	20-640
ONNV	WRAIR	0	30	2987	80-20480	692	20-5120
	USAMRIID	0	30	3452	40-20480	924	20-10240
MAYV	WRAIR	2	28	79	20-640	40	20-80
	USAMRIID	1	29	531	20-10240	79	20-640
UNAV	WRAIR	3	27	92	20-320	36	20-80
	USAMRIID	5	25	83	20-640	49	20-160
RRV	WRAIR	11	19	34	20-160	20	20
	USAMRIID	19	11	43	20-160	30	20-40

[1]Individuals having detectable (≥ 20) neutralizing Ab at either D22 or D182
[2]Of those individuals who had detectable neutralizing Ab. Average and range is from both D22 and D182 data.

Table 75. Summary of PRNT results against encephalitic alphaviruses

Virus	Cohort	Subjects with neutralizing Ab before VLP vaccination	GMT PRNT50	PRNT50 Range	GMT PRNT80	PRNT80 Range	Subjects with neutralizing Ab after VLP vaccination[1]	GMT PRNT50[2]	PRNT50 Range[2]	GMT PRNT80[2]	PRNT80 Range[2]
EEEV	WRAIR	0	<20	<20	<20	<20	2	30	20-40	20	20
	USAMRIID	19	2462	40-20480	271	20-640	23	6231	20-20480	1162	20-10240
WEEV	WRAIR	0	<20	<20	<20	<20	0	<20	<20	<20	<20
	USAMRIID	14	107	20-320	65	20-160	17	150	20-320	72	20-160

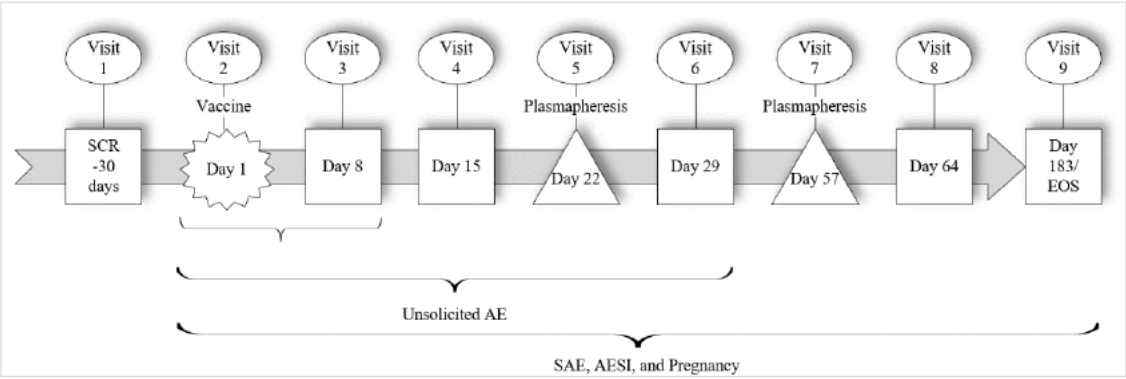
[1]Individuals having detectable neutralizing Ab at either D22 or D182
[2]Of those individuals who had detectable neutralizing Ab. Average and range is from both D22 and D182 data.

2.6.5.5.4. Phase 2 Study EBSI-CV-317-010

This was an open-label, single arm phase 2 study in healthy adults 18 to 45 years of age to assess safety and immunogenicity of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV VLP], alum-adjuvanted)

25 participants were planned to be enrolled. 51 participants were screened, with a total of 34 participants meeting the study eligibility criteria, however only 25 were enrolled and vaccinated, with the additional nine participants not being needed in the study. Of these nine additional participants not enrolled in the study, three decided to withdraw consent. Of the 25 participants that were enrolled and vaccinated, 23 (92.0%) completed the study.

Figure 51 ESBI-CV-317-010 Study design



Note: Days 29, 64, and 183 are phone calls.

Immunogenicity Results

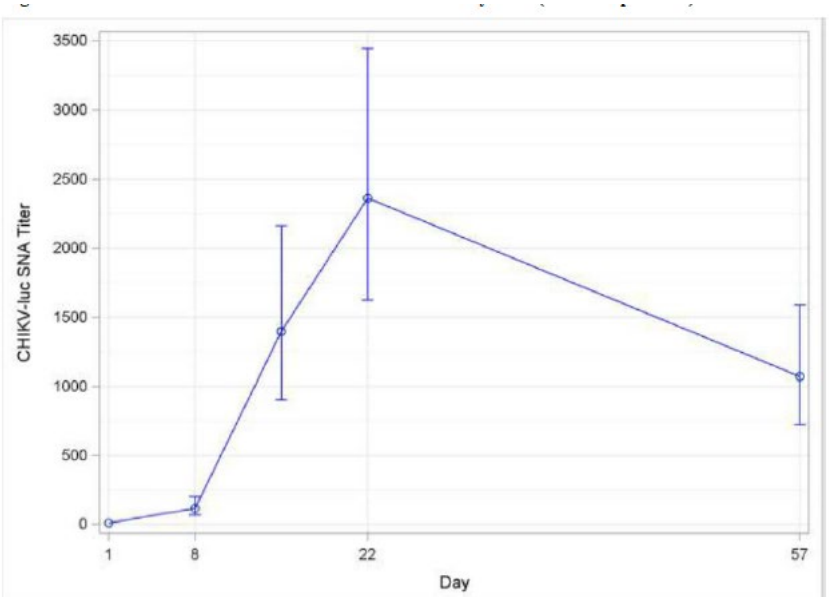
Induction of a CHIKV-specific immune response was determined following vaccination as measured by SNA, IgG, and IgM GMTs. Immune response was apparent as early as 7 days postvaccination in most participants, with a peak response at Day 22 overall. For the primary endpoints assessing SNA response up to Day 57, SNA titers ranged from 128.3 to 9720.9 at peak on Day 22 and dropped slightly by Day 57 with SNA titers ranging from 158.5 to 6000.5. At Day 15 and later, 100% of participants had reached SNA titers ≥40, as well as the more stringent titre ≥100 (SNA titers ranging from 146.9 to 7436.5 at Day 15).

By Day 8, all but one of the female participants (92.9%) had reached SNA titre ≥40, compared to 6 (54.5%) of the male participants by this visit. Female participants had roughly two-fold higher SNA GMTs than males at each timepoint. Although females did have a more pronounced increase in titers up to Day 22 (GMT of 1610.0 in males vs 3199.7 in females at Day 22), the SNA GMTs decreased more in females than in males over time, with females having an approximately 1.5-fold higher antibody titre by Day 57 (GMT of 872.7 in males vs 1270.8 in females).

Anti-CHIKV IgG and IgM levels measured by ELISA up to Day 57 showed similar kinetics to the SNA titers, peaking at Day 22, and decreasing at Day 57. Immunoglobulin M titers rose faster than IgG titers by Day 15, as expected based on a typical humoral immunity profile following antigen exposure.

Overall, SNA, IgG, and IgM titers remained high through Day 57 demonstrating an effective immune response was induced following vaccination with PXVX0317.

Figure 52. Geometric Mean CHIKV-luc SNA Titers by visit (mITT population)



2.6.5.6. Post approval effectiveness study

Title: A Phase 3b Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy, Safety, and Immunogenicity of an Adjuvanted Chikungunya Virus Virus-like Particle (CHIKV VLP) Vaccine for the Prevention of Chikungunya Disease in Adolescents (12 to <18 Years) and Adults (≥18 Years)

Protocol: Version 4.0, draft dated 22 January 2025

Rationale: Study EBSI-CV-317-007 is a field study to evaluate the efficacy, immunogenicity, and safety of CHIKV VLP. The study was designed using infectious disease models and advanced analytics to guide region and clinical site prioritization, define the timing of study activities, and optimize the study parameters to local epidemiological conditions for CHIKV disease to overcome the challenges of assessing efficacy for CHIKV VLP vaccine.

Study Centres: Multicentre, multiregional study

Study Duration for Each Participant: Minimum of 6 months, up to 3 years depending on date of enrolment.

Estimated Study Duration: Event driven; to be determined (TBD) by time to accumulate the number of acute CHIKV cases to assess vaccine efficacy, up to 4 years.

Estimated Enrolment Period: Event driven; TBD. Enrolment will proceed until the necessary number of acute CHIKV cases to assess vaccine efficacy have accrued.

Anticipated Start Date: TBD; as early as July 2025

Estimated End Date: Event driven; TBD. The study will terminate when the required number of confirmed cases have accumulated, and all participants have been followed for safety for at least 6 months after investigational product administration.

Study Design: EBSI-CV-317-007 is a phase 3b randomized, placebo-controlled, double-blind, event-driven efficacy study. It is a multicentre, multiyear clinical study. The study will enrol up to 6144 participants to be stratified (for balance) by age group (12 to <18, 18 to <46, 46 to <65, 65 to <75, and ≥75 years old) and site and randomized 1:1 to CHIKV VLP vaccine or placebo. Provided consent is not withdrawn, all participants will be followed after investigational product administration for a

minimum duration of 6 months and maximum of 3 years to identify cases of CHIKV disease and to monitor the safety of the vaccine. Study enrolment is event-driven, so enrolment will proceed until the necessary number of acute CHIKV cases to assess vaccine efficacy have accrued.

Active Study Start: The study will be initiated in populations (neighbourhoods, villages) at risk for local CHIKV transmission. Vaccination of participants will be triggered upon detection of a signal indicating the potential onset of a CHIKV disease outbreak in each geographic location (i.e., capture of a prespecified number of confirmed CHIKV cases by disease surveillance; this is not a fixed number and will require local PI input to determine if there is enough activity). Study participants will receive a single, intramuscular (IM) dose of investigational product, either CHIKV VLP or placebo. Participants will be monitored for new onset of acute clinical manifestations of CHIKV disease and those suspected of CHIKV disease will be tested for the virus. Enrolment in a location will depend on the detection of CHIKV cases by disease surveillance and will continue until transmission subsides (i.e., lack of new CHIKV cases for about 2 consecutive weeks identified by enhanced local surveillance) or a predetermined number of participants are enrolled (based on estimates of expected attack rates, demographic considerations, site capacity, and other factors identified during simulation modelling and site selection). Enrolment targets for each location will be determined by modelled transmission forecasts updated throughout the study, and site feasibility. In addition, to capture safety in the selected geographic locations, a subgroup of participants may receive investigational product before CHIKV transmission is detected (safety subset).The study will include an independent Data Monitoring Committee (DMC) and an independent Endpoint Adjudication Committee (EAC) that will review all potential cases for confirmation of case definition. The DMC, in addition to periodic review of safety data, will tally the total number of cases received and reviewed by the EAC.

Study Locations: The study will initially focus on multiple locations in arbovirus endemic regions in southeast Asia to increase the likelihood of observing new CHIKV transmission. If no or limited transmission is observed at the initial locations, additional locations in other regions will be considered.

Study Termination: This is an event-driven study that will continue until the target number of confirmed events is reached (n=64) and the DMC meets and makes a recommendation based on their tally of cases and estimates of efficacy and safety.

Number of Participants (Planned): Up to 6144; this is the projected number to enrol to achieve the target number of confirmed cases given the transmission and efficacy assumptions. If the event target of 64 acute CHIKV cases (or updated event target number) is achieved with lower enrolment or if futility or success are declared by the DMC, then study enrolment may be stopped earlier, subject to considerations about incidence of chronic CHIKV disease. If the event target of 64 acute CHIKV cases is not achieved with 6144 participants, then study feasibility will be evaluated, and additional participants may be enrolled.

Study Population: Males and nonpregnant females 12 years of age and older residing in areas at risk for CHIKV transmission.

Inclusion Criteria:

Participants must meet **all** the following criteria to be enrolled:

- Able and willing to provide informed consent (and assent, as applicable) voluntarily signed by participant (and guardian, as applicable). Must verbalize understanding of the reason for and the procedures needed for the study and be willing to stay in the study for its entire duration.
- Male or nonpregnant female 12 years of age or older.
- In stable health per the investigator, and no hospital admission or major surgical procedure in the last 30 days before investigational product administration.

- If not in the safety subset, negative for CHIKV antibodies as determined by CHIK IgM/IgG rapid diagnostic test (RDT).
- Women who are either:
    - Not of childbearing potential (CBP): premenarchal, surgically sterile (at least 6 weeks post bilateral tubal ligation, bilateral salpingectomy, bilateral oophorectomy, or hysterectomy); or postmenopausal (defined as a history of ≥12 consecutive months without menses prior to randomization in the absence of other pathologic or physiologic causes, following cessation of exogenous sex-hormonal treatment)

    or

    - Meeting **all** the criteria below:
        - Negative urine pregnancy test at screening visit
        - Negative urine pregnancy test immediately prior to dosing
        - Using an acceptable form of contraception per local standards and agree to use this for at least 8 weeks after investigational product administration

        Note: Contraception requirements do not apply for participants in exclusively same-sex relationships and these participants should have no plans to become pregnant by any other means for at least 8 weeks after investigational product administration.

Exclusion Criteria: Participants who meet **any** of the following criteria **cannot** be enrolled:

- Currently pregnant or breastfeeding.
- Participation or planned participation in an investigational clinical study within 30 days of Day 1 (investigational product administration) and for the duration of the study. Note: Participation in an observational trial/study or follow-up phase of a trial/study may be eligible; however, participation in any other study should be discussed with the medical monitor (MM) prior to enrolment.
- History of severe allergic reaction or anaphylaxis to any component of the investigational product.
- Prior receipt of any CHIKV vaccine (or therapeutic) or participation in a prior interventional CHIKV trial/study.
- Prior documented, laboratory confirmed CHIKV disease.
- History of any known congenital or acquired immunodeficiency or immunosuppressive condition that could impact response to investigational product administration (e.g., leukaemia, lymphoma, malignancy, functional or anatomic asplenia, alcoholic cirrhosis). Notes: i) history of basal cell and squamous cell carcinoma of the skin or carcinoma in situ of the cervix considered cured is not exclusionary; ii) history of malignancy considered cured from over 5 years from the date of screening with minimal risk of reoccurrence or relapse is not exclusionary; iii) documented controlled human immunodeficiency virus (HIV) infection (most recent tests show undetectable viral load and a CD4 cell count over 350 at the time of investigational product administration) is not exclusionary.
- Prior receipt or anticipated use of systemic immunomodulatory or immunosuppressive medications including hyperimmune products, monoclonal antibody therapies, systemic corticosteroids, and/or therapy with alkylating agents, antimetabolites, or radiation from 6

months prior to screening through Day 22 visit (21 days [-3/+5] after investigational product administration). **Note:** i) for systemic corticosteroid uses at a dose or equivalent dose of 20 mg of prednisone daily for 14 days or more within 90 days of screening through Day 22 visit is exclusionary, and ii) use of inhaled, intranasal, topical, or ocular steroids is not exclusionary.

- Receipt or anticipated receipt of any vaccine from 30 days prior to Day 1 through Day 22 visit.
- Unstable medical condition in the last 30 days prior to Day 1.
- Acute illness with or without fever (oral temperatures ≥38.0ºC [100.4°F]) within 14 days prior to investigational product administration.
- Medical or social condition (e.g., substance abuse) that could have an impact on the participant's ability to be compliant with study procedures/visits, as determined by the investigator.
- Plans to travel outside the study area or move away for more than 3 consecutive months and will not be available for follow up at the site.
- Identified as an investigator or employee of an investigator or study centre with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, spouse) of the investigator or employee with direct involvement in the proposed study, or identified as an employee of Bavarian Nordic, their families, contractors, agents, business partners, or anyone with a financial interest in the outcome of the study.
- Any other medical condition that, in the opinion of the investigator, could adversely impact the participant's participation or the conduct of the study.

Procedures and Assessments: The clinical study will be initiated at selected sites in populations (neighbourhoods, villages) at risk for local CHIKV transmission when a prespecified number of confirmed CHIKV cases are detected by enhanced surveillance and indicate the potential onset of a CHIKV disease outbreak within the site's geographic area of interest.

Study procedures and assessments will occur at: Screening (Visit 1), Day 1 (Visit 2, baseline, randomization, and administration of investigational product), Day 22 (Visit 3), Day 29 (Visit 4), Day 183 (Visit 5), semi-annually (by phone for safety follow-up), and annually. Confirmed acute CHIKV cases will be followed for at least 12 weeks for determination of chronic CHIKV disease and until symptom resolution or stabilization.

Blood samples will be drawn at Day 1 (prior to investigational product administration), Day 22, Day 183, annually to assess anti-CHIKV SNA levels, at EOS or Early Withdrawal Visit, and may be drawn periodically at additional times if the study is ongoing. A subset of these samples will be tested and constitute the basis for immunogenicity assessment (immunogenicity subset).

Participants will be monitored for new-onset febrile illnesses from Day 1. Study field teams will contact all participants twice weekly (with at least 2 days between contacts) if there is evidence of an outbreak to inquire whether they have experienced new onset of acute clinical manifestations of CHIKV disease.

Memory aids will be used to collect reactogenicity events and AEs following investigational product administration. Participants in the safety subset will record events in their memory aid daily for at least 28 days after investigational product administration.

Serious adverse events and AESI will be monitored and assessed for relation to investigational product for the duration of the study.

Figure 53 Schematic diagram of EBSI-CV-317-007 events

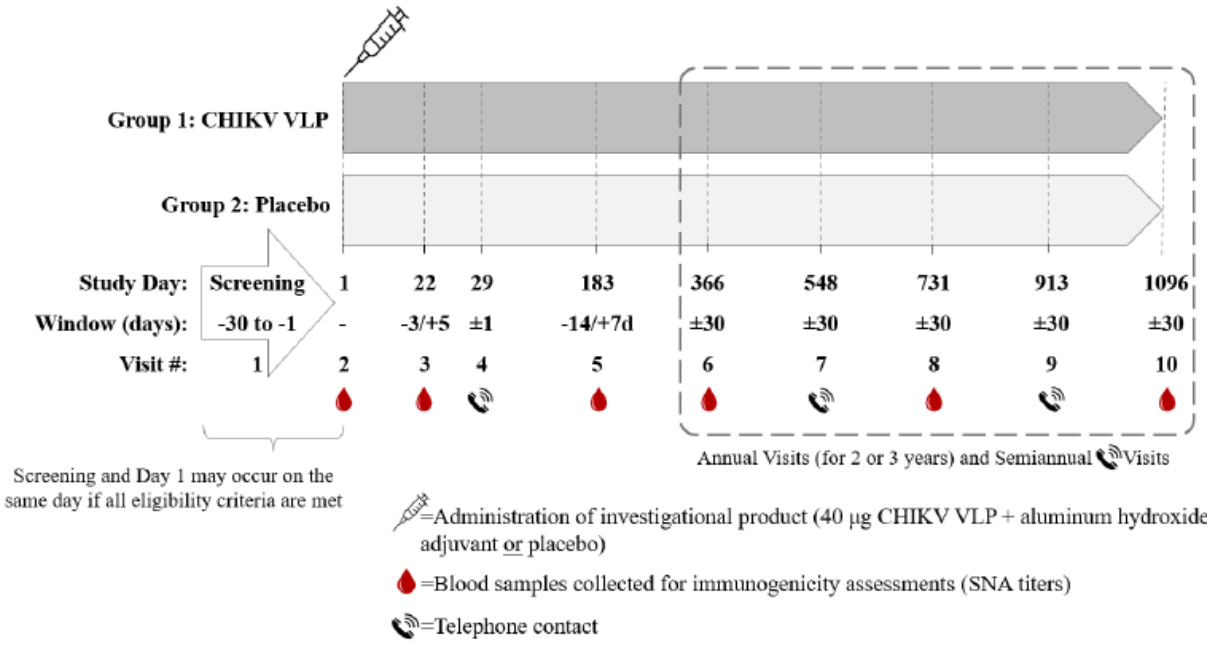


Table 76. Schedule of events

	Screening[1] Visit 1	Day 1[1] Visit 2	Day 22 Visit 3	Day 29 Visit 4 (phone)	Day 183 Visit 5	Annual Visits[2] (for 2 or 3 years)	Semiannual Visit[16] (phone)	EOS or Early Withdrawal Visit
Window (days)	**Within 30 days of Day 1**	**N/A**	**-3/+5d**	**±1d**	**-14/+7d**	**±30d**	**±30d**	
Informed consent/assent (as applicable)	X							
Eligibility criteria	X	*X						
Medical history[3]	X	*X[4]						
Demography[5]	X							
Physical examination[6]	X	*X[4]				X[4]		
Vital signs[7]	X	*X[8]				X		
Pregnancy test (urine sample)[9]	X	*X						
Viral markers (blood sample)[10]	X							
CHIKV IgM/IgG (blood sample)	X							
Randomization		*X						
Investigational product (CHIKV VLP or placebo) administration[11]		X						
Memory aid (paper) distribution and training[12]		X						
CHIKV fact sheet review		X	X	X	X			
Memory aid review[12]			X	X				
SAEs/AESI		X	X	X	X	X	X	X
AEs[12]		X	X	X				

	Screening[1] Visit 1	Day 1[1] Visit 2	Day 22 Visit 3	Day 29 Visit 4 (phone)	Day 183 Visit 5	Annual Visits[2] (for 2 or 3 years)	Semiannual Visit[16] (phone)	EOS or Early Withdrawal Visit
Window (days)	**Within 30 days of Day 1**	**N/A**	**-3/+5d**	**±1d**	**-14/+7d**	**±30d**	**±30d**	
Concomitant medications	X	X	X[13,14]	X[13,14]	X[13]	X[13]	X[13]	X[13]
Anti-CHIKV SNA (blood sample)		*X	X		X	X		X
Febrile illness observation follow-up[15]		X	X	X	X	X	X	X

AE = adverse event, AESI = adverse event of special interest, CHIKV = chikungunya virus, d = day, EOS = end of study, HBsAg = Hepatitis B surface antigen, HCV = hepatitis C virus, IgM = immunoglobulin M, IgG = immunoglobulin G, N/A = not applicable, SAE = serious adverse event, SNA = serum neutralizing antibodies.
*To be completed prior to investigational product administration.
[1]Screening and Day 1 may occur on the same day if all eligibility criteria are met.
[2]First annual visit will occur at 365 days after investigational product administration (Day 366), second annual visit will occur at 730 days after investigational product administration (Day 731), and third annual visit will occur at 1095 days after investigational product administration (Day 1096).
[3]Medical history information will be collected from participants at the Screening Visit and confirmed at the Day 1 Visit (ie, prior to investigational product administration) and will include (but not be limited to) relevant current and past medical conditions, recent relevant infections, surgical history, and prior medications.
[4]To be updated, if necessary.
[5]To include date of birth, race, ethnicity, and sex of participant.
[6]Complete physical examination will be performed on participants during the Screening Visit. The examination should include, general appearance, eyes-ears-nose-throat, head-neck, lungs-chest, heart, abdomen, musculoskeletal (including assessment of arthralgia: joint aches and/or pain and joint stiffness), lymph nodes, skin, extremities, and neurological assessment. Targeted physical exam may be performed on participants at additional time points if indicated by SAE, or AESI reporting or at the investigator's discretion.
[7]Vital signs collected from participants will include blood pressure, heart rate, respiratory rate, and oral temperature. Body weight, height for body mass index (BMI) calculations will be obtained at Screening Visit.
[8]To be taken before and after investigational product administration.
[9]Pregnancy tests (urine) will be performed at Screening Visit and Day1 for all women of CBP. Test result must be negative. Pregnancy tests are not required for female participants who are postmenopausal ≥12 months, or surgically sterilized.
[10]Blood samples for HBsAg, HCV antibody, HIV-1, and HIV-2 (HCV PCR will be performed in HCV antibody positive individuals only).
[11]Participants will be monitored by study staff for signs of an acute adverse reaction for at least 30 minutes after investigational product administration.
[12]Applies to safety subset only.
[13]Concomitant medications associated with SAE/AESI only.
[14]Concomitant medications associated with AEs will be collected for the safety subset only.
[15]Participants will be monitored for new-onset febrile illnesses from Day 1. Study field teams will contact participants twice weekly (with at least 2 days between contacts) when there is evidence of an outbreak to inquire whether they have experienced new onset of acute clinical manifestations of CHIKV disease (eg, high fever, and at least one of the following, rash, myalgia, or arthralgia). Query for new or ongoing SAEs and AESI.
[16]Semiannual visits will occur at 547 and 912 days after investigational product administration (Day 548 and Day 913).

Case identification & monitoring symptomatic participants

If a CHIKV positive case is suspected and it has been less than 7 days since symptom onset, an acute blood sample will be obtained for rapid diagnostic testing conducted locally, including a CHIKV-specific RT-qPCR. Participants who are RT-qPCR CHIKV positive, CHIKV RDT positive, or with a strong clinical suspicion of CHIKV disease will be followed (see table below). Participants will be provided an ePRO device for recording their symptoms and symptom severity. In addition, a serum sample will be submitted to a central laboratory for RT-qPCR confirmation and a serological assessment. Participants with RT-qPCR negative central laboratory results will return to regular study visits. For participants with RT-qPCR positive central laboratory results, a convalescent blood sample will be drawn 21 days (±5) after symptom onset for measurement of CHIKV IgG and IgM, and anti-CHIKV antibodies by human SNA assay.

The DMC will monitor periodic safety data reports and CHIKV positive cases.

Table 77. Acute febrile cases and chronic disease follow-up schedule of events

	Day of CHIKV symptom onset[1]	Day of Reporting		
		7 days post symptom onset	21 days post symptom onset	Post symptom onset follow-up, as needed[5]
Window (days)	**+6**	**±3**	**±5**	
Physical examination	X[2]	X[3]		
Vital Signs	X	X		
Blood sample for ZIKV/DENV/Malaria RDT, CHIKV RDT	X			
CHIKV strain identification or other tests (blood sample)	X			
CHIKV IgG and IgM (blood sample)	X		X	
CHIKV RT-qPCR (local laboratory, blood sample)	X			
CHIKV RT-qPCR (central laboratory, blood sample)	X			
Anti-CHIKV SNA (blood sample)	X		X	
Symptom follow-up[4]	X	X	X	X
Memory aid (ePRO) distribution and training[6]	X			

CHIKV = chikungunya virus, DENV = dengue virus, ePRO = electronic patient-reported outcomes, IgG = immunoglobulin G, IgM = immunoglobulin M, RDT = rapid diagnostic test, RT-qPCR = reverse transcription-quantitative polymerase chain reaction, SNA = serum neutralizing antibodies, ZIKV = zika virus.
[1]Visit closest to the day of CHIKV symptom onset is preferred.
[2]A complete physical examination. The examination should include, general appearance, eyes-ears-nose-throat, head-neck, lungs-chest, heart, abdomen, musculoskeletal (including assessment of arthralgia and stiffness), lymph nodes, skin, extremities, and neurological assessment.
[3]A targeted physical exam may be performed on participants if indicated by AE, SAE, or AESI reporting or at the investigator's discretion.
[4]Daily for first 7 days post-CHIKV symptom onset, weekly from 7 to 21 days post symptom onset, then monthly (at a minimum) until disease is stable or resolved as deemed by the investigator.
[5]Phone visits; in-person visits may be requested at the investigator's discretion.
[6]ePRO will be used by participants until disease is stable or resolved as deemed by the investigator.

Objectives:

Primary Efficacy Objective:

- To evaluate the vaccine efficacy (VE) of CHIKV VLP compared to placebo in the prevention of laboratory confirmed acute CHIKV disease in adolescents and adults (12 years of age and older).

Safety Objective:

- To evaluate the safety of CHIKV VLP in adolescents and adults (12 years of age and older).

Secondary Objectives:

- To evaluate the VE of CHIKV VLP in the prevention of chronic CHIKV disease.

Note: Chronic CHIKV disease will be defined as the persistence of at least one of the articular manifestations (continuous or recurrent pain, rigidity, oedema) for >12 weeks after the onset of laboratory confirmed acute CHIKV disease.

- To evaluate CHIKV VLP vaccine for prevention of severe CHIKV disease cases.

Note: Severe CHIKV disease is a clinical case of laboratory confirmed chikungunya presenting with dysfunction of at least one organ or system that threatens life and requires hospitalization.

- To evaluate the immunogenicity of CHIKV VLP vaccine.

Exploratory Objectives:

- To estimate anti-CHIKV serum neutralizing antibody (SNA) titter as an immune correlate of protection against CHIKV disease.
- To descriptively evaluate CHIKV disease cases, acute and chronic, by strain.
- To develop a post hoc severity score based on symptom severity and duration and use it to analyse VE according to disease severity.

Endpoints and Outcome Measures

Primary Efficacy Endpoint:

- Vaccine efficacy calculated from the incidence of laboratory confirmed acute CHIKV disease ≥14 days after investigational product administration in vaccine and placebo recipients.

Secondary Efficacy Endpoints:

- Vaccine efficacy in the prevention of chronic CHIKV disease calculated for the incidence of chronic CHIKV disease for the duration of the study.
- Vaccine efficacy in the prevention of severe CHIKV disease calculated for the incidence of severe CHIKV disease for the duration of the study.

Safety Endpoints:

- In the safety subset, frequency and percentage of participants with local and systemic reactogenicity events through Day 8 and adverse events (AEs) within 28 days after vaccination.
- For all participants who receive investigational product, frequency and percentage of participants with serious adverse events (SAEs) and adverse events of special interest (AESI) for the duration of the study. For participants in the safety subset, frequency and percentage of participants with medically attended adverse event (MAAE) for the duration of the study.

Note: All participants will be followed for a minimum duration of 6 months following investigational product administration.

Note: AESI for this study will be the occurrence of new onset or worsening arthralgia that is medically attended. Medically attended visits include hospital, emergency room (ER), urgent care clinic, or other visits to or from medical personnel. Routine scheduled study visits will not be considered medically attended visits.

Secondary Immunogenicity Endpoint:

- Immune response, defined as geometric mean titter (GMT) and percentage of participants with seroresponse; measured as SNA tested in specimens collected 3 weeks after investigational product administration (Day 22 [-3/+5 days]) in a subset of participants.

Note: Seroresponse rate (considered the presumptive seroprotection rate) is defined as the percentage of participants who achieve an anti-CHIKV SNA titter ≥100.

Exploratory Endpoints:

- Vaccine efficacy calculated from the incidence of laboratory confirmed acute CHIKV disease ≥7 days after investigational product administration in vaccine and placebo recipients.
- Determination of the anti-CHIKV SNA titter correlated with protection based on the most recent titter prior to onset in participants with CHIKV disease (cases) compared to matched controls (subset population) selected from participants without acute CHIKV disease.
- Frequency and percentage of participants with acute and chronic CHIKV disease by CHIKV strain.

- Post hoc severity score of CHIKV cases identified in the study based on the intensity and duration of symptoms and signs present in participants with acute CHIKV disease and VE according to disease severity score.

Statistical Methods

Sample Size Considerations:

Clinical study enrolment will be determined by observation of local CHIKV transmission and could vary considerably across sites and years. Based on preliminary sample size calculations assuming 70% VE for laboratory confirmed acute CHIKV disease and 1:1 CHIKV VLP vaccine to placebo control allocation, it is estimated that there is a need to observe a total of 64 cumulative cases of laboratory confirmed acute CHIKV disease across all sites to demonstrate efficacy with 90% power to rule out a 95% confidence interval (CI) lower bound of 25%. With 6144 subjects, an attack rate of 2% in the placebo arm would provide the target number of cases, which is considered conservative based on attack rates observed in known past CHIKV outbreaks.

Table 2 Sample Size Assumptions and Calculations[1]

Vaccine Efficacy	Required Total No. of Events	Cumulative Attack Rate in Placebo Arm	Cumulative Attack Rate in CHIKV VLP Arm	Sample Size in Placebo Arm	Sample Size in CHIKV VLP Arm	Total Sample Size
70%	64	0.5%	0.15%	12 303	12 303	24 606
		1%	0.30%	6149	6149	12 298
		2%	0.60%	3072	3072	6144
		4%	1.22%	1534	1534	3068
80%	36	0.5%	0.10%	7498	7498	14 996
		1%	0.20%	3748	3748	7496
		2%	0.40%	1873	1873	3746
		4%	0.81%	935	935	1870
90%	21	0.5%	0.05%	4772	4772	9554
		1%	0.10%	2386	2386	4772
		2%	0.20%	1193	1193	2386
		4%	0.41%	596	596	1192

[1]Total sample size requirements to achieve 90% power for testing that the lower bound of a 95% CI for VE as estimated by a Cox proportional hazards model is greater than 25%. Sample size is calculated using a 1:1 vaccine to control allocation and assuming a worst case 20% lost to follow-up over the duration of the study.

Analysis Populations:

Modified Intention-to-Treat (mITT) Population: Includes all randomized participants who receive investigational product and will be analysed according to their randomized investigational product group assignment. This is the primary analysis population for VE analyses.

Clinically important subgroup populations among the mITT analysis set will also be examined. These subsets will consist of categorization by age group, race, sex, geography, baseline CHIKV SNA serostatus, and immunosuppression status, among others.

Acute CHIKV Disease Population: Includes mITT participants who meet the case definition for acute CHIKV disease, analysed according to their randomized investigational product group assignment. This is the primary analysis population for strain analyses.

Immunogenicity Subset (IS): The IS includes the first 500 mITT participants in the study, analysed according to their randomized investigational product group assignment. If a participant meets the case definition for acute CHIKV disease at or before their Day 22 visit, that participant will be excluded

from the IS and replaced. Immunogenicity samples will be collected for all participants, but the samples will only be tested for the IS. This is the primary analysis population for immunogenicity analyses.

Correlate of Protection Subset (CoPS): The CoPS includes all acute CHIKV disease population participants and, additionally, a set of matched controls for the cases (5:1 ratio). The matched controls will be based on the following:

- Matched on location, investigational product administration date (within 2 months), and age (within 5 years) to acute CHIKV disease cases
- Requires negative CHIKV SNA at baseline

The CoPS analysis will use the actual investigational product received by each participant.

Safety Population: Includes all randomized participants who received investigational product and will be analysed according to the investigational product received. This is the primary analysis set for safety analyses.

Safety Subset: To provide more detailed safety postvaccination in these geographic regions, a subset of 500 participants from the safety population will comprise the first participants randomized in each geography (e.g., the first 125 per location if 4 locations are initiated). Safety subset participants will have local and systemic reactogenicity events collected through Day 8 and all AEs collected for 28 days after investigational product administration in addition to the collection of SAEs and AESI. Participants will be analysed according to the investigational product they received.

Considerations for Endpoints:

Vaccine efficacy is defined as the percent reduction in the hazard of the primary endpoint for the CHIKV VLP group relative to placebo in the mITT population. The study will be event driven, with the target number of events determined by the assumed level of VE for laboratory confirmed acute CHIKV disease, allocation ratio (1:1 vaccine to control), type I error (one-sided 0.025), target CI lower bound (25%), and desired power (90%). The study will be considered to meet the primary efficacy objective if the lower bound of the two- sided 95% CI of VE is >25%. In the primary analysis of VE, cases will be counted starting 14 days after vaccination. A Cox proportional hazards model including investigational product group and site will be used to assess the hazard ratio at a one-sided 0.025 significance level.

With an assumed, conservative VE target for laboratory confirmed acute CHIKV disease of 70%, an estimated 64 events will be required to confirm VE. This calculation assumes an underlying one-sample binomial test for the event split across the 2 arms, adapted from an approach used for dengue vaccines. The total sample size will be highly dependent upon the observed attack rate, so the target event number may be updated. Estimates of arbovirus attack rates during outbreaks can be over 10% in affected populations.

Assuming a conservative attack rate of ≥2% will be observed in the placebo group, with a 70% reduction in the vaccinated group, a total sample size of up to 6144 will achieve 90% power. However, based on immunogenicity data collected during phase 1 and 2 clinical studies showing 100% seroresponse (SNA titter ≥100) 21 days after vaccination (Day 22), it is possible that VE will be higher. At 90% VE, 21 events will be required to be observed among 2386 participants for a power of 90%. A Lan-DeMets alpha spending strategy using an O'Brien Fleming function will enable early stopping for success while preserving overall type I error.

A secondary efficacy objective is VE of CHIKV VLP vaccine to prevent chronic CHIKV disease in the mITT population. For this objective, the null hypothesis is that the lower bound of the one-sided 97.5% CI for chronic VE ≤0% with success therefore defined as the lower bound of the one-sided 97.5% CI

for chronic VE being >0%. In the analysis of VE against chronic CHIKV disease, participants presenting as acute cases with ongoing symptoms will be considered chronic starting 3 months (12 weeks) after the onset of symptomatic CHIKV disease. If 40% of acute cases are subsequently chronic, the sample size provided above will be sufficient to estimate VE against chronic CHIKV disease of 80% with 90% power. While chronic CHIKV disease is a very clinically relevant endpoint, its incidence is highly variable by virus strain and population, ranging from 20% to 80%. For this reason, VE against chronic CHIKV disease is a secondary efficacy objective but will be incorporated into the decision rules for the interim analysis. Ultimately, the recommendation by the DMC regarding study continuance will be based on a combination of the efficacy against acute CHIKV and observations regarding the magnitude of efficacy against chronic disease.

The assessment of VE for severe CHIKV disease in the mITT population will be determined in an analogous manner as the primary VE endpoint for acute CHIKV disease and the secondary VE endpoint for chronic CHIKV disease. The exploratory endpoint of VE for laboratory confirmed acute CHIKV disease ≥7 days after vaccination will be derived similarly but will use the earlier 7-day start period for acute CHIKV disease.

Immunogenicity, as measured by SNA GMT and percentage of participants with seroresponse using samples collected 3 weeks after investigational product administration (Day 22 [-3/+5 days]) will be assessed in a subset of participants as a secondary endpoint. As an exploratory endpoint, determination of an immune marker based on SNA as a correlate of protection will be attempted. The analysis will use a matched case-control design utilizing the most recent SNA titter obtained before the infection for those who become cases vs matched controls at a 5:1 ratio of controls for each case (see CoPS above). Another exploratory endpoint will consist of a descriptive analysis of participants with acute and chronic CHIKV disease by CHIKV strain. As a final exploratory endpoint, a post hoc severity score for CHIKV cases identified in the study will be developed based on the intensity and duration of symptoms and signs present in participants with acute CHIKV disease. Vaccine efficacy according to disease severity score will be estimated.

For the primary endpoint of acute VE, a sensitivity analysis will be included to assign any mITT population participant lost to follow-up as a CHIKV case. It is possible that an acute CHIKV disease case may be missed due to inadequately frequent monitoring. If a participant is RT-qPCR-negative (i.e., not a laboratory confirmed case), then they may be considered a "suspected" case, particularly if a 4-fold rise in titers between acute and convalescent samples is evident.

For the primary endpoint of acute VE, 2 additional sensitivity analyses will be performed for the following:

- The inclusion of all occurrences of laboratory confirmed acute CHIKV disease at any time
- The inclusion of all occurrences of laboratory confirmed acute CHIKV disease regardless of the occurrence of any intercurrent event

Preparatory Research (Epidemiological and Modelling Activities)
This clinical study will be conducted in populations at risk for local CHIKV transmission.

Because CHIKV transmission is characterized by unpredictable, explosive outbreaks, the selection of populations for the clinical study will be based on predictions of disease transmission derived from both i) analysis of prospective serological and surveillance data, and ii) mathematical modelling. Sero-epidemiological and modelling activities will be conducted prior to this study; they will be leveraged to identify locations most likely to support CHIKV transmission as well as guide the targeting (i.e., selection of subpopulations at high risk for CHIKV transmission) and optimization (i.e., target sample size) of the clinical study population.

Surveillance for CHIKV outbreaks

Surveillance for CHIKV outbreaks will be conducted under a parallel surveillance protocol to inform study EBSI-CV-317-007 decisions. Surveillance will be continual at the selected sites to detect signals indicating the potential onset of a CHIKV disease outbreak (i.e., a prespecified number of confirmed CHIKV cases within the site's geographic area of interest). The clinical study will be initiated in populations (neighbourhoods, villages) at risk for local CHIKV transmission.

Study site personnel will contact all participants twice weekly (with at least 2 days between contacts) if there is evidence of an outbreak to inquire whether they have experienced new onset of acute clinical manifestations of CHIKV disease. In addition, the participants will be continuously reminded to contact the study site when they experience CHIKV symptoms.

CHIKV Disease Symptom Severity Scale

A severity score for CHIKV cases identified in the study based on the intensity and duration of symptoms and signs present in participants with acute CHIKV disease will be developed post hoc and used to assess VE according to disease severity score.

Chronic CHIKV Disease

Chronic CHIKV disease in this protocol is defined as the persistence of at least one of the articular manifestations: continuous or recurrent pain, rigidity, oedema for >12 weeks after the onset of laboratory confirmed acute CHIKV disease. Clinical symptoms associated with acute and chronic disease will be captured in eCRFs.

Confirmed acute CHIKV cases will be followed for at least 12 weeks for determination of chronic CHIKV disease and until symptom resolution or stabilization.

2.6.6. Discussion on clinical efficacy

The application comprises data from 7 completed studies that investigated immunogenicity and safety of in total three formulations of the CHIKV VLP vaccine. 5 of those are considered of supportive value, these are 2 studies from early clinical development (VRC 311, VRC 704) conducted in healthy adults with the 1st formulation, and 3 studies for Phase 2 clinical development (PXVX-CV-317-001, EBSI-CV-317-002, and EBSI-CV-317-010) conducted in healthy adults with the 2nd formulation, of which PXVX-CV-317-001 represents the main dose-finding evidence. The main, pivotal evidence is provided by 2 Phase 3 studies, EBSI-CV-317-004 and EBSI-CV-317-005, in which the 3rd formulation was investigated in healthy adolescents and adults, and in elderly subjects, respectively.

Since a field efficacy trial in a chikungunya-endemic area is considered a formidable challenge and in addition, there is no established immunological correlate of protection against Chikungunya disease, clinical efficacy is inferred by use of a surrogate immunogenicity parameter in form of an anti-CHIKV SNA titre of at least 100 (using the CHIKV-Luc NT80 assay) that can be considered reasonably likely to predict protection from CHIKV disease, and was agreed in the scientific advice. The evidence to support this titre threshold stems from a prospective sero-epidemiologic study (published by Yoon et al., PLoS Negl Trop Dis. 2015) in subjects with prior exposure to CHIKV and from a non-human primate (NHP) passive transfer/challenge study (PAS-NHP-CHIK-003) using pooled sera from humans vaccinated with the CHIKV VLP vaccine. In detail, a logistic regression model (binary outcome: viraemia/no viraemia) of the NHP data predicted that a titre of 23.6 would correspond to 80% probability of protection from CHIKV viraemia. However, in consideration of the lower bound of the estimated 95% CI, complete protection from viremia at probabilities of at least 80% and 90% were attained with sufficient confidence (95%) at titres of 50 and 75, respectively. Consequently, to account

for potential uncertainties, the threshold of an anti-CHIKV SNA titre of at least 100 was considered appropriate.

Dose selection

The dose and regimen that was chosen to be examined further in the pivotal studies was a single intramuscularly administered dose of 40 µg CHIKV VLP adjuvanted with 300 µg aluminium hydroxide (40/300). Dose selection was primarily based on the humoral immune response results (anti-CHIKV SNA and binding IgG & IgM) of the dose-finding study PXVX-CV-317-001 that featured an intricate design to investigate various combinations of doses and regimens, adjuvanted and unadjuvanted.

Although other tested doses elicited initially higher titres than the 40/300 attempt, long-term data (Day 182, Day 365, Day 547) indicated that antibody-mediated immune responses waned noticeable slower with the latter. Moreover, the effect of the alum adjuvant was investigated with smaller doses of CHIKV VLP (e.g. 20 µg) and not directly with 40 µg but demonstrated an adding benefit for antibody persistence.

Design and conduct of clinical studies

As regards endpoints, for both pivotal studies, differences (CHIKV VLP vaccine minus placebo) in seroresponse rates (SRRs) (considered the presumptive seroprotection rate, defined as the percentage of participants who achieve an anti-CHIKV SNA titre ≥100, which is the threshold titre considered reasonably likely to predict protection) are considered most valuable as a they provide information about the percentage of subjects achieving the threshold titre and thus likelihood of protection in a population of vaccinees. To conclude superiority over placebo, the lower bound of the 95% CI had to be ≥70%. In contrast, other parameters, e.g. anti-CHIKV SNA GMTs are of minor value due to being difficult to interpret in terms of clinical relevance as they represent the collective titres of the respective analysis group. Moreover, analyses of GMFI and participants with an anti-CHIKV SNA titre ≥15 and 4-fold rise over baseline are also considered of limited value because of a link to clinical relevance being absent. Clinical lot-to-lot consistency was evaluated by the Applicant in study EBSI-CV-317-004, however, this is not required for the EU MAA as this is assessed on the quality level. The hierarchical order of the key secondary endpoints (differences in SRRs on Day 15 and Day 183 for EBSI-CV-317-005, and additionally Day 8 for EBSI-CV-317-004) in connection with the primary analysis on Day 22 is indeed reasonable in light of the vaccine's assumed application in case of an outbreak when information about how long after vaccination a certain percentage of vaccinated subjects might reach a titre likely to confer protection is of major significance.

EBSI-CV-317-004

This trial was a randomized, placebo-controlled, double-blind, parallel-group Phase 3 study to investigate safety, immunogenicity, and clinical lot consistency of a single IM 40 µg dose CHIKV VLP vaccine adjuvanted with 300 µg aluminium hydroxide given per pre-filled syringe (PFS) in 3258 healthy adults and adolescents 12 to <65 yoa, of which 3254 were treated (2790 CHIKV VLP vaccine, 464 placebo). Participants were randomized in a 2:2:2:1 ratio within each of the three age strata (ages 12 to <18 years, 18 to <46 years, and 46 to <65 years) to receive one of three consecutively manufactured lots of CHIKV VLP vaccine or placebo.

The study was conducted in 47 sites in the US and thus in a population with a relatively low rate of prior natural exposure to Chikungunya, as agreed in the scientific advice. Study design aspects were overall acceptable.

Apparently, no estimand was defined in either the protocol or SAP; definition of estimand elements can therefore only be implicitly deduced based on corresponding elements of the study design and analysis

methods. This mainly pertains to exclusion of subjects from the primary analysis set (IEP) and handling of corresponding missing information in the statistical analysis. Considering that only a moderate number of subjects were excluded from the IEP in combination with immunogenicity results clearly favouring the treatment group, however, no concern is raised.

Overall, statistical methods are considered appropriate. Use of the Newcombe hybrid score method to assess differences in seroresponse rate is acceptable. Use of Wilson CIs for individual proportions is acceptable. Comparison of log GMTs using ANOVA with stratification factors applied is considered adequate. While randomization was stratified by centre and age-group, the primary analysis did not adjust for the corresponding factors. Considering, however, that primary endpoint results were clearly in favour of superior immunogenicity with only minor differences in seroresponse rates between age strata no concern is raised.

EBSI-CV-317-005

This was a Phase 3, randomized, double-blind, placebo-controlled, parallel-group study with two treatment groups. A total of 400 healthy participants ≥65 years of age were planned for enrolment and stratified in two age subgroups (≥65 to <75 and ≥75 years of age). Participants were randomized in a 1:1 ratio to receive either a single IM dose of CHIKV VLP vaccine or placebo at Day 1.

The study was conducted in 10 sites in the US. The study design was overall acceptable, however, as noted in the SA, a different allocation rate in favour of the treatment arm (i.e., 3:1) would have been beneficial as this had increased the safety database of CHIKV VLP subjects.

No specific estimand was pre-specified in the protocol. Consequently, estimand components (especially intercurrent events and their handling) would need to be deduced from design and analysis. However, considering the moderate number of subjects that had to be excluded from analysis (for various reasons) and given that immunogenicity results clearly favour treatment, no substantial impact on results and conclusions are expected and no concern is raised.

Statistical methods to evaluate immunogenicity are by and large acceptable. Use of the Newcombe hybrid score method to estimate the confidence interval for the lower bound of the two-sided 95% difference in sero-response rates and conclusion of superiority if that bound is larger than 70% is considered appropriate. Similarly, the use of ANOVA to estimate GMT ratios is acceptable.

It appears that treatment allocation was stratified by age-group. Consequently, use of a corresponding stratification factor in the primary analysis would be expected. Considering that immunogenicity seems to be less strong in the >75 yoa cohort re-analysis of the two co-primary endpoints (difference in SRR, GMT ratio) using methods that stratify for age was requested. The corresponding stratified analysis confirmed the primary conclusion of superior immunogenicity.

Efficacy data and additional analyses

Immunogenicity data are limited to anti-CHIKV SNA titres, or analyses thereof. Analyses of other parameters of an immune response, for instance, binding antibodies or cell-mediated responses were not provided. However, considering that results of such additional immune response analyses would be – in absence of a suitable link to clinical relevance – hard to interpret, further analyses are not requested.

EBSI-CV-317-004

Baseline characteristics were overall acceptable, notably, the number of subjects in each age group (stratum) differed remarkably: The age group 12 to <18 years included 254 (7.8%), the age group 18 to <46 years 1906 (58.5%), and the age group 46 to <65 years 1098 (33.7%) subjects.

Difference in SRRs

The differences in SRRs on Day 22 and Day 15 were superior in the pooled CHIKV VLP groups compared to placebo for the IEP (96.6%, and 96.0%, respectively), all three age strata, and analysed subgroups. Moreover, no meaningful or critical differences were observed within the three age strata and each subgroup for Day 22 and Day 15, respectively. The lower bound of the 95% CI (difference in SRRs) of the age strata 12 to <18 years (adolescents) was 81.2% for Day 22 and 80.9% for Day 15, those of all other strata/groups were around 90% or even higher, however, no concern is raised as this is considered a consequence of the lower sample size. Overall, SRRs and differences thereof on Day 15 were only marginally lower than that reported for Day 22, which is an important information as in case of an endemic when the knowledge of the period until a considerable percentage of vaccinees is likely protected is crucial.

Overall, differences in SRRs on Day 183 were considerably lower than those of Day 15 and Day 22 (e.g., 84.0% in the IEP). Furthermore, the difference in SRRs of female subjects was considerably higher than that of male subjects (90.6% vs. 76.7%, respectively), in contrast to Day 22 and Day 15, were this gap was noticeable but not as pronounced; however, no concern is raised as this is not unexpected and the study was not designed to evaluate differences within this subgroup. Among the age strata, the highest difference in SRRs was reported in subjects 12 to <18 years, followed by subjects 18 to <46 years, and finally, subjects 46 to <65 years, with 94.8%, 83.2%, and 82.7%, respectively; this is expected as it is known that the immune system's reactivity decreases with age.

In contrast to Day 22, Day 15, and also Day 183, differences in SRRs at Day 8 were remarkably low, e.g. 46.1% in the IEP, which is not concerning as generally antibody responses in naïve subjects, in particular neutralizing antibody responses, are not readily available. As regards the three age strata, the highest difference in SRRs was reported in subjects 12 to <18 years, followed by subjects 18 to <46 years, and finally, subjects 46 to <65 years, with 67.8%, 50.8%, and 33.4%, respectively; alike Day 22, Day 15, and Day 183, this trend is expected as aforementioned.

Differences within subgroups race and ethnicity were observed, however, those were not considered to be of concern as the study was not designed to evaluate effects within these subgroups.

Anti-CHIKV SNA GMT

For the most part, anti-CHIKV SNA GMT data supported the observation of the differences in SRRs; GMTs of the IEP were 1618.05 on Day 22, 1095.83 on Day 15, 337.73 on Day 183, and 93.36 on Day 8. As mentioned before, those GMTs are difficult to interpret in terms of clinical relevance as they represent the titres of the entire analysis group, in contrast to the SRRs and difference in SRRs, which provide information about the percentage of subjects achieving the threshold titre. In order to allow a better view on titre distribution, the Applicant was requested to provide reverse cumulative distribution curves of each stratum and analysed subgroup on Day 22, Day 15, Day 183, and Day 8. The results of these data were overall in accordance with those of SRRs.

Other parameters

No meaningful difference between the three examined lots in terms of GMTs (coprimary endpoint) and difference in SRRs (exploratory endpoint) were reported. Concerning GMFIs, those increased from Day 1 to Day 8 to Day 15, peaked at Day 22, and declined afterwards (Day 183). The numbers and percentages of participants with an anti-CHIKV SNA titre ≥15 [≥LLOQ] (and thus considered seropositive) increased rapidly after CHIKV VLP administration and were at 99% or higher from Day 15 until study end. The number and percentage of participants with an anti-CHIKV SNA titre 4-fold rise over baseline peaked on Day 15 and Day 22 around 99% of subjects and declined afterward (92.9% on Day 183). This is acknowledged, however, alike the GMTs, a reliable connection to protection is challenging to be inferred.

Data of subjects seropositive at baseline

Only few (n=63) subjects were seropositive at baseline (defined as Day 1 pre-dose anti-CHIKV titre ≥15 [≥LLOQ]), which is expectable since the study was performed in the US and thus in a population with a relatively low rate of prior natural exposure to Chikungunya. The GMTs of those were considerably higher than the GMTs of subjects seronegative at baseline, throughout the entire study duration. This is expected as recurrence of an adjuvanted antigen usually elicits a substantial immune response. Of note, seronegative subjects mounted a higher GMFI than seropositive subjects.

EBSI-CV-317-005

Difference in SRRs

The difference in SRRs on Day 22 in the IEP was 86.2%. Superiority over placebo was demonstrated also in the age stratum ≥65 to <75 years, in female subjects, white subjects, Hispanic or Latino subjects, subjects not Hispanic or Latino but not in age stratum ≥75, male subjects, and non-white subjects. The finding that the lower bound of the 95% CI of the difference in SRRs of subjects ≥75 years was below 70 is not seen critical as the sample size in this group was rather low (n=40 for PXVX0317/CHIKV VLP vaccine and for placebo) and the differences in SRRs were quite high (≥65 to <75 years: 87.2%, ≥75 years: 82.5%) and within expectations for aging immune systems. The difference in SRRs of female subjects was considerably higher than that of male subjects (89.7% vs. 80.4%, respectively), however, not of concern as this is not unexpected and the study was not designed to evaluate differences within this subgroup.

The difference in SRRs on Day 15 was 79.5% in the IEP. Within the subgroup sex, similar to Day 22, a significant gap was observed as differences in SRRs were higher in female subjects than in male subjects, 86.3% vs. 68.9%, respectively. In addition, the gap between the difference in SRRs of subjects ≥65 to <75 years and that of subjects ≥75 years (81.1% and 73.0%, respectively) is, similarly to the finding on Day 22, not deemed critical, as the reactivity in terms of immune responses decreases with age.

The difference in SRRs on Day 183 was 74.4% in the IEP. A significant gap within the subgroup sex was observed (similar to Day 22 and Day 15) as differences in SRRs were higher in female subjects than in male subjects, 83.2% vs. 60.1%, respectively. Unlike to Day 22 (were the difference in SRRs in subjects ≥65 to <75 years was higher than that of subjects ≥75 years; 87.2% vs. 82.5%), the Day 183 differences in SRRs were relatively comparable between those age groups (74.7% and 73.0%, respectively).

Similar to adolescents and adults, differences within subgroups race and ethnicity were observed in the elderly, however, those did not raise concerns as the study was not designed to evaluate effects within these subgroups.

Anti-CHIKV SNA GMT

Overall, GMT data supported the findings of the differences in SRRs. GMTs of the IEP were 723.93 on Day 22, 378.42 on Day 15, and 233.02 on Day 183. In order to ease comprehensive analyses of anti-CHIKV SNA titre distribution, the Applicant was requested to provide reverse cumulative distribution curves of the anti-CHIKV SNA GMTs of each stratum and analysed subgroup on Day 22, Day 15, and Day 183. The results of these data were overall in accordance with those of SRRs.

Other parameters

Results for GMFIs, numbers and percentages of participants with an anti-CHIKV SNA titre ≥15 [≥LLOQ] (and thus considered seropositive) and with an anti-CHIKV SNA titre 4-fold rise over baseline

had similar trends to those of EBSI-CV-317-004 but with lower or slightly lower magnitude, explainable with the considerably higher age.

Data of subjects seropositive at baseline

The GMTs of the 5 subjects seropositive at baseline (defined as Day 1 pre dose anti-CHIKV titre ≥15 [≥LLOQ]) were considerably higher than the GMTs of subjects seronegative at baseline, throughout the entire study duration. This is expected as recurrence of an adjuvanted antigen usually elicits a substantial immune response.

Cross-protection/-neutralization data CHIKV

The CHIKV VLP vaccine is based on the chikungunya virus (CHIKV) Senegal strain 37997 from the West African (WA) lineage.

As agreed in the PRIME submission readiness meeting, cross-protection data of the supportive study EBSI-CV-317-002 (<65 yoa) were provided with the current application: The 3 major lineages Asian, WA, and ECSA were tested for CHIKV VLP vaccine cross- neutralization using the following 4 strains: 15561 (Asian – Thailand 1962), 181/25 (Asian – attenuated strain of 15561), LR (ECSA/IOL – La Reunion 2006), and PM2951 (WA – Senegal 1966). At Day 22, GMTs for strains 181/25, 15561, LR, and PM2951 were 584, 551, 905, and 2584, respectively, and seroconversion (titre ≥20) was seen in 100% of participants. At Day 182, GMTs for strains 181/25, 15561, LR, and PM2951 were 204, 184, 148, and 686, respectively, and seroconversion remained high (100% for strains 181/25 and PM2951 and 98% and 95% for strains 15561 and LR, respectively).

Cross-neutralization data across strains covering the main CHIKV lineages in the elderly will be provided post-authorization (assessed as exploratory endpoint in study EBSI-CV-317-005).

Cross-neutralization data other alphaviruses

Limited data on cross neutralization of other alphaviruses were generated in 30 alphavirus-naïve individuals and 30 individuals who had previously received investigational alphavirus vaccines (exploratory endpoint in study EBSI-CV-317-002).

The highest titres of cross-neutralizing antibodies upon CHIKV vaccination were observed against O'nyong Nyong virus (ONNV), the closest phylogenetically related alphavirus to CHIKV. All subjects had neutralizing Ab against ONNV. More than 90% of subjects had neutralizing Ab titre against MAYV and more than 83% against UNAV (across both groups). The most distantly related arthritogenic alphavirus, Ross River virus (RRV), had the lowest levels of cross-neutralizing antibodies titres. Only two participants from the alphavirus-naïve cohort had detectable transient neutralizing antibodies on Day 22 post-vaccination against equine encephalitis virus (EEEV) and none had detectable western equine encephalitis virus (WEEV)-specific neutralizing antibody titres. These results suggest that some protective effect against the more closely related alphaviruses may be expected. Still, this is based on limited, exploratory data and remains to be confirmed.

Persistence and booster data

Anti-CHIKV SNA titres of an adjuvanted formulation of the CHIKV VLP vaccine beyond Day 183 were only investigated in study PXVX-CV-317-001. However, because of its dose-finding, proof-of-concept purpose and the thereby limited sample size, and also the restricted age range (18 to ≤45 yoa), PXVX-CV-317-001 is only considered as supportive to inform about immune responses in general. A booster was examined also in one group of that study but after a primary series with two doses of adjuvanted 20 µg CHIKV VLP 4 weeks apart, on top of the aforementioned limitations. Therefore, to shed light into persistence of immune responses beyond 6 months and the appropriate time of booster dose, data of study EBSI-CV-317-008, which serves as rollover study for the pivotal studies and plans to investigate

anamnestic response induced by booster administrations (after 3, 4, or 5 years post-initial vaccination), is of significant importance and expected to be provided by the Applicant via future variations.

Supportive studies

The supportive data package includes 2 studies (1 Phase 1, 1 Phase 2) conducted early in the development of CHIKV VLP that investigated a non-adjuvanted formulation of the vaccine at various doses (between 10-40 µg) given at week 0 and 4 (both studies) or additionally at week 20 (Phase 1 only). Results demonstrate that an anamnestic response could be induced with the numerically highest humoral immune response seen after the third (booster) dose in the Phase 1 study. This will be further evaluated for the clinically relevant dose in study EBSI-CV-317-008 post-MA.

Supportive data evaluating the clinically relevant 40/300 µg CHIKV VLP dose were generated in alphavirus-vaccine naïve vs. experienced individuals (study EBSI-CV-317-002, 18-65 yoa, n=30 per group, immunogenicity data up to day 183) and in a single arm study (study EBSI-CV-317-010, 18-45 yoa, n=30, immunogenicity data up to day 57). Immunogenicity data are overall in line with those obtained in the Phase 3 studies and no safety findings of concern became apparent.

Immunocompromised individuals

No data are available. Given that this vaccine could potentially be used in immunocompromised individuals (in contrast to the approved LAIV), such data would be of interest. Hence, the Applicant was initially asked to present their plans on investigating immunogenicity (and safety) in this population. In their response, the Applicant noted that there are no established plans to conduct a study in immunocompromised individuals, however, participants with chronic hepatitis B and C as well as individuals with controlled HIV disease are permitted to be enrolled in planned post-approval effectiveness study EBSI-CV-317-007 and thus it is likely that the study will generate information on the use of the vaccine in these immunocompromised participants.

Concomitant vaccination

No data on concomitant vaccination with any other vaccines are available. Although not a requirement for B/R assessment in the scope of this MAA, such data would be of interest for future use of this vaccine. The Applicant was therefore asked to present their plans how to investigate immunogenicity (and safety) of concomitant vaccination. In their response, the Applicant argued that at this time a study to investigate concomitant vaccination is not feasible due to the varying recommendations for vaccine administration to CHIKV-endemic regions, and the multiple combinations for co-administration of travel vaccines; moreover, concomitant vaccination is planned to be monitored via routine pharmacovigilance activities but co-administration of other vaccines within a certain period is an exclusion criterion of the PAES EBSI-CV-317-007. Based on that, information as regards concomitant vaccination will be likely scarce and not be subject to any planned study analyses. Consequently, it is recommended to investigate co-administration with other relevant vaccines (e.g., traveller vaccines) post-marketing.

Post-approval effectiveness study

Irrespective of the validity of the immunogenicity results of the pivotal studies, these are based on a neutralizing antibody titre threshold reasonably likely to predict protection (supported by animal and sero-epidemiological data) and not on an established immune correlate of protection. Uncertainties remain around how this threshold actually translates into protection against disease and/or infection. Therefore, the actual level of protection of CHIKV VLP vaccine remains to be confirmed, and effectiveness data are needed as soon as possible to substantiate the clinical benefit of this vaccine.

In order to address this, the Applicant plans to conduct a single post-approval Phase 3b randomized, double-blind, placebo-controlled field study (EBSI-CV-317-007) to evaluate the effectiveness, safety, and immunogenicity of the CHIKV VLP vaccine for the prevention of chikungunya disease in individuals ≥ 12 yoa. A study protocol (V 4.0, draft dated 22 January 2025) was submitted, and a detailed feasibility evaluation was provided upon request (see below).

The study will enrol up to 6144 participants in different arbovirus endemic locations in Southeast Asia to be stratified (for balance) by age group (12 to <18, 18 to <46, 46 to <65, 65 to <75, and ≥75 years old) and site and randomized 1:1 to CHIKV VLP vaccine or placebo. Participants are planned to be followed-up for a minimum of 6 months and maximum of 3 years to identify cases of CHIKV disease and to monitor the safety of the vaccine. The study will be initiated in populations (neighbourhoods, villages) at risk for local CHIKV transmission, vaccinations will be triggered upon capture of a prespecified number of confirmed CHIKV cases by disease surveillance in consultation with local PI input.

Study site personnel will contact all participants twice weekly (with at least 2 days between contacts) if there is evidence of an outbreak to inquire whether they have experienced new onset of CHIKV disease. In addition, the participants will be continuously reminded to contact the study site when they experience CHIKV symptoms. An independent Data Monitoring Committee (DMC) and an independent Endpoint Adjudication Committee (EAC) will review all potential cases for confirmation of case definition.The primary study objective is to evaluate the vaccine effectiveness of CHIKV VLP compared to placebo in the prevention of laboratory confirmed acute CHIKV disease in individuals ≥ 12 yoa. Secondary study objectives will evaluate the VE of CHIKV VLP in the prevention of chronic CHIKV disease (persistence of at least one of the articular manifestations (continuous or recurrent pain, rigidity, oedema) for >12 weeks after the onset of laboratory confirmed acute CHIKV disease), evaluate CHIKV VLP vaccine for prevention of severe CHIKV cases (laboratory confirmed chikungunya with dysfunction of at least one organ or system that threatens life and requires hospitalization) and immunogenicity.

Exploratory study objectives will evaluate anti-CHIKV serum neutralizing antibody (SNA) titter as an immune correlate of protection against CHIKV disease and CHIKV disease cases, acute and chronic, by strain. Furthermore, development of a post hoc severity score based on symptom severity and duration and use it to analyse VE according to disease severity is planned.

Overall, EBSI-CV-317-007 is expected to generate the highest level of evidence, as it is a placebo-controlled randomized trial. Key study aspects including the study population, definitions of endpoints, monitoring and identification of CHIKV cases are overall considered adequate to deliver meaningful results. The study may provide estimates of VE against chronic chikungunya.

However, the study will be challenging on the feasibility point of view. Due to inclusion of a placebo-arm, the study poses question in terms of ethical feasibility, particularly in an outbreak setting. The views of local Ethics Committee are not known at this stage. Conducting this study will be challenging due to the sporadic and short-lived nature of chikungunya outbreaks, along with the season-dependent and geographically inconsistent transmission, with very high spatial-temporal heterogeneity of transmission.

Upon request, the Applicant provided more specific plans for the planned study conduct, including evidence on the feasibility and evidence of support from and interactions with public health institutions in the various Southeast Asian countries.

In addition, the Applicant clarified that an observational, epidemiological study (CHIKPREP, CHIKV-VLP-011) will be conducted to provide serological and disease surveillance data. CHIKPREP is planned to be conducted before and in parallel with EBSI-CV-317-007 to inform the conduct of the latter. A draft

protocol (in ethics review at the time of writing) for CHIKPREP was provided. Together with mathematical modelling CHIKPREP results will be used to target and time enrolment of individuals at greatest risk of CHIKV transmission. This study will be conducted at different locations in Thailand and the Philippines (and potentially other countries) with high endemic Aedes-borne disease burden (dengue, Zika, chikungunya) and a history of local CHIKV transmission. CHIKPREP is planned to be initiated in Q1 2025.

The Applicant furthermore provided relevant information on (positive) interactions with Thai and Philippines ethics committees, local investigators and community officials to initiate EBSI-CV-317-007 (planned start Q2 2025).

As regards site feasibility and selection, the Applicant states that the selected sites for EBSI-CV-317-007 have a history of both dengue virus and CHIKV transmission. In addition, most sites previously participated in dengue vaccine trials, thus providing operational expertise and experience to the overall research programme. From an operational point of view this strategy is considered reasonable.

Overall, evidence on feasibility and interactions with public health institutions have been provided. Based on the outlined plans it is anticipated that relevant data on CHIKV VLP vaccine effectiveness in case of a CHIKV outbreak can be generated.

EBSI-CV-317-007 is included as an Annex II D condition in the SmPC with proposed timelines based on reasonable assumptions (CSR due August 2030). Amendments to proposed timelines may be needed in the post-marketing period depending on (lack of) CHIKV outbreaks and can be handled via Type II variations.

Moreover, several additional aspects were raised and were clarified as follows:

- The Applicant was requested to confirm that time from CHIKV outbreak to vaccination and especially time from vaccination to the end of the outbreak will be recorded. The Applicant clarified that there will be 2 stages of enrolment: 1) to enrol a cohort for safety and immunogenicity studies and 2) to enrol a cohort to evaluate efficacy once a new chikungunya outbreak is detected. Measures will be in place that are expected to contextualize the time from CHIKV outbreak to vaccination and time from vaccination to the end of the outbreak.
- The Applicant’s judgement concerning the urgency to detect strong signals for efficacy as early as possible was not shared. Upon request, the Applicant clarified aspects of the planned group sequential design in Version 4.0 (draft Dec 2024) of the study protocol, with further details to be specified in an eventual SAP. This was considered acceptable. Corresponding plans, also include options to continue recruitment in case insufficient events to address secondary objectives (e.g. with respect to chronic and severe disease) have been accrued at the time of interim analysis
- Initially, it was not entirely clear if and for what purpose an antigen-based diagnostic would have been employed, particularly if RT-PCR is the (more sensitive) method of choice for case identification. The Applicant explained that a rapid diagnostic test (RDT) will be used to help diagnose febrile cases (CHIKV IgM/ IgG) and it was further stated that “The RDT result will be provided to patients as a benefit and used by the study team to help identify suspect CHIKV disease cases for follow-up until these can be verified by a validated reverse transcription quantitative polymerase chain reaction (RT-qPCR) test at a central lab. All suspected CHIKV cases will be confirmed by RT-qPCR.” In this context it was initially unclear why this would constitute a benefit to patients and more importantly, whether the RDT will be employed as a sort of pre-screening measure that may not detect all CHIKV cases, which would otherwise be selected for the more sensitive RT-PCR testing. Thus, the Applicant was asked to confirm that also suspected CHIKV cases that are RDT negative, will be further tested with RT-PCR. In their

response, the Applicant clarified that all suspected CHIKV infections (including those with a negative RDT result) will be tested by the validated RT qPCR at a central lab. Moreover, the Applicant noted that site staff will be trained to consider the chikungunya RDT result in their diagnosis, but to not exclude a negative sample from additional testing if the Principal Investigator still suspects chikungunya (i.e., symptoms are present, antigen-based RDT for dengue/Zika is negative), and, additionally, that the RDT results generally (Zika, Dengue, CHIKV) may support clinical diagnosis and provide a benefit to study participants.

The Applicant acknowledged issues about interpretation of results from the planned study.

Concerning target population, the Applicant noted that some proportion of cases can be expected to emerge from safety and immunogenicity study populations and thereby should provide evidence also with respect to a non-reactive setting. It remains, however, unclear whether this will yield sample sizes (case numbers) sufficient to draw corresponding conclusions. Moreover, it may be prudent to reflect different treatment modalities (reactive vs. non-reactive) in the analysis model to address potential treatment effect heterogeneity (e.g. arising from waning protection), which should also improve sensitivity of the planned statistical model. Similar considerations apply to cases treated as a reaction to an outbreak in an earlier season.

Considering the long follow-up period for each subject, it can be expected that following the confirmatory analysis further cases accrue which may provide critical information on secondary objectives as e.g., long-term protection. Pre specification of corresponding updated analyses would improve the credibility of eventual results.

Aspects concerning the handling of intercurrent events remained unclear and no analysis considering all cases, regardless of onset, was foreseen in the protocol Version 4.0 (draft Dec 2024). Consequently, the Applicant was requested to clarify the handling of intercurrent events and especially for cases emerging after vaccination in the estimand definition. In addition, a supplementary analysis including all cases regardless of time of onset should have been added. With the response to that request, an updated draft version (Jan 2025) of the protocol Version 4.0 was submitted, in which the requested changes (Removal of the sentence "Participants in the population will be analysed regardless of any intercurrent event occurring while on study." and addition of two sensitivity analyses for the primary endpoint of acute VE ["The inclusion of all occurrences of laboratory confirmed acute CHIKV disease at any time.", "The inclusion of all occurrences of laboratory confirmed acute CHIKV disease regardless of the occurrence of any intercurrent event."]) were implemented.

2.6.7. Conclusions on the clinical efficacy

In summary, the provided immunogenicity data indicate that administration of a single dose of CHIKV VLP vaccine elicits a humoral immune response that is likely to confer protection in the majority of vaccinees. However, some concerns are raised that preclude a final conclusion on clinical efficacy.

2.6.8. Clinical safety

The safety profile of CHIKV VLP vaccine was established based on pooled data gathered from five clinical studies conducted with CHIKV VLP vaccine in healthy volunteers, i.e., three phase 2 studies (**PXVX-CV-317-001, EBSI-CV-317-002 and EBSI-CV-317-010**) and two phase 3 studies (**EBSI-CV-317-004** and **EBSI-CV-317-005**).

The safety population on which pooled safety analyses were based on included all participants who received the vaccine and provided safety assessment data.

Different dosages and vaccination schedules for CHIKV VLP vaccine, ranging from 6 to 40 µg CHIKV VLP with or without aluminium hydroxide adjuvant, were evaluated in study PXVX-CV-317-001, after which the single dose of 40 µg CHIKV VLP and 300 µg aluminium as aluminium hydroxide adjuvant (40/300 µg) was selected for further investigations in phase 2 and phase 3 studies.

Local and systemic solicited AEs were collected from Day 1 (day of vaccination) until 7 days post-vaccination (Day 8) using a memory aid. Solicited AEs included local events of pain, redness, and swelling at the injection site and systemic events of oral temperature ≥38.0°C (≥100.4°F), chills, fatigue, headache, myalgia, arthralgia, and nausea (and malaise for studies PXVX-CV-317-001 and EBSI-CV-317-002). Unsolicited AEs (AEs not listed in the memory aid) were collected from Day 1 through 28 days post-vaccination (Day 29). Serious adverse events (SAEs) were collected for all participants from Day 1 through Day 183 End of study (EOS) visit, as well as adverse events of special interest (AESI) (new onset or worsening arthralgia that was medically attended) in studies EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005, and medically attended adverse events (MAAEs) in studies EBSI-CV-317-004 and EBSI-CV-317-005.

2.6.8.1. Patient exposure

A total of 3522 participants were exposed to at least one IM dose of CHIKV VLP vaccine: 526 (14.9%) participants in phase 2, and 2996 (85.1%) participants in phase 3. Overall, 3141 participants received a single dose of 40/300 µg CHIKV VLP vaccine.

Among the 3141 participants who were exposed to a single dose of 40/300 µg CHIKV VLP vaccine, 217 participants were adolescents (12 to less than 18 years of age), and 206 participants were 65 years of age and older.

Table 78, 79 and 80 summarises the clinical trial exposure.

Table 78.Patient exposure

	Patients enrolled	**Patients exposed[1]**	**Patients exposed to the proposed dose range**	**Patients with long term[2] safety data**
Blinded studies (placebo-controlled)[3]	3667	2996	2996	2724
Blinded studies (active-controlled)[4]	445	441	60	391
Open studies[5]	85	85	85	84
Post marketing	0	0	0	0
Compassionate use	0	0	0	0

[1] Received at least 1 dose of active treatment.
[2] Received at least 1 dose of active treatment and was followed for safety through 6 months. Participants were included if they received any dose of CHIKV VLP vaccine and were in the respective trial for at least 168 days, calculated as 6 months with a lower 2 week window.
[3] ESBI-CV-317-004 and ESBI-CV-317-005.
[4] ESBI-CV-317-001 explored different regimens of CHIKV VLP vaccine administration, with CHIKV VLP vaccine being the active control.
[5] ESBI-CV-317-010 and ESBI-CV-317-002.

Table 79. Participant safety population by study phase, study, and treatment group- All studies, safety population, all ages pooled

		CHIKV VLP Vaccine				
Study Phase	**Study**	**Single Dose 40/300 µg[a] (N=3141) n (%)**	**Other CHIKV VLP Dose[b] (N=381) n (%)**	**Total CHIKV VLP (N=3522) n (%)**	**Pooled Placebo (N=675) n (%)**	**Total Safety Population All Ages (N=4197) n (%)**
Phase 2	PXVX-CV-317-001	60 (1.9)	381 (100)	441 (12.5)	4 (0.6)	445 (10.6)
	EBSI-CV-317-002	60 (1.9)	0	60 (1.7)	0	60 (1.4)
	EBSI-CV-317-010	25 (0.8)	0	25 (0.7)	0	25 (0.6)
Total Phase 2		**145 (4.6)**	**381 (100)**	**526 (14.9)**	**4 (0.6)**	**530 (12.6)**
Phase 3	EBSI-CV-317-004	2790 (88.8)	0	2790 (79.2)	464 (68.7)	3254 (77.5)
	EBSI-CV-317-005	206 (6.6)	0	206 (5.8)	207 (30.7)	413 (9.8)
Total Phase 3		**2996 (95.4)**	**0**	**2996 (85.1)**	**671 (99.4)**	**3667 (87.4)**
Total Both Phases		**3141 (100)**	**381 (100)**	**3522 (100)**	**675 (100)**	**4197 (100)**

N = number of participants per group; n = number of participants per parameter

Note: Safety population = Exposed participants who provided safety assessment data; participants in CHIKV VLP treatment groups who received only placebo and not CHIKV VLP vaccine are counted in the placebo group only. Percentages are based on the number of safety population participants in each column.

[a] Study PXVX-CV-317-001 Groups 8 and 10: 40 µg dose with 300 µg adjuvant single dose; studies EBSI-CV-317-002, EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005.

[b] Study PXVX-CV-317-001 Groups 1-7 and 9: 6, 10, or 20 µg dose with or without 300 µg adjuvant administered twice separated by 14 or 28 days (includes participants who received only one dose).

Table 80. Participant safety population by age group, study and treatment group – all studies, safety population

		CHIKV VLP Vaccine				
Age Group	**Study**	**Single Dose 40/300 µg[a] (N=3141) n (%)**	**Other CHIKV VLP Dose[b] (N=381) n (%)**	**Total CHIKV VLP (N=3522) n (%)**	**Pooled Placebo (N=675) n (%)**	**Total Safety Population All Ages (N=4197) n (%)**
12 to <18 years	EBSI-CV-317-004	217 (6.9)	0	217 (6.2)	37 (5.5)	254 (6.1)
18 to <46 years	PXVX-CV-317-001	60 (1.9)	381 (100)	441 (12.5)	4 (0.6)	445 (10.6)
	EBSI-CV-317-002	27 (0.9)	0	27 (0.8)	0	27 (0.6)
	EBSI-CV-317-010	25 (0.8)	0	25 (0.7)	0	25 (0.6)
	EBSI-CV-317-004	1634 (52.0)	0	1634 (46.4)	270 (40.0)	1904 (45.4)
Total 18 to <46 years		**1746 (55.6)**	**381 (100)**	**2127 (60.4)**	**274 (40.6)**	**2401 (57.2)**
46 to <65 years	EBSI-CV-317-002	33 (1.1)	0	33 (0.9)	0	33 (0.8)
	EBSI-CV-317-004	939 (29.9)	0	939 (26.7)	157 (23.3)	1096 (26.1)
Total 46 to <65 years		**972 (30.9)**	**0**	**972 (27.6)**	**157 (23.3)**	**1129 (26.9)**
65 to <75 years	EBSI-CV-317-005	159 (5.1)	0	159 (4.5)	159 (23.6)	318 (7.6)
≥75 years	EBSI-CV-317-005	47 (1.5)	0	47 (1.3)	48 (7.1)	95 (2.3)
18 to 65 years	PXVX-CV-317-001	60 (1.9)	381 (100)	441 (12.5)	4 (0.6)	445 (10.6)
	EBSI-CV-317-002	60 (1.9)	0	60 (1.7)	0	60 (1.4)
	EBSI-CV-317-010	25 (0.8)	0	25 (0.7)	0	25 (0.6)
	EBSI-CV-317-004	2573 (81.9)	0	2573 (73.1)	427 (63.3)	3000 (71.5)
	EBSI-CV-317-005	20 (0.6)	0	20 (0.6)	18 (2.7)	38 (0.9)
Total 18 to 65 years		**2738 (87.2)**	**381 (100)**	**3119 (88.6)**	**449 (66.5)**	**3568 (85.0)**
>65 years	EBSI-CV-317-005	186 (5.9)	0	186 (5.3)	189 (28.0)	375 (8.9)
Total All Ages		**3141 (100)**	**381 (100)**	**3522 (100)**	**675 (100)**	**4197 (100)**

N = number of participants per group; n = number of participants per parameter
Note: Safety population = Exposed participants who provided safety assessment data; participants in CHIKV VLP treatment groups who received only placebo and not CHIKV VLP vaccine are counted in the placebo group only. Percentages are based on the number of safety population participants in each column.
[a] Study PXVX-CV-317-001 Groups 8 and 10: 40 µg dose with 300 µg adjuvant single dose; studies EBSI-CV-317-002, EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005.
[b] Study PXVX-CV-317-001 Groups 1-7 and 9: 6, 10, or 20 µg dose with or without 300 µg adjuvant administered twice separated by 14 or 28 days (includes participants who received only one dose).

2.6.8.2. Adverse events

Solicited AEs (reactogenicity)

Local and systemic solicited AEs were collected from Day 1 (day of vaccination) until 7 days post-vaccination (Day 8) using a memory aid. Solicited AEs included local events of pain, redness, and swelling at the injection site and systemic events of oral temperature ≥38.0°C (≥100.4°F), chills, fatigue, headache, myalgia, arthralgia, and nausea (and malaise for studies PXVX-CV-317-001 and EBSI-CV-317-002).

Table 81. Reactogenicity within 7 days post vaccination by maximum severity and treatment group- all studies, Safety population

Reactogenicity Parameter Severity	CHIKV VLP Vaccine Single Dose[a] 40/300 µg (N=3141) n (%)	CHIKV VLP Vaccine Other CHIKV VLP Vaccine Dose[b] (N=381) n (%)	CHIKV VLP Vaccine Total CHIKV VLP Vaccine (N=3522) n (%)	Pooled Placebo (N=675) n (%)
Any Reaction	**1156/3115 (37.1)**	**162/379 (42.7)**	**1318/3494 (37.7)**	**154/661 (23.3)**
Grade ≥3	50/3115 (1.6)	8/379 (2.1)	58/3494 (1.7)	3/661 (0.5)
Any Local Reaction	**730/3114 (23.4)**	**112/379 (29.6)**	**842/3493 (24.1)**	**53/661 (8.0)**
Grade ≥3	6/3114 (0.2)	0	6/3493 (0.2)	0
Injection Site Pain	725/3114 (23.3)	112/379 (29.6)	837/3493 (24.0)	52/661 (7.9)
Grade ≥3	5/3114 (0.2)	0	5/3493 (0.1)	0
Injection Site Redness	11/3114 (0.4)	1/379 (0.3)	12/3493 (0.3)	1/661 (0.2)
Grade ≥3[c]	1/3114 (0.0)	0	1/3493 (0.0)	0
Injection Site Swelling	10/3114 (0.3)	1/379 (0.3)	11/3493 (0.3)	0
Grade ≥3[c]	0	0	0	0
Any Systemic Reaction	**956/3115 (30.7)**	**102/379 (26.9)**	**1058/3494 (30.3)**	**143/661 (21.6)**
Grade ≥3	48/3115 (1.5)	8/379 (2.1)	56/3494 (1.6)	3/661 (0.5)
Fever	26/3109 (0.8)	1/379 (0.3)	27/3488 (0.8)	3/660 (0.5)
Grade ≥3[c]	7/3109 (0.2)	0	7/3488 (0.2)	0
Chills	246/3114 (7.9)	12/379 (3.2)	258/3493 (7.4)	21/661 (3.2)
Grade ≥3	5/3114 (0.2)	3/379 (0.8)	8/3493 (0.2)	0
Fatigue	582/3114 (18.7)	41/379 (10.8)	623/3493 (17.8)	90/661 (13.6)
Grade ≥3	23/3114 (0.7)	4/379 (1.1)	27/3493 (0.8)	1/661 (0.2)
Headache	530/3115 (17.0)	52/379 (13.7)	582/3494 (16.7)	92/661 (13.9)
Grade ≥3	12/3115 (0.4)	2/379 (0.5)	14/3494 (0.4)	2/661 (0.3)
Myalgia	523/3114 (16.8)	52/379 (13.7)	575/3493 (16.5)	58/661 (8.8)
Grade ≥3	13/3114 (0.4)	5/379 (1.3)	18/3493 (0.5)	3/661 (0.5)
Arthralgia/Joint Pain	230/3114 (7.4)	25/379 (6.6)	255/3493 (7.3)	41/661 (6.2)
Grade ≥3	7/3114 (0.2)	3/379 (0.8)	10/3493 (0.3)	1/661 (0.2)
Nausea	219/3114 (7.0)	21/379 (5.5)	240/3493 (6.9)	34/661 (5.1)
Grade ≥3	12/3114 (0.4)	1/379 (0.3)	13/3493 (0.4)	0
Malaise[d]	7/120 (5.8)	28/379 (7.4)	35/499 (7.0)	0
Grade ≥3	0	3/379 (0.8)	3/499 (0.6)	0

N = number of participants per group; n = number of participants per parameter.
Note: Percentages are based on the number of safety population participants in each treatment group who have completed any diary anytime. Participants are counted at most once per sign or symptom at the highest severity reported within 7 days (Days 1-8) following vaccination. For study PXVX-CV-317-001 CHIKV VLP vaccine participants, only events occurring after the first CHIKV VLP vaccine dose (not placebo) are counted.

The majority of solicited AEs lasted for a single day. Among participants in the 40/300 µg CHIKV VLP vaccine group, 49% of participants reported symptoms lasting 1 day and 26% of participants reported symptoms lasting 2 days. A similar trend was seen in the placebo group. The mean duration of symptoms for solicited AEs in the 40/300 µg CHIKV VLP vaccine group was 2.0 days (SD = 1.4) with a median of 2.0 days (range 1 to 8 days). Duration of symptoms in the placebo group was a mean of 1.8 days (SD = 1.4) with a median of 1.0 day (range 1 to 7 days).

Unsolicited AEs

Unsolicited AEs (AEs not listed in the memory aid) were collected from Day 1 through 28 days post-vaccination (Day 29).

Unsolicited AEs were reported by 492/3141 (15.7%) of single dose 40/300 µg CHIKV VLP vaccine recipients and 97/675 (14.4%) of placebo recipients. The most common (>5%) SOC for unsolicited AEs was Infections and Infestations (5.8% in the 40/300 µg CHIKV VLP vaccine group and 5.8% in the placebo group). By PT, the most common unsolicited AE was COVID-19 (2.0% in the 40/300 µg CHIKV VLP vaccine group and 1.6% in the placebo group) and upper respiratory tract infection (0.5% in the 40/300 µg CHIKV VLP vaccine group and 1.0% in the placebo group).

Grade ≥3 unsolicited AEs were reported by 1.3% of 40/300 µg CHIKV VLP vaccine recipients and by 0.6% of placebo recipients. Grade 4 unsolicited AEs included an anaphylactic reaction from food allergy , influenza, road traffic accident (fatal), diabetic ketoacidosis, cerebrovascular accident, and respiratory failure in the 40/300 µg CHIKV VLP vaccine group, a gastroenteritis viral in the other CHIKV VLP

vaccine dose group and worsening of glaucoma and lung neoplasm (fatal) in the placebo group, none of which were related to study vaccine.

Unsolicited AEs considered treatment-related by the investigator occurred in 2.4% of single dose 40/300 µg CHIKV VLP vaccine recipients and 1.9% of placebo recipients. The most common (≥0.1%) treatment-related unsolicited AEs in order of decreasing frequency for the single dose 40/300 µg CHIKV VLP vaccine group were headache (0.3%), arthralgia (0.3%), dizziness (0.2%), fatigue (0.2%), rash (0.2%), injection site pain (0.1%), and myalgia (0.1%).

Some of these AEs are included in the list of solicited AEs however were considered as unsolicited since they occurred outside of the 7-day window post-vaccination.

Grade ≥3 unsolicited AEs that were considered treatment-related by the investigator included a grade 3 dehydration (assessed as probably related) and a grade 3 fatigue (assessed as possibly related), both in the 40/300 µg CHIKV VLP vaccine group.

Table 82. Treatment related unsolicited treatment emergent adverse events through 28 days post-vaccination by System Organ Class, Preferred Term and treatment group- All studies, safety population. All ages pooled

	CHIKV VLP Vaccine			
System Organ Class Preferred Term (MedDRA 26.0)	Single Dose 40/300 µg[a] (N=3141) n (%)	Other CHIKV VLP Dose[b] (N=381) n (%)	Total CHIKV VLP (N=3522) n (%)	Pooled Placebo (N=675) n (%)
Any Treatment-related Unsolicited AE	74 (2.36)	19 (4.99)	93 (2.64)	13 (1.93)
Blood and lymphatic system disorders	2 (0.06)	0	2 (0.06)	0
Lymphadenopathy	2 (0.06)	0	2 (0.06)	0
Ear and labyrinth disorders	1 (0.03)	0	1 (0.03)	0
Vertigo	1 (0.03)	0	1 (0.03)	0
Eye disorders	2 (0.06)	0	2 (0.06)	0
Blepharospasm	1 (0.03)	0	1 (0.03)	0
Retinal detachment	1 (0.03)	0	1 (0.03)	0
Gastrointestinal disorders	7 (0.22)	1 (0.26)	8 (0.23)	2 (0.30)
Diarrhoea	3 (0.10)	0	3 (0.09)	0
Nausea	2 (0.06)	0	2 (0.06)	1 (0.15)
Lip swelling	2 (0.06)	0	2 (0.06)	0
Abdominal discomfort	0	1 (0.26)	1 (0.03)	0
Vomiting	0	0	0	1 (0.15)
General disorders and administration site conditions	16 (0.51)	9 (2.36)	25 (0.71)	4 (0.59)
Injection site bruising	2 (0.06)	7 (1.84)	9 (0.26)	1 (0.15)
Fatigue	5 (0.16)	0	5 (0.14)	3 (0.44)
Injection site pain	4 (0.13)	1 (0.26)	5 (0.14)	0
Chills	1 (0.03)	0	1 (0.03)	1 (0.15)

System Organ Class Preferred Term (MedDRA 26.0)	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a] (N=3141) n (%)	Other CHIKV VLP Dose[b] (N=381) n (%)	Total CHIKV VLP (N=3522) n (%)	Pooled Placebo (N=675) n (%)
Injection site erythema	2 (0.06)	0	2 (0.06)	0
Pyrexia	1 (0.03)	0	1 (0.03)	0
Chest pain	1 (0.03)	0	1 (0.03)	0
Injection site discomfort	0	1 (0.26)	1 (0.03)	0
Vessel puncture site pain	1 (0.03)	0	1 (0.03)	0
Infections and infestations	3 (0.10)	1 (0.26)	4 (0.11)	0
Gastroenteritis	1 (0.03)	1 (0.26)	2 (0.06)	0
Sinusitis	1 (0.03)	0	1 (0.03)	0
Upper respiratory tract infection	1 (0.03)	0	1 (0.03)	0
Injury, poisoning and procedural complications	1 (0.03)	0	1 (0.03)	0
Joint injury	1 (0.03)	0	1 (0.03)	0
Metabolism and nutrition disorders	1 (0.03)	0	1 (0.03)	0
Dehydration	1 (0.03)	0	1 (0.03)	0
Musculoskeletal and connective tissue disorders	11 (0.35)	3 (0.79)	14 (0.40)	4 (0.59)
Arthralgia	8 (0.25)	0	8 (0.23)	2 (0.30)
Myalgia	4 (0.13)	2 (0.52)	6 (0.17)	2 (0.30)
Pain in extremity	1 (0.03)	1 (0.26)	2 (0.06)	0
Musculoskeletal discomfort	0	0	0	1 (0.15)
Nervous system disorders	21 (0.67)	4 (1.05)	25 (0.71)	4 (0.59)
Headache	9 (0.29)	1 (0.26)	10 (0.28)	1 (0.15)
Dizziness	6 (0.19)	1 (0.26)	7 (0.20)	1 (0.15)
Paraesthesia	2 (0.06)	1 (0.26)	3 (0.09)	0
Migraine	1 (0.03)	0	1 (0.03)	1 (0.15)
Brain fog	1 (0.03)	0	1 (0.03)	0
Dysgeusia	0	1 (0.26)	1 (0.03)	0
Lethargy	1 (0.03)	0	1 (0.03)	0
Sciatica	1 (0.03)	0	1 (0.03)	0
Seizure	1 (0.03)	0	1 (0.03)	0
Tension headache	0	0	0	1 (0.15)
Psychiatric disorders	2 (0.06)	0	2 (0.06)	0

System Organ Class Preferred Term (MedDRA 26.0)	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a] (N=3141) n (%)	Other CHIKV VLP Dose[b] (N=381) n (%)	Total CHIKV VLP (N=3522) n (%)	Pooled Placebo (N=675) n (%)
Anxiety	1 (0.03)	0	1 (0.03)	0
Depression	1 (0.03)	0	1 (0.03)	0
Reproductive system and breast disorders	1 (0.03)	0	1 (0.03)	0
Dysmenorrhoea	1 (0.03)	0	1 (0.03)	0
Respiratory, thoracic and mediastinal disorders	11 (0.35)	2 (0.52)	13 (0.37)	1 (0.15)
Nasal congestion	3 (0.10)	1 (0.26)	4 (0.11)	0
Oropharyngeal pain	3 (0.10)	0	3 (0.09)	0
Rhinorrhoea	2 (0.06)	0	2 (0.06)	1 (0.15)
Cough	0	1 (0.26)	1 (0.03)	0
Dyspnoea	1 (0.03)	0	1 (0.03)	0
Pharyngeal swelling	1 (0.03)	0	1 (0.03)	0
Sinus congestion	1 (0.03)	0	1 (0.03)	0
Sneezing	1 (0.03)	0	1 (0.03)	0
Upper-airway cough syndrome	1 (0.03)	0	1 (0.03)	0
Skin and subcutaneous tissue disorders	10 (0.32)	0	10 (0.28)	1 (0.15)
Rash	5 (0.16)	0	5 (0.14)	0
Pruritus	1 (0.03)	0	1 (0.03)	1 (0.15)
Eczema	1 (0.03)	0	1 (0.03)	0
Hyperhidrosis	1 (0.03)	0	1 (0.03)	0
Night sweats	1 (0.03)	0	1 (0.03)	0
Rash vesicular	1 (0.03)	0	1 (0.03)	0
Vascular disorders	2 (0.06)	0	2 (0.06)	0
Hot flush	1 (0.03)	0	1 (0.03)	0
Hypertension	1 (0.03)	0	1 (0.03)	0

AE = adverse event; N = number of participants per group; n = number of participants per parameter
Note: Percentages are based on the number of safety population participants in each treatment group. Participants are counted once within each system organ class and preferred term. Preferred terms are ordered by descending total frequency within alphabetic system organ class. For study PXVX-CV-317-001, events occurring within 28 days of the first CHIKV VLP dose on Day 1 are counted for Groups 1-4 and 9-10. For Groups 5-8, events occurring within 28 days of the first CHIKV VLP dose, Day 15 (Groups 5-7) or Day 29 (Group 8), are counted; participants in Groups 5-7 received a second CHIKV VLP dose 14 days later on Day 29 that fell within the reporting period.
[a] Study PXVX-CV-317-001 Groups 8 and 10: 40 µg dose with 300 µg adjuvant single dose; studies EBSI-CV-317-002, EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005.

2.6.8.3. Serious adverse event/deaths/other significant events

Serious adverse events (SAEs) were collected for all participants from Day 1 through Day 183 End of study (EOS) visit, as well as adverse events of special interest (AESI) (new onset or worsening arthralgia that was medically attended) in studies EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005, and medically attended adverse events (MAAEs) in studies EBSI-CV-317-004 and EBSI-CV-317-005.

Serious adverse events (SAEs)

Overall, SAEs were reported by 41 participants. SAEs occurred in 1.1% (n=37) of all CHIKV VLP vaccine recipients (40/300 µg CHIKV VLP vaccine: n=31, other CHIKV VLP vaccine dose: n=6) and 0.6% (n=4) of placebo recipients. SAEs were mostly related to the SOC Infections and Infestations.

There was 1 SAE (PT: retinal detachment) that was considered possibly treatment-related by a study investigator and was assessed by the sponsor and independent SMC Chair as unrelated due to prior medical history of seeing 'black spots' in the same eye in which the retinal detachment occurred 1 month pre-study. One SAE (PT:), a rare congenital condition which was newly diagnosed in an adult participant, should have been recorded as pre-existing medical history rather than an AE.

Table 83. Serious unsolicited Treatment-emergent adverse events throughout study by System Organ Class, Preferred Term and Treatment Group- All studies, safety population, all ages pooled

System Organ Class Preferred Term (MedDRA 26.0)	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a] (N=3141) n (%)	Other CHIKV VLP Dose[b] (N=381) n (%)	Total CHIKV VLP (N=3522) n (%)	Pooled Placebo (N=675) n (%)
Any Serious Unsolicited AE	31 (0.99)	6 (1.57)	37 (1.05)	4 (0.59)
Congenital, familial and genetic disorders	2 (0.06)	0	2 (0.06)	0
Arnold-Chiari malformation[c]	1 (0.03)	0	1 (0.03)	0
Encephalocele	1 (0.03)	0	1 (0.03)	0
Ear and labyrinth disorders	1 (0.03)	0	1 (0.03)	0
Vertigo	1 (0.03)	0	1 (0.03)	0
Eye disorders	1 (0.03)	0	1 (0.03)	1 (0.15)
Glaucoma	0	0	0	1 (0.15)
Retinal detachment	1 (0.03)	0	1 (0.03)	0
Gastrointestinal disorders	1 (0.03)	0	1 (0.03)	0
Vomiting	1 (0.03)	0	1 (0.03)	0
Hepatobiliary disorders	2 (0.06)	0	2 (0.06)	0
Biliary dyskinesia	1 (0.03)	0	1 (0.03)	0
Cholecystitis	1 (0.03)	0	1 (0.03)	0
Immune system disorders	1 (0.03)	1 (0.26)	2 (0.06)	0
Anaphylactic reaction	1 (0.03)	1 (0.26)	2 (0.06)	0
Infections and infestations	7 (0.22)	1 (0.26)	8 (0.23)	2 (0.30)
Appendicitis	1 (0.03)	0	1 (0.03)	1 (0.15)
Cellulitis	1 (0.03)	0	1 (0.03)	0
Clostridium difficile infection	0	0	0	1 (0.15)
COVID-19	0	0	0	1 (0.15)
Device related infection	1 (0.03)	0	1 (0.03)	0
Gastroenteritis	0	1 (0.26)	1 (0.03)	0
Influenza	1 (0.03)	0	1 (0.03)	0
Pneumonia	1 (0.03)	0	1 (0.03)	0
Pyelonephritis	1 (0.03)	0	1 (0.03)	0
Staphylococcal infection	0	0	0	1 (0.15)
Tubo-ovarian abscess	1 (0.03)	0	1 (0.03)	0

System Organ Class Preferred Term (MedDRA 26.0)	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a] (N=3141) n (%)	Other CHIKV VLP Dose[b] (N=381) n (%)	Total CHIKV VLP (N=3522) n (%)	Pooled Placebo (N=675) n (%)
Urosepsis	0	0	0	1 (0.15)
Injury, poisoning and procedural complications	4 (0.13)	3 (0.79)	7 (0.20)	0
Road traffic accident	1 (0.03)	1 (0.26)	2 (0.06)	0
Craniocerebral injury	1 (0.03)	0	1 (0.03)	0
Femoral neck fracture	1 (0.03)	0	1 (0.03)	0
Gun shot wound	1 (0.03)	0	1 (0.03)	0
Induced abortion haemorrhage	0	1 (0.26)	1 (0.03)	0
Laryngeal injury	0	1 (0.26)	1 (0.03)	0
Skin laceration	1 (0.03)	0	1 (0.03)	0
Metabolism and nutrition disorders	3 (0.10)	0	3 (0.09)	0
Diabetic ketoacidosis	2 (0.06)	0	2 (0.06)	0
Dehydration	1 (0.03)	0	1 (0.03)	0
Musculoskeletal and connective tissue disorders	1 (0.03)	0	1 (0.03)	0
Pain in extremity	1 (0.03)	0	1 (0.03)	0
Neoplasms benign, malignant and unspecified (including cysts and polyps)	2 (0.06)	0	2 (0.06)	1 (0.15)
Breast cancer	1 (0.03)	0	1 (0.03)	0
Lung neoplasm malignant	0	0	0	1 (0.15)
Malignant melanoma	1 (0.03)	0	1 (0.03)	0
Nervous system disorders	3 (0.10)	0	3 (0.09)	0
Basal ganglia infarction	1 (0.03)	0	1 (0.03)	0
Cerebrovascular accident	1 (0.03)	0	1 (0.03)	0
Neuropathy peripheral	1 (0.03)	0	1 (0.03)	0
Transient ischaemic attack	1 (0.03)	0	1 (0.03)	0
Pregnancy, puerperium and perinatal conditions	0	2 (0.52)	2 (0.06)	0
Hyperemesis gravidarum	0	1 (0.26)	1 (0.03)	0
Premature separation of placenta	0	1 (0.26)	1 (0.03)	0
Threatened labour	0	1 (0.26)	1 (0.03)	0
Psychiatric disorders	1 (0.03)	0	1 (0.03)	0
Bipolar disorder	1 (0.03)	0	1 (0.03)	0
Depression	1 (0.03)	0	1 (0.03)	0

System Organ Class Preferred Term (MedDRA 26.0)	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a] (N=3141) n (%)	Other CHIKV VLP Dose[b] (N=381) n (%)	Total CHIKV VLP (N=3522) n (%)	Pooled Placebo (N=675) n (%)
Renal and urinary disorders	2 (0.06)	0	2 (0.06)	0
Nephrolithiasis	1 (0.03)	0	1 (0.03)	0
Urinary retention	1 (0.03)	0	1 (0.03)	0
Reproductive system and breast disorders	0	1 (0.26)	1 (0.03)	0
Vaginal haemorrhage	0	1 (0.26)	1 (0.03)	0
Respiratory, thoracic and mediastinal disorders	2 (0.06)	0	2 (0.06)	0
Chronic obstructive pulmonary disease	1 (0.03)	0	1 (0.03)	0
Respiratory failure	1 (0.03)	0	1 (0.03)	0

AE = adverse event; N = number of participants per group; n = number of participants per parameter
Note: Percentages are based on the number of safety population participants in each treatment group. Participants are counted once within each system organ class and preferred term. Preferred terms are ordered by descending total frequency within alphabetic system organ class.
[a] Study PXVX-CV-317-001 Groups 8 and 10: 40 µg dose with 300 µg adjuvant single dose; studies EBSI-CV-317-002, EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005.
[b] Study PXVX-CV-317-001 Groups 1-7 and 9: 6, 10, or 20 µg dose with or without 300 µg adjuvant administered twice separated by 14 or 28 days (includes participants who received only one dose).
[c] Inadvertently documented as an AE rather than medical history.
Source: Table 10.1.1

Deaths

A total of 3 deaths occurred during the clinical studies, none of which were assessed as related to study vaccine. There was 1 death in study EBSI-CV-317-004 in a >30-year-old participant (PT: road traffic incident; 40/300 µg CHIKV VLP vaccine group). There were 2 deaths in study EBSI-CV-317-005, one was a >70-year-old participant (PT: lung neoplasm malignant; placebo group) and the other one was a >70-year-old participant (PT: respiratory failure; 40/300 µg CHIKV VLP vaccine group).

Adverse events of special interest (AESI)

There were 6 (0.2%) participants that reported an AESI in the 40/300 µg CHIKV VLP vaccine group and 2 (0.3%) participants in the placebo group. All AESI were non-serious, grade 1 or grade 2 in severity. Four AESI were assessed as related to study treatment (3 in the CHIKV VLP vaccine group and 1 in the placebo group).

Table 84. Solicited and Unsolicited Treatment-Emergent Adverse events of Special interest throughout the study by System Organ Class, Preferred Term and treatment group- All studies, safety population, all ages pooled

System Organ Class Preferred Term	CHIKV VLP Single Dose 40/300 µg[a] (N=2996) n (%)	Pooled Placebo (N=671) n (%)
Any Solicited and Unsolicited AESI	6 (0.20)	2 (0.30)
Injury, poisoning and procedural complications	0	1 (0.15)
Joint dislocation	0	1 (0.15)
Musculoskeletal and connective tissue disorders	6 (0.20)	1 (0.15)
Arthralgia	5 (0.17)	1 (0.15)
Spinal osteoarthritis	1 (0.03)	0

AESI = adverse event of special interest; N = number of participants per group; n = number of participants per parameter

Note: Percentages are based on the number of safety population participants in each treatment group. Participants are counted once within each system organ class and preferred term. Preferred terms are ordered by descending total frequency within alphabetic system organ class.

[a] Studies EBSI-CV-317-004 and EBSI-CV-317-005.

2.6.8.4. Laboratory findings

Clinical laboratory tests throughout the clinical development program were performed at screening to test for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, HIV-1/HIV-2 (human immunodeficiency virus) and pregnancy (also done before study vaccine administration). Vital signs (blood pressure, heart rate, respiratory rate, and temperature) were recorded before and after study vaccine administration and a complete physical examination was performed at screening at the discretion of the study investigator, if indicated by medical history or AE follow-up. In study EBSI-CV-317-002 a complete blood count, creatinine, and liver associated enzymes (AST/ALT) were evaluated at screening and Days 1 and 8. No abnormalities were noted. These laboratory parameters were not collected in Phase 3 studies.

No trends in vital signs or physical examination abnormalities were noted.

2.6.8.5. Safety in special populations

The below section presents safety data stratified by:

- Age range
- Sex
- Baseline serostatus

In addition, available data in special populations such as pregnant and breastfeeding women is presented.

Age range

Table 95 presents a summary of the safety data for adolescents, adults and older adults. Solicited and unsolicited data are presented in Table 96 and Table 97.

Table 85. Summary of adverse events for adolescents, adults and older adults- All studies, safety population, CHIK VLP Single dose 40/300 µg Group and Placebo

	Age 12 to <18 (N=254) n (%)		Age 18 to 65 (N=3187) n (%)		Age >65 (N=375) n (%)		All Ages Pooled (N=3816) n (%)	
Parameter	**CHIKV VLP (N=217)**	**Placebo (N=37)**	**CHIKV VLP (N=2738)**	**Placebo (N=449)**	**CHIKV VLP (N=186)**	**Placebo (N=189)**	**CHIKV VLP (N=3141)**	**Placebo (N=675)**
Any Solicited AE	94/213 (44.13)	8/37 (21.62)	1042/2717 (38.35)	120/ 441 (27.21)	20/ 185 (10.81)	26/ 183 (14.21)	1156/3115 (37.11)	154/ 661 (23.30)
≥Grade 3	4/213 (1.88)	1/37 (2.70)	45/2717 (1.66)	2/441 (0.45)	1/185 (0.54)	0	50/3115 (1.61)	3/661 (0.45)
Mean duration in days (SD)	2.2 (1.73)	1.6 (1.19)	2.0 (1.40)	1.7 (1.27)	2.5 (1.40)	2.3 (1.62)	2.0 (1.43)	1.8 (1.35)
Any Unsolicited AE	41 (18.89)	3 (8.11)	425 (15.52)	61 (13.59)	26 (13.98)	33 (17.46)	492 (15.66)	97 (14.37)
≥Grade 3	3 (1.38)	0	34 (1.24)	1 (0.22)	4 (2.15)	3 (1.59)	41 (1.31)	4 (0.59)
Treatment-Related	2 (0.92)	0	68 (2.48)	8 (1.78)	4 (2.15)	5 (2.65)	74 (2.36)	13 (1.93)
Any Unsolicited SAE	1 (0.46)	0	26 (0.95)	1 (0.22)	4 (2.15)	3 (1.59)	31 (0.99)	4 (0.59)
Any AESI[a]	0	0	6 (0.23)	1 (0.22)	0	1 (0.53)	6 (0.20)	2 (0.30)
Any MAAE[b]	24 (11.06)	2 (5.41)	229 (8.75)	40 (8.99)	19 (10.22)	23 (12.17)	272 (9.00)	65 (9.69)
Deaths	0	0	1	0	1	1	2	1

AE = adverse event; AESI = adverse event of special interest; MAAE = medically attended adverse event; N= number of participants per group; n = number of participants per parameter; SAE = serious adverse event; SD = standard deviation

Note: Percentages are based on the number of safety population participants in each treatment group.

[a]AESI counts are based on studies EBSI-CV-317-004 and EBSI-CV-317-005 only.

[b] MAAE counts are based on studies EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005 only.

Solicited AEs

Table 86. Reactogenicity within 7 days post-vaccination by maximum severity and age group- All studies, safety population, CHIKV VLP single dose 40/300 µg group and placebo

	Age 12 to <18 (N=254)		Age 18 to <46 (N=2020)		Age 46 to <65 (N=1129)		Age 65 to <75 (N=318)		Age ≥75 (N=95)	
Reactogenicity Parameter Severity	**CHIKV VLP (N=217)** n/N (%)	**Placebo (N=37)** n/N (%)	**CHIKV VLP (N=1746)** n/N (%)	**Placebo (N=274)** n/N (%)	**CHIKV VLP (N=972)** n/N (%)	**Placebo (N=157)** n/N (%)	**CHIKV VLP (N=159)** n/N (%)	**Placebo (N=159)** n/N (%)	**CHIKV VLP (N=47)** n/N (%)	**Placebo (N=48)** n/N (%)
Any Reaction	94/213 (44.13)	8/37 (21.62)	735/1731 (42.46)	85/271 (31.37)	302/966 (31.26)	33/153 (21.57)	18/159 (11.32)	22/154 (14.29)	7/46 (15.22)	6/46 (13.04)
Grade 4	0	0	1/1731 (0.06)	0	0	0	0	0	0	0
Grade 3[a]	4/213 (1.88)	1/37 (2.70)	30/1731 (1.73)	2/271 (0.74)	14/966 (1.45)	0	1/159 (0.63)	0	0	0
Grade 2	49/213 (23.00)	5/37 (13.51)	250/1731 (14.44)	40/271 (14.76)	108/966 (11.18)	11/153 (7.19)	6/159 (3.77)	6/154 (3.90)	4/46 (8.70)	3/46 (6.52)
Grade 1	41/213 (19.25)	2/37 (5.41)	454/1731 (26.23)	43/271 (15.87)	180/966 (18.63)	22/153 (14.38)	11/159 (6.92)	16/154 (10.39)	3/46 (6.52)	3/46 (6.52)
None	119/213 (55.87)	29/37 (78.38)	996/1731 (57.54)	186/271 (68.63)	664/966 (68.74)	120/153 (78.43)	141/159 (88.68)	132/154 (85.71)	39/46 (84.78)	40/46 (86.96)
Any ≥Grade 3	4/213 (1.88)	1/37 (2.70)	31/1731 (1.79)	2/271 (0.74)	14/966 (1.45)	0	1/159 (0.63)	0	0	0
Mean duration in days (SD)	2.2 (1.73)	1.6 (1.19)	2.0 (1.40)	1.7 (1.28)	1.9 (1.40)	1.7 (1.16)	1.7 (1.02)	2.2 (1.48)	3.4 (1.51)	3.0 (2.28)
Local Reaction	60/213 (28.17)	5/37 (13.51)	502/1731 (29.00)	31/271 (11.44)	157/965 (16.27)	13/153 (8.50)	8/159 (5.03)	1/154 (0.65)	3/46 (6.52)	3/46 (6.52)
Grade 4	0	0	0	0	0	0	0	0	0	0
Grade 3[a]	1/213 (0.47)	0	1/1731 (0.06)	0	4/965 (0.41)	0	0	0	0	0
Grade 2	13/213 (6.10)	0	43/1731 (2.48)	3/271 (1.11)	9/965 (0.93)	0	0	0	0	1/46 (2.17)
Grade 1	46/213 (21.60)	5/37 (13.51)	458/1731 (26.46)	28/271 (10.33)	144/965 (14.92)	13/153 (8.50)	8/159 (5.03)	1/154 (0.65)	3/46 (6.52)	2/46 (4.35)
None	153/213 (71.93)	32/37 (86.49)	1229/1731 (71.00)	240/271 (88.56)	808/965 (83.73)	140/153 (91.50)	151/159 (94.97)	153/154 (99.35)	43/46 (93.48)	43/46 (93.48)

	Age 12 to <18 (N=254)		Age 18 to <46 (N=2020)		Age 46 to <65 (N=1129)		Age 65 to <75 (N=318)		Age ≥75 (N=95)	
Reactogenicity Parameter Severity	**CHIKV VLP (N=217)** n/N (%)	**Placebo (N=37)** n/N (%)	**CHIKV VLP (N=1746)** n/N (%)	**Placebo (N=274)** n/N (%)	**CHIKV VLP (N=972)** n/N (%)	**Placebo (N=157)** n/N (%)	**CHIKV VLP (N=159)** n/N (%)	**Placebo (N=159)** n/N (%)	**CHIKV VLP (N=47)** n/N (%)	**Placebo (N=48)** n/N (%)
≥Grade 3 Local	1/213 (0.47)	0	1/1731 (0.06)	0	4/965 (0.41)	0	0	0	0	0
Mean duration in days (SD)	1.5 (0.93)	1.0 (0.00)	1.7 (1.10)	1.1 (0.43)	1.4 (0.79)	1.1 (0.28)	1.3 (0.46)	1.0 (NE)	2.3 (1.15)	1.7 (1.15)
Systemic Reaction[b]	86/213 (40.38)	8/37 (21.62)	590/1731 (34.08)	78/271 (28.78)	258/966 (26.71)	30/153 (19.61)	16/159 (10.06)	22/154 (14.29)	6/46 (13.04)	5/46 (10.87)
Grade 4	0	0	1/1731 (0.06)	0	0	0	0	0	0	0
Grade 3[a]	4/213 (1.88)	1/37 (2.70)	30/1731 (1.73)	2/271 (0.74)	12/966 (1.24)	0	1/159 (0.63)	0	0	0
Grade 2	46/213 (21.60)	5/37 (13.51)	237/1731 (13.69)	38/271 (14.02)	106/966 (10.97)	11/153 (7.19)	6/159 (3.77)	6/154 (3.90)	4/46 (8.70)	3/46 (6.52)
Grade 1	36/213 (16.90)	2/37 (5.41)	322/1731 (18.60)	38/271 (14.02)	140/966 (14.49)	19/153 (12.42)	9/159 (5.66)	16/154 (10.39)	2/46 (4.35)	2/46 (4.35)
None	127/213 (59.62)	29/37 (78.38)	1141/1731 (65.92)	193/271 (71.22)	708/966 (73.29)	123/153 (80.39)	143/159 (89.94)	132/154 (85.71)	40/46 (86.96)	41/46 (89.13)
≥Grade 3 Systemic	4/213 (1.88)	1/37 (2.70)	31/1731 (1.79)	2/271 (0.74)	12/966 (1.24)	0	1/59 (0.63)	0	0	0
Mean duration in days (SD)	2.2 (1.76)	1.6 (1.19)	2.0 (1.43)	1.7 (1.31)	1.9 (1.46)	1.7 (1.15)	1.8 (1.06)	2.2 (1.48)	3.3 (1.37)	3.4 (2.30)

N = number of participants per group; n = number of participants per parameter; SD = standard deviation

Note: Percentages are based on the number of safety population participants in each treatment group who completed a memory aid. Participants are counted at most once per sign or symptom at the highest severity reported within 7 days (Days 1-8) following vaccination. For study PXVX-CV-317-001, only events occurring after the first CHIKV VLP dose (not placebo) are counted.

[a] Grade 3 (severe) redness or swelling is >100 mm diameter; grade 3 (severe) fever is ≥39.0°C.

[b] Malaise was assessed in studies PXVX-CV-317-001 and EBSI-CV-317-002 only.

Source: Table 13.2.1, Table 14.2.1, Table 15.2.1

Unsolicited AEs

Table 87. Unsolicited treatment emergent adverse events through 28 days post-vaccination occurring in ≥0.1% (n=5) of total participants by descending preferred term and age group- All studies, safety population, CHIKV VLP single dose 40/300 µg group and placebo

	Age 12 to <18 (N=254) n (%)		Age 18 to <46 (N=2020) n (%)		Age 46 to <65 (N=1129) n (%)		Age 65 to <75 (N=318) n (%)		Age ≥75 (N=95) n (%)	
Preferred Term (MedDRA 26.0)	**CHIKV VLP (N=217)**	**Placebo (N=37)**	**CHIKV VLP (N=1746)**	**Placebo (N=274)**	**CHIKV VLP (N=972)**	**Placebo (N=157)**	**CHIKV VLP (N=159)**	**Placebo (N=159)**	**CHIKV VLP (N=47)**	**Placebo (N=48)**
Any Unsolicited AE	41 (18.89)	3 (8.11)	268 (15.35)	44 (16.06)	157 (16.15)	16 (10.19)	23 (14.47)	23 (14.47)	3 (6.38)	11 (22.92)
≥Grade 3	3 (1.38)	0	23 (1.32)	1 (0.36)	11 (1.13)	0	3 (1.89)	1 (0.63)	1 (2.13)	2 (4.17)
Treatment-Related	2 (0.92)	0	43 (2.46)	6 (2.19)	25 (2.57)	1 (0.64)	4 (2.52)	6 (3.77)	0	0
COVID-19	1 (0.46)	0	31 (1.78)	5 (1.82)	30 (3.09)	1 (0.64)	2 (1.26)	2 (1.26)	0	3 (6.25)
Headache	2 (0.92)	0	20 (1.15)	1 (0.36)	2 (0.21)	1 (0.64)	0	0	0	1 (2.08)
Arthralgia	0	0	13 (0.74)	3 (1.09)	3 (0.31)	0	1 (0.63)	1 (0.63)	0	0
Upper respiratory tract infection	4 (1.84)	1 (2.70)	6 (0.34)	2 (0.73)	4 (0.41)	1 (0.64)	0	3 (1.89)	0	0
Sinusitis	1 (0.46)	0	7 (0.40)	2 (0.73)	6 (0.62)	1 (0.64)	1 (0.63)	0	0	1 (2.08)
Fatigue	0	0	9 (0.52)	1 (0.36)	3 (0.31)	0	1 (0.63)	2 (1.26)	0	0
Back pain	0	0	4 (0.23)	2 (0.73)	8 (0.82)	1 (0.64)	0	0	0	0
Hypertension	0	0	6 (0.34)	0	4 (0.41)	2 (1.27)	3 (1.89)	0	0	0
Anxiety	2 (0.92)	0	9 (0.52)	2 (0.73)	1 (0.10)	0	0	0	0	0
Oropharyngeal pain	2 (0.92)	0	11 (0.63)	0	0	0	0	1 (0.63)	0	0
Nausea	0	0	10 (0.57)	1 (0.36)	2 (0.21)	0	0	0	0	0
Rash	4 (1.84)	0	5 (0.29)	0	4 (0.41)	0	0	0	0	0
Cough	1 (0.46)	0	4 (0.23)	1 (0.36)	5 (0.51)	0	0	0	0	0
Depression	2 (0.92)	2 (5.41)	2 (0.11)	2 (0.73)	3 (0.31)	0	0	0	0	0
Myalgia	0	0	6 (0.34)	1 (0.36)	1 (0.10)	1 (0.64)	1 (0.63)	1 (0.63)	0	0
Dizziness	0	0	4 (0.23)	1 (0.36)	5 (0.51)	0	0	0	0	0

	Age 12 to <18 (N=254) n (%)		Age 18 to <46 (N=2020) n (%)		Age 46 to <65 (N=1129) n (%)		Age 65 to <75 (N=318) n (%)		Age ≥75 (N=95) n (%)	
Preferred Term (MedDRA 26.0)	CHIKV VLP (N=217)	Placebo (N=37)	CHIKV VLP (N=1746)	Placebo (N=274)	CHIKV VLP (N=972)	Placebo (N=157)	CHIKV VLP (N=159)	Placebo (N=159)	CHIKV VLP (N=47)	Placebo (N=48)
Diarrhoea	0	0	4 (0.23)	0	3 (0.31)	0	1 (0.63)	0	0	1 (2.08)
Nasal congestion	0	0	5 (0.29)	2 (0.73)	2 (0.21)	0	0	0	0	0
Rhinorrhoea	1 (0.46)	0	4 (0.23)	0	1 (0.10)	0	1 (0.63)	2 (1.26)	0	0
Bronchitis	0	0	5 (0.29)	0	0	0	1 (0.63)	0	0	2 (4.17)
Nasopharyngitis	1 (0.46)	0	4 (0.23)	1 (0.36)	2 (0.21)	0	0	0	0	0
Urinary tract infection	0	0	3 (0.17)	1 (0.36)	4 (0.41)	0	0	0	0	0
Influenza	1 (0.46)	1 (2.70)	2 (0.11)	2 (0.73)	1 (0.10)	0	0	0	0	0
Muscle strain	0	0	4 (0.23)	1 (0.36)	1 (0.10)	0	1 (0.63)	0	0	0
Attention deficit hyperactivity disorder	1 (0.46)	0	4 (0.23)	1 (0.36)	0	0	0	0	0	0
Gastroenteritis	1 (0.46)	0	3 (0.17)	0	1 (0.10)	1 (0.64)	0	0	0	0
Gastrooesophageal reflux disease	0	0	4 (0.23)	0	1 (0.10)	1 (0.64)	0	0	0	0
Skin laceration	0	0	4 (0.23)	0	1 (0.10)	0	0	1 (0.63)	0	0
Viral infection	1 (0.46)	0	3 (0.17)	0	2 (0.21)	0	0	0	0	0
Ear infection	1 (0.46)	0	4 (0.23)	0	0	0	0	0	0	0
Injection site bruising	0	0	1 (0.06)	1 (0.36)	2 (0.21)	1 (0.64)	0	0	0	0
Injection site pain	0	0	5 (0.29)	0	0	0	0	0	0	0
Insomnia	0	0	3 (0.17)	0	2 (0.21)	0	0	0	0	0
Ligament sprain	2 (0.92)	0	3 (0.17)	0	0	0	0	0	0	0
Neck pain	0	0	2 (0.11)	0	2 (0.21)	1 (0.64)	0	0	0	0
Presyncope	1 (0.46)	0	3 (0.17)	0	1 (0.10)	0	0	0	0	0
Respiratory tract infection	0	0	5 (0.29)	0	0	0	0	0	0	0

	Age 12 to <18 (N=254) n (%)		Age 18 to <46 (N=2020) n (%)		Age 46 to <65 (N=1129) n (%)		Age 65 to <75 (N=318) n (%)		Age ≥75 (N=95) n (%)	
Preferred Term (MedDRA 26.0)	CHIKV VLP (N=217)	Placebo (N=37)	CHIKV VLP (N=1746)	Placebo (N=274)	CHIKV VLP (N=972)	Placebo (N=157)	CHIKV VLP (N=159)	Placebo (N=159)	CHIKV VLP (N=47)	Placebo (N=48)
Vomiting	0	0	2 (0.11)	1 (0.36)	2 (0.21)	0	0	0	0	0

N = number of participants per group; n = number of participants per parameter
Note: Percentages are based on the number of safety population participants in each treatment group. Participants are counted once within preferred term. Preferred terms are ordered by descending total frequency. For study PXVX-CV-317-001, events occurring within 28 days of the first CHIKV VLP dose on Day 1 are counted for Groups 10. For Groups 8, events occurring within 28 days of the first CHIKV VLP dose on Day 29, are counted.
Source: Table 6.2.1, Table 7.2.1, Table 8.2.1

Table below presents data for the older age group populations.

Table 88. Adverse event by age range

MedDRA Terms	Active				Comparator (Placebo)			
	Age <65 (N=3316) n (%)	Age 65-74 (N=159) n (%)	Age 75-84 (N=41) n (%)	Age 85+ (N=6) n (%)	Age <65 (N=468) n (%)	Age 65-74 (N=159) n (%)	Age 75-84 (N=48) n (%)	Age 85+ (N=0) n (%)
Total AEs	552 (16.6)	23 (14.5)	3 (7.3)	0	63 (13.5)	23 (14.5)	11 (22.9)	0
Serious AEs – Total	33 (1.0)	3 (1.9)	1 (2.4)	0	1 (0.2)	1 (0.6)	2 (4.2)	0
– Fatal	1 (0.0)	0	1 (2.4)	0	0	1 (0.6)	0	0
– Hospitalisation/prolong existing hospitalisation	26 (0.8)	3 (1.9)	0	0	0	1 (0.6)	1 (2.1)	0
– Life-threatening	5 (0.2)	1 (0.6)	0	0	0	1 (0.6)	0	0
– Other (medically significant)	13 (0.4)	2 (1.3)	0	0	0	1 (0.6)	2 (4.2)	0

MedDRA Terms	Active				Comparator (Placebo)			
	Age <65 (N=3316) n (%)	Age 65-74 (N=159) n (%)	Age 75-84 (N=41) n (%)	Age 85+ (N=6) n (%)	Age <65 (N=468) n (%)	Age 65-74 (N=159) n (%)	Age 75-84 (N=48) n (%)	Age 85+ (N=0) n (%)
AEs leading to drop-out	3 (0.1)	0	1 (2.4)	0	0	1 (0.6)	0	0
Psychiatric disorders	34 (1.0)	0	0	0	7 (1.5)	0	0	0
Nervous system disorders	66 (2.0)	2 (1.3)	0	0	6 (1.3)	0	2 (4.2)	0
Accidents and injuries[1]	76 (2.3)	2 (1.3)	0	0	11 (2.4)	4 (2.5)	0	0
Cardiac disorders	7 (0.2)	2 (1.3)	0	0	0	1 (0.6)	1 (2.1)	0
Vascular disorders	16 (0.5)	3 (1.9)	0	0	3 (0.6)	0	0	0
Cerebrovascular disorders[2]	2 (0.1)	0	0	0	0	0	0	0
Infections and infestations	196 (5.9)	9 (5.7)	1 (2.4)	0	26 (5.6)	7 (4.4)	6 (12.5)	0
Anticholinergic syndrome[3]	0	0	0	0	0	0	0	0
Quality of life decreased[4]	0	1 (0.6)	0	0	0	0	1 (2.1)	0
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures[5]	30 (0.9)	2 (1.3)	0	0	4 (0.9)	0	0	0
Other AEs appearing more frequently in older patients[6]								
– Arthralgia	0	1 (0.6)	0	0	0	1 (0.6)	0	0
– Bronchitis	0	1 (0.6)	0	0	0	0	2 (4.2)	0
– COVID-19	0	2 (1.3)	0	0	0	2 (1.3)	3 (6.3)	0
– Fatigue	0	1 (0.6)	0	0	0	2 (1.3)	0	0
– Hypertension	0	3 (1.9)	0	0	0	0	0	0
– Joint swelling	0	0	0	0	0	1 (0.6)	1 (2.1)	0
– Rhinorrhoea	0	1 (0.6)	0	0	0	2 (1.3)	0	0
– Upper respiratory tract infection	0	0	0	0	0	3 (1.9)	0	0

AE = adverse event; COVID-19 = coronavirus disease 2019; MedDRA = Medical Dictionary for Regulatory Affairs v26.0; PT = preferred term; SMQ = Standardised MedDRA Query; SOC = System Organ Class.
[1] Accidents and injuries were queried using the SOC 'Injury, poisoning and procedural complications.
[2] Cerebrovascular disorders were queried using the MedDRA 'Central nervous system vascular disorders' SMQ 20000060. These terms were also counted with the 'Nervous system disorders' SOC.
[3] Anticholinergic syndrome PT was not reported in the integrated safety database. Safety data were also queried using the MedDRA 'Anticholinergic syndrome' SMQ 20000048 and no participants were qualified based on this query.
[4] Event counts based on medical review of the safety data. Note, psychiatric events are not included here as they were counted in the 'Psychiatric disorders' category.
[5] No cases of postural hypotension (queried as 'Orthostatic hypertension' per MedDRA), falls, black outs, or ataxia were observed in the programme. The remaining events included cases of syncope, presyncope, dizziness, and fractures.
[6] Most frequent PTs appearing in older patients are based on trial EBSI-CV-317-005 which enrolled only subjects 65 years of age and older.

Sex

The below tables present the available safety data by sex sub-groups.

Solicited AEs

Table 89. Summary of reactogenicity within 7 days post-vaccination by maximum severity and treatment group – All studies, safety population, all ages pooled by sex subgroups

Reactogenicity Parameter Parameter	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a]	Other CHIKV VLP Dose[b]	Total CHIKV VLP	Pooled Placebo
Male				
N	1520	152	1672	324
Any Reaction n/N (%)	447/1500 (29.80)	51/ 150 (34.00)	498/1650 (30.18)	67/ 314 (21.34)
≥Grade 3, n/N (%)	16/1500 (1.07)	1/ 150 (0.67)	17/1650 (1.03)	1/ 314 (0.32)
Mean duration in days (SD)	2.0 (1.46)	2.1 (1.48)	2.0 (1.46)	1.7 (1.46)
Local Reaction[c] n/N (%)	273/1500 (18.20)	33/ 150 (22.00)	306/1650 (18.55)	21/ 314 (6.69)
≥Grade 3, n/N (%)	4/1500 (0.27)	0	4/1650 (0.24)	0
Mean duration in days (SD)	1.5 (1.00)	1.5 (0.76)	1.5 (0.97)	1.1 (0.44)
Systemic Reaction[c,d] n/N (%)	361/1500 (24.07)	31/ 150 (20.67)	392/1650 (23.76)	59/ 314 (18.79)
≥Grade 3, n/N (%)	15/1500 (1.00)	1/ 150 (0.67)	16/1650 (0.97)	1/ 314 (0.32)
Mean duration in days (SD)	2.0 (1.49)	2.3 (1.74)	2.0 (1.52)	1.8 (1.53)

Reactogenicity Parameter Parameter	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a]	Other CHIKV VLP Dose[b]	Total CHIKV VLP	Pooled Placebo
Female				
N	1621	229	1850	351
Any Reaction n/N (%)	709/1615 (43.90)	111/ 229 (48.47)	820/1844 (44.47)	87/ 347 (25.07)
≥Grade 3, n/N (%)	34/1615 (2.11)	7/ 229 (3.06)	41/1844 (2.22)	2/ 347 (0.58)
Mean duration in days (SD)	2.1 (1.41)	2.1 (1.36)	2.1 (1.41)	1.9 (1.25)
Local Reaction[c] n/N (%)	457/1614 (28.31)	79/ 229 (34.50)	536/1843 (29.08)	32/ 347 (9.22)
≥Grade 3, n/N (%)	2/1614 (0.12)	0	2/1843 (0.11)	0
Mean duration in days (SD)	1.7 (1.04)	1.7 (0.94)	1.7 (1.03)	1.2 (0.45)
Systemic Reaction[c,d] n/N (%)	595/1615 (36.84)	71/ 229 (31.00)	666/1844 (36.12)	84/ 347 (24.21)
≥Grade 3, n/N (%)	33/1615 (2.04)	7/ 229 (3.06)	40/1844 (2.17)	2/ 347 (0.58)
Mean duration in days (SD)	2.0 (1.45)	2.1 (1.54)	2.0 (1.46)	1.9 (1.25)

Grade 3 = severe; N = number of participants per group; n = number of participants per parameter; SD = standard deviation

Note: Percentages are based on the number of safety population participants in each treatment group who have completed any diary anytime. Participants are counted at most once per sign or symptom at the highest severity reported within 7 days (Days 1-8) following vaccination. For study PXVX-CV-317-001 CHIKV VLP participants, only events occurring after the first CHIKV VLP dose (not placebo) are counted.

[a] Study PXVX-CV-317-001 Groups 8 and 10: 40 µg dose with 300 µg adjuvant single dose; studies EBSI-CV-317-002, EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005.

[b] Study PXVX-CV-317-001 Groups 1-7 and 9: 6, 10, or 20 µg dose with or without 300 µg adjuvant administered twice separated by 14 or 28 days (includes participants who received only one dose).

[c] Grade 3 (severe) redness or swelling is >100 mm diameter; grade 3 (severe) fever is ≥39.0°C.

[d] Malaise was assessed in studies PXVX-CV-317-001 and EBSI-CV-317-002 only.

Unsolicited AEs

Table 90. Unsolicited Treatment-emergent adverse events through 28 days post-vaccination by descending preferred term and treatment group- all studies, safety population. All ages pooled by sex subgroups

Preferred Term (MedDRA 26.0)	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a] n (%)	Other CHIKV VLP Dose[b] n (%)	Total CHIKV VLP n (%)	Pooled Placebo n (%)
Male				
N	1520	152	1672	324
Any Unsolicited AE	199 (13.09)	37 (24.34)	236 (14.11)	38 (11.73)
≥Grade 3	19 (1.25)	1 (0.66)	20 (1.20)	1 (0.31)
Treatment-related	33 (2.17)	10 (6.58)	43 (2.57)	4 (1.23)
Most Common Unsolicited AEs (≥0.5%):				
COVID-19	19 (1.25)	0	19 (1.14)	6 (1.85)
Headache	11 (0.72)	0	11 (0.66)	2 (0.62)
Upper respiratory tract infection	6 (0.39)	5 (3.29)	11 (0.66)	1 (0.31)
Oropharyngeal pain	9 (0.59)	1 (0.66)	10 (0.60)	0
Female				
N	1621	229	1850	351
Any Unsolicited AE	293 (18.08)	49 (21.40)	342 (18.49)	59 (16.81)
≥Grade 3	22 (1.36)	11 (4.80)	33 (1.78)	3 (0.85)
Treatment-related	41 (2.53)	9 (3.93)	50 (2.70)	9 (2.56)
Most Common Unsolicited AEs (≥0.5%):				
COVID-19	45 (2.78)	0	45 (2.43)	5 (1.42)
Upper respiratory tract infection	8 (0.49)	4 (1.75)	12 (0.65)	6 (1.71)
Headache	13 (0.80)	3 (1.31)	16 (0.86)	1 (0.28)
Sinusitis	12 (0.74)	0	12 (0.65)	4 (1.14)
Arthralgia	12 (0.74)	1 (0.44)	13 (0.70)	1 (0.28)

N = number of participants per group; n = number of participants per parameter

Note: Percentages are based on the number of safety population participants in each treatment group. Participants are counted once within each preferred term. Preferred terms are ordered by descending total frequency. For study PXVX-CV-317-001, events occurring within 28 days of the first CHIKV VLP dose on Day 1 are counted for Groups 1-4 and 9-10. For Groups 5-8, events occurring within 28 days of the first CHIKV VLP dose, Day 15 (Groups 5-7) or Day 29 (Group 8), are counted; participants in Groups 5-7 received a second CHIKV VLP dose 14 days later on Day 29 that fell within the reporting period.

[a] Study PXVX-CV-317-001 Groups 8 and 10: 40 µg dose with 300 µg adjuvant single dose; studies EBSI-CV-317-002, EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005.

[b] Study PXVX-CV-317-001 Groups 1-7 and 9: 6, 10, or 20 µg dose with or without 300 µg adjuvant administered twice separated by 14 or 28 days (includes participants who received only one dose).

Source: Table 6.1.2, Table 7.1.2, Table 8.1.2

Baseline serostatus

The below tables present safety data by baseline serostatus.

Table 91. Summary of reactogenicity within 7 days post-vaccination by maximum severity and treatment group- All studies, Safety population, all ages pooled by baseline serostatus subgroups

Reactogenicity Parameter Parameter	CHIKV VLP Vaccine			
	Single Dose 40/300 µg[a]	Other CHIKV VLP Dose[b]	Total CHIKV VLP	Pooled Placebo
Seronegative				
N	3067	379	3446	658
Any Reaction n/N (%)	1131/3042 (37.18)	162/377 (42.97)	1293/3419 (37.82)	150/645 (23.26)
≥Grade 3, n/N (%)	47/3042 (1.55)	8/377 (2.12)	55/3419 (1.61)	3/645 (0.47)
Mean duration in days (SD)	2.0 (1.44)	2.1 (1.39)	2.0 (1.43)	1.8 (1.29)
Local Reaction[c] n/N (%)	716/3041 (23.54)	112/377 (29.71)	828/3418 (24.22)	51/645 (7.91)
≥Grade 3 n/N (%)	5/3041 (0.16)	0	5/3418 (0.15)	0
Mean duration in days (SD)	1.6 (1.03)	1.6 (0.89)	1.6 (1.01)	1.1 (0.36)
Systemic Reaction[c,d] n/N (%)	931/3042 (30.60)	102/377 (27.06)	1033/3419 (30.21)	140/645 (21.71)
≥Grade 3, n/N (%)	45/3042 (1.48)	8/377 (2.12)	53/3419 (1.55)	3/645 (0.47)
Mean duration in days (SD)	2.0 (1.47)	2.1 (1.60)	2.0 (1.48)	1.8 (1.31)
Seropositive				
N	72	2	74	16
Any Reaction n/N (%)	24/71 (33.80)	0	24/73 (32.88)	4/15 (26.67)
≥Grade 3, n/N (%)	3/71 (4.23)	0	3/73 (4.11)	0
Mean duration in days (SD)	1.9 (1.19)	-	1.9 (1.19)	2.8 (2.87)
Local Reaction[c] n/N (%)	14/71 (19.72)	0	14/73 (19.18)	2/15 (13.33)
≥Grade 3, n/N (%)	1/71 (1.41)	0	1/73 (1.37)	0
Mean duration in days (SD)	1.6 (1.08)	-	1.6 (1.08)	2.0 (1.41)
Systemic Reaction[c,d] n/N (%)	24/71 (33.80)	0	24/73 (32.88)	3/15 (20.00)
≥Grade 3, n/N (%)	3/71 (4.23)	0	3/73 (4.11)	0
Mean duration in days (SD)	1.7 (1.20)	-	1.7 (1.20)	3.3 (3.21)

Grade 3 = severe; N = number of participants per group; n = number of participants per parameter; SD = standard deviation

Note: Percentages are based on the number of safety population participants in each treatment group who have completed any diary anytime. Participants are counted at most once per sign or symptom at the highest severity reported within 7 days (Days 1-8) following vaccination. For study PXVX-CV-317-001 CHIKV VLP participants, only events occurring after the first CHIKV VLP dose (not placebo) are counted.

[a] Study PXVX-CV-317-001 Groups 8 and 10: 40 µg dose with 300 µg adjuvant single dose; studies EBSI-CV-317-002, EBSI-CV-317-010, EBSI-CV-317-004, and EBSI-CV-317-005.

Pregnancy, pregnancy outcomes and lactation

There were 11 pregnancies that occurred during the studies.

In study PXVX-CV-317-001, there were 9 pregnancies reported: 3 pregnancies were complicated by SAEs (hypertensis gravidarium, vaginal bleeding, with an additional 6 uncomplicated pregnancies. The pregnancy outcomes reported were (n=2), (n=2), (n=2), (n=1,), (n=1) and (n=1).

In study EBSI-CV-317-004, there were two pregnancy outcomes in the CHIK VLP single dose 40/300: One was a SAE complicated pregnancy (major congenital anomaly:) and the other an uncomplicated pregnancy.

None of the pregnancy SAEs were considered related to study vaccine.

There was no apparent CHIKV VLP vaccine pregnancy-related safety signals in any of the clinical studies with CHIKV VLP vaccine.

Due to the limited number of pregnant study participants, the safety of CHIKV VLP vaccine in pregnant participants and during breastfeeding could not be established.

Hepatic or renal impairment

The protocol of the clinical trials included in the application excluded individuals with hepatic or renal impairment.

2.6.8.6. Immunological events

Immunogenicity represents the pharmacodynamic effect of the vaccine and represents the main evidence for this application. Results are described and assessed in 2.6.5.

2.6.8.7. Safety related to drug-drug interactions and other interactions

The concomitant use of CHIKV VLP vaccine with other vaccines or drugs was not studied.

The analysis of concomitant medication usage in the Phase 3 studies revealed no meaningful difference in the proportion of participants who had prior and concomitant medications in the CHIKV VLP vaccine group compared with the placebo group. No significant AEs, trends or safety concerns attributable to concomitant medication interactions were identified.

2.6.8.8. Discontinuation due to adverse events

There were 5 AEs that led to study withdrawal, none of which were related to study vaccine.

A participant in other CHIKV VLP dose group experienced a worsening of nephrocalcinosis. The participant had medical history of nephrocalcinosis which got worse and became severe, which was not considered related to study vaccine.

Three participants (2 in study vaccine group and 1 in placebo group) withdraw from study due to fatal events not related to study vaccination (refer to deaths section).

A participant who received 40/300 µg CHIKV VLP vaccine group in study EBSI-CV-317-004 completed the study but her pregnancy outcome was classified as an AE leading to withdrawal from the study despite the fact that the study day of this participant's AE was Day 399, well past the 182-day duration of the study.

2.6.8.9. Post marketing experience

Not applicable.

2.6.9. Discussion on clinical safety

Background

The clinical safety profile of CHIKV VLP vaccine was mainly established in the Phase 3 study EBSI-CV-317-004 (pivotal, randomized, double-blind, placebo-controlled) in individuals 12-64 years of age. Further safety data were collected in individuals ≥ 65 years of age in the Phase 3 study EBSI-CV-317-005 (pivotal, randomized, double-blind, placebo-controlled). In both studies the clinically relevant dose and formulation (40 µg CHIKV VLP adjuvanted with aluminium hydroxide, 300 µg aluminium) was used. The safety data are supported by data from Phase 2 studies (PXVX-CV-317-001, EBSI-CV-317-002, and EBSI-CV-317-010) that evaluated various vaccine formulations ranging from 6 to 40 µg CHIKV VLP with or without adjuvants.

The methods for collection of safety data were similar across studies and pertained to evaluation of local and systemic solicited AEs (day of vaccination until 7 days postvaccination), unsolicited AEs (day of vaccination until 28 days post vaccination), SAEs, AESI and MAAEs (day of vaccination until day 183 EOS).

Safety data were pooled across Phase 2 and 3 studies and presented throughout the dossier for the clinically relevant 40/300 µg CHIKV VLP vaccine dose, other CHIKV VLP doses, total CHIKV VLP (i.e. combining the first 2 categories) and pooled placebo.

Overall, relevant CHIKV VLP vaccine safety parameters have been adequately defined, the pooling strategy is considered appropriate, and the data have been presented comprehensibly to enable a thorough assessment of clinical safety. Also, the methods for collection of safety data are considered adequate.

Exposure and baseline characteristics

The safety population on which analyses were based on is comprised of all participants who received the vaccine and provided safety assessment data.

A total of **3522 participants were exposed** to at least one IM dose of CHIKV VLP vaccine (total CHIKV VLP): 526 (14.9%) participants in phase 2, and 2996 (85.1%) participants in phase 3. Overall, 3141 participants received a single dose of 40/300 µg CHIKV VLP vaccine (n=2996 in Phase 3 and n= 145 in Phase 2 studies) of which approx. 200 individuals each were 12-17 years of age (subset in Phase 3 study EBSI-CV-317-004) or ≥65 years of age (entire Phase 3 study EBSI-CV-317-005). A total of 381 participants received other CHIKV VLP doses (all Phase 2). The pooled placebo group consisted of 675 individuals (of which 671 were from Phase 3). Thus, the safety database is considered of sufficient size to describe common and uncommon adverse events. Henceforth the group of participants who received the clinically relevant 40/300 µg CHIKV VLP vaccine dose is referred to as "CHIKV VLP vaccine group".

In terms of **baseline characteristics**, the mean (SD) age of the safety population was 41.2 (16.5) years ranging from 12 to 95 years. The pooled placebo group had a comparatively higher mean age (48.5 years) than the CHIKV VLP vaccine group (40.7 years) and the other CHIKV VLP dose (31.5 years) group, due to the relatively higher contribution of older adults from study EBSI-CV-317-005. This is not considered problematic as vaccine reactogenicity would be expected to decrease with age following general immunological considerations. This would result in a more conservative safety comparison of vaccine versus placebo. No imbalances were seen when comparing the CHIKV VLP vaccine and placebo groups for other relevant baseline parameters, including sex (in both groups approx. 52% females, 75% white, 80% non-hispanic or latino, mean height of 170 cm, mean BMI of 27). This was similar for the total CHIKV VLP group. Ultimately, seropositive individuals were recorded as 72/3141 (2.3%) in the CHIKV VLP group compared to 16/675 (2.4%) in the placebo group and 2/381 (0.5%) in the other CHIKV VLP dose group. No issue ensues and the study population is considered to adequately reflect the intended target population.

Study completion rate was approximately 90% for both the 40/300 µg CHIKV VLP and placebo group. Reasons for termination were provided (mainly lost to follow-up or participant withdrawal) and can be followed.

The mean and median **duration of follow-up** was approximately 6 months in both the 40/300 µg CHIKV VLP and placebo group (ranges from 1-763 days) and approx. 12-13 months for individuals in the other CHIKV VLP dose group (range 1-824 days). This is considered acceptable to enable a reasonable estimate of the short to mid-term safety profile.

Of note, for subsequent sections on the evaluation of adverse events, the clinically relevant CHIKV VLP vaccine group (n=3141) was chosen. Results in the total CHIKV VLP dose group (n=3522) were not considered to differ meaningfully.

Solicited AEs

Solicited AEs were comprised of local reactions (injection site pain, redness, and swelling) and systemic reactions (fever, chills, fatigue, headache, myalgia, arthralgia, and nausea).

A total of 37.1% of CHIKV VLP vaccinated individuals experienced any solicited AE compared to 23.3% in the placebo group that were mostly Grade 1 or 2 in severity. Grade ≥3 solicited AEs were reported as 1.6% and 0.5%, respectively. A single grade 4 solicited AE (40.6°C fever) was recorded in the electronic diary by a CHIKV VLP vaccine recipient, but the site deemed this entry an error. Based on further details on this event (direct contact with the sites PI and participant), it is considered reasonable that this entry was recorded erroneously.

Local solicited AE rates were approximately 4-times higher in the CHIKV VLP group (23.4%) compared to placebo (8.0%) and were mainly due to injection site pain (very common), whereas injection site swelling or redness were uncommon (≤ 0.4% across groups).

Systemic solicited AE rates were approx. 1.5-times higher in the CHIKV VLP group (30.7%) compared to placebo (21.6%) and were mainly due to the very common events of fatigue (18.7% vs. 13.6%), headache (17.0% vs. 13.9%) and myalgia (16.8% vs. 8.8%). Arthralgia, chills and nausea were commonly reported, whereas pyrexia was uncommon (≤0.8%) across groups.

All solicited AEs usually resolved within a median of 2.0 days (range 1 to 8 days) in the 40/300 µg CHIKV VLP vaccine group and within a median of 1.0 day (range 1 to 7 days) in the placebo group. Approximately 50% of participants reported resolution of solicited AEs after 1 day and 25% after 2 days.

Overall, no concerning findings became apparent. All solicited local and systemic AEs are reflected in section 4.8 of the SmPC.

While the majority of solicited events were considered treatment-related, a total of 26/3494 (0.74) events up to Grade 3 in severity following vaccination with CHIKV VLP vaccine (any dose) were not considered treatment related (none in placebo group). However, based on the criteria to evaluate relationship with treatment it remains unclear why these events were not considered treatment related. Consequently, the Applicant was asked to provide further explanations on why 26/3494 (0.74%) events up to Grade 3 in severity following vaccination with CHIKV VLP vaccine (any dose) had not been considered treatment related and short narratives for these events were requested. The Applicant responded that upon review of their submitted table, "it was discovered that among the 26 previously identified participants with unrelated solicited adverse events (AEs), 10 participants with solicited AEs marked as "not related" did not have corresponding unsolicited AEs explaining the solicited symptoms. These 10 participants were thus considered as having experienced related solicited AEs." Since there is no consequence for the data depicted in the sections above and, more importantly, section 4.8 of the SmPC, this aspect is considered being of minor relevance.

It is unexpected for a vaccine and underlying immunological mechanisms that rates of injection site swelling, redness and pyrexia were reported with uncommon frequency. The Applicant argued that the unexpectedly low frequencies of these events may have to do with the aluminium hydroxide adjuvant concentration that is lower compared to a selection of vaccines with the same adjuvant (exemplified by Hepatitis B or Human papilloma virus (HPV) vaccines). However, the Applicant acknowledged that no definitive explanation can be given and the root cause of this finding remains unexplained.

Unsolicited AEs

Unsolicited AEs were AEs reported spontaneously by the participant or discovered by the investigator.

Frequencies of unsolicited AEs were comparable between 40/300 µg CHIKV VLP vaccine recipients (492/3141; 15.7%) and placebo recipients (97/675; 14.4%). By SOC, Infections and Infestations were

most frequently reported (5.8% for both groups). Frequencies of AEs categorized to all other SOCs were <2%. By PT (frequencies shown for placebo vs. CHIKV VLP) the most frequently reported unsolicited AEs were COVID-19 (1.6 vs. 2.0%) headache (0.44 vs. 0.76%), upper respiratory tract infection (1.04 vs. 0.45%) and arthralgia (0.59% vs. 0.54%). No imbalances as regards frequencies of unsolicited AEs between treatment groups could be identified. While approx. twice as many individuals in the CHIKV VLP vaccine group experienced a Grade ≥3 unsolicited AEs compared to placebo, frequencies were low in both groups (1.3% vs. 0.6%).

Frequencies of **unsolicited AEs considered treatment-related** were also comparable between participants receiving 40/300 µg CHIKV VLP vaccine (2.4%) or placebo (1.9%). By PT, the most frequently reported treatment-related unsolicited AEs in the CHIKV VLP vaccine group were headache (0.3%), arthralgia (0.3%), dizziness (0.2%), fatigue (0.2%) and rash (0.2%). In the placebo group fatigue (0.4%), arthralgia (0.3%) and myalgia (0.3%) were most frequently reported.

Grade ≥3 unsolicited AEs considered treatment-related included a grade 3 dehydration (assessed as probably related) and a grade 3 fatigue (assessed as possibly related), both in the CHIKV VLP vaccine group. The event of fatigue is contained in SmPC table 4.8 (fatigue occurring at very common frequency).The Applicant argued that the Grade 3 event of dehydration should not be included in SmPC section 4.8 because it occurred only in a single individual with a history of type II diabetes and associated concomitant medication and because the principal investigator deemed it likely that this event was caused by the associated solicited symptoms (such as fatigue, chills, nausea) that began at the same time. This was considered acceptable.

A grade 2 lymphadenopathy assessed as "probably related" was reported. After review, the case concerned a >30-year-old participant who experienced at Day 1 after vaccination an unsolicited AE (grade 1) of lymphadenopathy. The event resolved on Day 8 after vaccination. Overall, 2 cases of mild lymphadenopathy were deemed as related to CHIKV VLP vaccine, therefore the Applicant updated the SmPC to include lymphadenopathy as an ADR occurring with a 'rare' frequency, which is endorsed.

While listings for individual events have been provided, the Applicant was asked to also submit a summary table on the duration of unsolicited AEs (unrelated and treatment-related) and state how many events have not resolved to date. The Applicant submitted the summary table as requested; however, it was unclear which events that were considered treatment-related were unresolved. Therefore, the Applicant was asked to provide event narratives for the 11 (1.3%) treatment-related unsolicited events that were unresolved. With the responses, the Applicant provided a listing of these 11 unresolved treatment-related unsolicited AEs (experienced by 9 participants) and the respective narratives, resolving this issue. Of these 11 events, 5 were grade 1 and 6 were grade 2; 4 events were first experienced within 7 days, and within 2 weeks after vaccination, respectively, and 3 later (44, 92, and 128 days after vaccination).

Furthermore, it had become clear from the initially submitted above-mentioned table that the time to resolution of treatment-related unsolicited AEs was up to 432 days post-vaccination. Hence, the Applicant was asked to provide further information on all treatment-related unsolicited AEs that lasted longer than the respective median for each category (e.g. treatment-related unsolicited AEs of Arthralgia that took longer to resolve than the median of 57.0 days, or treatment-related unsolicited AEs of headache that lasted longer than 4.0 days to resolve, etc.) including summary tables and/or short event narratives as appropriate. With the responses, the Applicant provided a listing of treatment-related unsolicited AEs that lasted longer than the respective median for each category and the respective narratives, as requested. All of these events were either mild (grade 1) or moderate (grade 2) in severity, and all were recovered/resolved by the end of the trial.

Overall, no findings of concern regarding unsolicited AEs could be identified.

Adverse events of special interest (AESIs)

AESI were defined as the occurrence of new onset or worsening arthralgia that was medically attended. A total of 8 AESI were reported by 8 participants (6 (0.2%) in the CHIKV VLP vaccine group and 2 (0.3%) in the placebo group). All AESI were non-serious, grade 1 or grade 2 in severity. Four AESI were assessed as related to study treatment (3 in the CHIKV VLP vaccine group and 1 in the placebo group). This is adequately reflected in SmPC table 4.8. The review of the cases did not raise concerns.

Medically attended adverse events (MAAEs)

MAAEs occurred at comparable frequencies in participants in the single dose CHIKV VLP vaccine (9.0%) and placebo (9.7%) groups and overall align with the safety findings as described above. No issues were identified.

Serious adverse events (SAEs)

SAES were defined as any untoward medical occurrence that resulted in death, was life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions, or was a congenital anomaly or birth defect.

A total of 53 SAEs occurred in 41 participants: 1.1% (n=37) of all CHIKV VLP vaccine recipients (40/300 µg CHIKV VLP vaccine: n=31, other CHIKV VLP vaccine dose: n=6) and 0.6% (n=4) of placebo recipients reported at least 1 SAE. By SOC these SAEs were reported with highest frequency for Infections and Infestations (n=8 for all CHIKV VLP vaccine recipients vs. n= 2 for placebo). By PT no SAE was reported more than twice, thus no clear pattern emerged.

There was 1 SAE (PT: retinal detachment) that was considered possibly treatment-related by a study investigator and was assessed by the sponsor and independent SMC Chair as unrelated due to prior medical history of seeing 'black spots' in the same eye in which the retinal detachment occurred 1 month pre-study. It is agreed that this event should be considered as unrelated due to the prior medical history.

The Applicant further reported that one SAE (PT:) newly diagnosed in an adult participant should have been recorded as pre-existing medical history rather than an AE. This is supported by the narrative and agreed.

The assessment of all SAEs does not raise concerns.

Therefore, no SAEs could be identified that could be attributed to CHIKV VLP vaccination.

Deaths

A total of 3 deaths occurred during the clinical development program: A >30-year-old participant died in a road traffic incident (40/300 µg CHIKV VLP vaccine group; study EBSI-CV-317-004), a >70-year-old participant died of a malignant lung neoplasm malignant (placebo group; study EBSI-CV-317-005) and a >70-year-old participant died of respiratory failure (40/300 µg CHIKV VLP vaccine group, study EBSI-CV-317-005). The review of the cases did not raise concerns. It is agreed with the Applicant that no event of death should be considered treatment related.

Events leading to study withdrawal

A total of 5 AEs in 5 participants led to study withdrawal: worsening of nephrocalcinosis (other CHIKV VLP dose group); a pregnancy outcome classified as an AE leading to withdrawal from the study

despite the fact that the study day of this participant's AE was Day 399, well past the 182-day duration of the study (40/300 µg CHIKV VLP vaccine group); 3 deaths as described previously. The Applicant states that none of these events were related to study vaccine. This is considered reasonable based on review of the provided narratives.

Laboratory and other findings

Clinical laboratory tests throughout the clinical development program were performed at screening to test for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody, HIV-1/HIV-2 (human immunodeficiency virus) and pregnancy (also done before study vaccine administration). Vital signs (blood pressure, heart rate, respiratory rate, and temperature) were recorded before and after study vaccine administration and a complete physical examination was performed at screening at the discretion of the study investigator, if indicated by medical history or AE follow-up. In study EBSI-CV-317-002 a complete blood count, creatinine, and liver associated enzymes (AST/ALT) were evaluated at screening and Days 1 and 8. It is stated that no abnormalities were noted. These laboratory parameters were not collected in Phase 3 studies, which is considered acceptable in the context of the overall safety evaluation and findings.

Safety in special populations

Age

The Applicant provided safety analyses comparing for the following age groups (number of individuals receiving 40/300 µg CHIKV VLP vaccine/ total number including 40/300 µg CHIKV VLP + placebo): 12 to <18 yoa (n= 217/254), 18 to <46 yoa (1746/2020), 46 to <65 yoa (972/1129), 65 to <75 yoa (159/318) and ≥75-year-olds (47/95). The youngest and oldest age strata were comparatively less populated, thus low sample sizes need to be taken into account. The following overall picture emerged:

Any solicited AE after vaccination with 40/300 µg CHIKV VLP occurred with frequencies of approximately 40% both in individuals 12 to <18 yoa and 18 to <46 yoa, which declined for older age groups reaching 15% in ≥75-year-olds. Severities followed a similar pattern, with Grade ≥3 AEs of approximately 2% in the younger age groups declining to 0.0-0.5% in the older age groups. Mean duration of approximately 2 days was more comparable between age groups.

Any unsolicited AE after vaccination with 40/300 µg CHIKV VLP occurred with frequencies of 19% among 12 to <18 yoa, 15% among 18 to <46 yoa, 16% among 46 to <65 yoa, 14% among 65 to <75 yoa, and 6% among ≥75 yoa. Frequencies of unsolicited AEs of ≥grade 3 severity slightly increased with age (1.4% among 12 to <18 yoa and 2.1% among ≥75 yoa). No connection between the nature or frequency of treatment-related unsolicited AEs and age can be discerned due to the overall low incidence and partially small group size (e.g. 2 participants 12 to <18 yoa experiencing a treatment-related unsolicited AE vs. 4 participants 65 to <75 yoa vs. 0 participants ≥75 yoa).

Frequencies of solicited or unsolicited AEs in the respective placebo groups did not always follow the same trend of declining frequency/intensity as for 40/300 µg CHIKV VLP. The underlying reason was not further discussed by the Applicant. No further issue is made as it is acknowledged that low sample sizes specifically for the youngest and oldest individuals (n=37 for 12 to <18 yoa and n=48 for ≥75 yoa individuals receiving placebo) make further discussions difficult. Moreover, no impact on the general conclusion on the safety profile of 40/300 µg CHIKV VLP by age is suspected.

In summary, the safety profile of 40/300 µg CHIKV VLP appears similar for adolescents 12-17 yoa and individuals 18 to <46 yoa. For other age groups a decrease in reactogenicity with increasing age was seen, whereas unsolicited AE rates were more comparable across age groups. Frequencies of unsolicited AEs of ≥grade 3 severity slightly increased with age (the latter to be interpreted with caution due to overall low frequencies and sample sizes for respective age strata).

Sex, Race, Ethnicity

By **sex**, solicited AE rates after 40/300 µg CHIKV VLP vaccination were higher in females (44%) compared to males (30%) with higher frequencies of solicited AEs ≥grade 3 (2.1% vs. 1.1%) and 0.6%). Similarly, more unsolicited AEs were reported in females compared to males (18% vs. 13%) with rates of unsolicited AEs ≥grade 3 being more comparable (1.4 vs. 1.3 %). No clear trend for treatment-related unsolicited AEs became apparent (2.5 vs. 2.2%).

By **race** White or Asian participants were more likely to report any AE. By **ethnicity** this was true for non-Hispanic or Latino participants. Otherwise, no findings that would be of concern in terms of frequency/duration/severity of AEs by race or ethnicity could be identified.

Baseline serostatus

The number of CHIKV seropositive individuals at baseline who received the 40/300 µg CHIKV VLP vaccine (n=72) was too low to draw any meaningful conclusions on the safety profile by serostatus.

Pregnancy and outcomes

Pregnancy was an exclusion criterion, however, 11 pregnancies occurred during the CHIKV VLP clinical development program: In study -001 3/9 pregnancies were complicated by SAEs (subject 1: hyperemesis gravidarum; subject 2:, Vaginal Bleeding,; subject 3: abortion). In study EBSI-CV-317-004, there was 1 SAE complicated pregnancy) and 1 uncomplicated pregnancy. None of the pregnancy SAEs were considered related to study vaccine which appears plausible based on the provided narratives and/or the lack of preclinical findings for reproductive toxicity.

However, the number of pregnant study participants is too limited to conclude on the safety of CHIKV VLP vaccine in pregnant individuals. SmPC section 4.6 contains respective information on fertility, pregnancy and lactation.

Concomitant medication

Systemic immunomodulatory or immunosuppressive medication (e.g. mAb therapy, corticosteroids, radiation therapy etc.) were not permitted 6 months prior to study entry up to 3 weeks post CHIKV VLP vaccination. Lack of safety and efficacy data in immunocompromised individuals is adequately reflected in SmPC section 4.4.

In the Phase 3 studies, the most common prior and concomitant medications were balanced between placebo and CHIKV VLP groups. More than two thirds of participants in these studies reported any prior or concomitant medication. The most commonly given concomitant medications were: selective serotonin reuptake inhibitors (approx. 13-14%), propionic acid derivatives (approx. 11-13%) and other antidepressants (approx. 10%) in study EBSI-CV-317-004 and HmG CoA reductase inhibitors (approx. 35%), ACE inhibitors plain (approx. 17-19%), Angiotensin II receptor blockers plain (approx. 16-17%).

Concomitant vaccines (mainly against COVID-19) were given to approx. 3-5% of participants in study EBSI-CV-317-004 and 12% in study EBSI-CV-317-005 which is not unexpected due to the higher age of participants in the latter.

Overall, based on the data provided, no safety issue related to concomitant medication interactions became immediately apparent. Of note, however, the clinical development program of CHIKV VLP did not aim at specifically studying concomitant medication or vaccination. Thus, additional studies would be needed to further inform on concomitant vaccination (not required for initial MAA). SmPC section 4.5 states that no interaction studies with other medicinal products have been performed and that concomitant administration of CHIKV VLP with other vaccines has not been studied. This is acceptable.

2.6.10. Conclusions on the clinical safety

The clinical safety profile of CHIKV VLP vaccine was mainly characterised by mild to moderate AEs of short duration. The most frequently reported AEs (frequency ≥1:10) were injection site pain, fatigue, headache and myalgia. Frequencies of unsolicited AEs considered treatment-related were comparable between CHIKV VLP vaccine and placebo recipients.

The safety profile of CHIKV VLP appears similar for adolescents 12-17 yoa and individuals 18 to <46 yoa. For other age groups a decrease in reactogenicity with increasing age was seen, whereas unsolicited AE rates were more comparable across age groups. Frequencies of unsolicited AEs of ≥grade 3 severity slightly increased with age (the latter to be interpreted with caution due to overall low frequencies and sample sizes for respective age strata).

No SAEs or deaths could be attributed to CHIKV VLP vaccination.

In conclusion, CHIKV VLP vaccine is well tolerated in individuals ≥12 years of age.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 92. Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	None
Missing information	Use during pregnancy and breastfeeding

2.7.2. Pharmacovigilance plan

Table 93. On-going and planned additional pharmacovigilance activities

Study / Status	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Not applicable				
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				
Not applicable				
Category 3 - Required additional pharmacovigilance activities				
VIMKUNYA Pregnancy Registry: An Observational Prospective Study	To evaluate pregnancy outcomes in women immunised with VIMKUNYA up to 28 days before	Use during pregnancy and breastfeeding	Protocol submission	30-May-2025
			Annual updates	Progress reports on enrolment

Study / Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
of the Safety of VIMKUNYA Vaccine Exposure in Pregnant Women and their Offspring	conception or at any time during pregnancy.			and intermediate analysis results will be provided yearly.
Planned			Final report	28-Feb-2031

2.7.3. Risk minimisation measures

Table 94. Summary Table of Pharmacovigilance Activities and Risk Minimisation Activities by Safety Concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important identified risks		
None		
Important potential risks		
None		
Missing information		
Use during pregnancy and breastfeeding	**Routine risk minimisation measures:** • Specific statement in SmPC regarding use of VIMKUNYA during pregnancy and breastfeeding if the potential benefits outweigh any potential risk to the mother and foetus/baby: • SmPC Section 4.6 • PL Section 2 • Vaccine product subject to medical prescription and administration by a licensed HCP. **Additional risk minimisation measures:** None.	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection**: Annual safety analysis in periodic aggregate safety reports of exposures to VIMKUNYA during pregnancy and breastfeeding, and adverse reactions that may be associated with exposure to VIMKUNYA. **Additional pharmacovigilance activities**: VIMKUNYA Pregnancy Registry; final study report: 28-Feb-2031.

2.7.4. Conclusion

The CHMP considers that the risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Quick Response (QR) code

A request to include a QR code in the labelling and package leaflet for the purpose of providing statutory information has been submitted by the applicant and has been found acceptable.

The following elements have been agreed to be provided through a QR code: The Package Leaflets (PL) and Summary of Product Characteristics (SmPC) in all local languages for all countries of the MA in PDF format with Annex V details and status date.

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Chikungunya vaccine (recombinant, adsorbed) is included in the additional monitoring list as it contains a new active substance, which on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

The target indication of this application is active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 12 years and older.

The aim of the new treatment is to protect from CHIKV disease.

Based on epidemiological data, there is a trend for increasing populations at risk for local transmission due to a number of different factors such as warmer than average temperatures, a continued spread of the mosquito vectors and travel of infected individuals into receptive areas with established and active competent vectors (*Aedes mosquitoes*). More specifically, the virus has spread worldwide over the past two decades from Asia and Africa to Europe and the Americas. Although CHIK is historically a tropical disease, currently all regions with established populations of *Aedes aegypti* or *Aedes albopictus* mosquitoes have experienced local mosquito-borne transmission.

Diagnosis of CHIKV infection can be based either on the presence of the CHIKV or on serology (IgM or IgG). RT-qPCR or isothermal amplification can be used to detect viral RNA during the acute phase of the disease with high levels viremia. However, CHIKV infection can be misdiagnosed as dengue and access to diagnostic tests is limited in some endemic areas.

CHIKV infection can result in severe disabling arthralgia, which occurs as recurrent joint pain that can persist for months to years post-infection in a large proportion of infected individuals

3.1.2. Available therapies and unmet medical need

The treatment of CHIKV disease is symptomatic, including non-salicylate analgesics and non-steroid anti-inflammatory therapy. There are no specific antiviral drugs against CHIKV. Prevention of CHIKV infection is mostly restricted to protection against mosquito bites.

Recently, an EU market-authorisation was issued on 28/06/2024 for a monovalent chikungunya live-attenuated vaccine (LAV) (Ixchiq, manufactured by Valneva), indicated for active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older after receiving a positive opinion from the European Medicines Agency's (EMA) Committee for Medicinal Products for Human Use (CHMP) on 30/05/2024.

3.1.3. Main clinical studies

The current application is supported mainly by the data from two completed Phase 3 studies, namely EBSI-CV-317-004 and EBSI-CV-317-005. Data of other studies (VRC 311, VRC 704, EBSI-CV-317-002, EBSI-CV-317-010, and PXVX-CV-317-001) were considered as supportive evidence.

For both pivotal studies EBSI-CV-317-004 and EBSI-CV-317-005, co-primary endpoints included the difference in anti-CHIKV SNA SRR and anti-CHIKV SNA GMT at Day 22 for CHIKV VLP vaccine and placebo. Anti-CHIKV SNA GMT at Day 22 was kept as co-primary endpoint by the Applicant (as it was required to be considered as such by the FDA) whereas it should have been downgraded to secondary endpoint based on scientific advice.

EBSI-CV-317-004 was a randomized, placebo-controlled, double-blind, parallel-group Phase 3 study to investigate safety, immunogenicity, and clinical lot consistency of a single IM 40 µg dose CHIKV VLP vaccine adjuvanted with 300 µg aluminium hydroxide given per pre-filled syringe (PFS) in 3258 healthy adults and adolescents 12 to <65 yoa, of which 3254 were treated (2790 CHIKV VLP vaccine, 464 placebo).

EBSI-CV-317-005 was a Phase 3, randomized, double-blind, placebo-controlled, parallel-group study with two treatment groups. A total of 400 healthy participants ≥65 years of age were planned for enrolment and stratified in two age subgroups (≥65 to <75 and ≥75 years of age). Participants were randomized in a 1:1 ratio to receive either a single IM dose of CHIKV VLP vaccine or placebo at Day 1.

3.2. Favourable effects

In study EBSI-CV-317-004 in adolescents and adults (12 to 65 yoa), the primary endpoint was met as the difference (CHIKV VLP vaccine minus placebo) in SRRs (defined as the percentage of participants who achieve an anti-CHIKV SNA titre ≥100, which is the threshold titre considered reasonably likely to predict protection) on Day 22 was 96.6% in the primary analysis population, the IEP, and superior (defined as the lower bound of the 95% CI being above 70%) in the seronegative CHIKV VLP recipients compared to placebo. The difference in SRRs on Day 15 was 96.0% in the IEP, only marginally lower than that reported for Day 22, and met also the superiority criteria. Furthermore, the difference in SRRs on Day 183 was 84.0% in the IEP and thus considerably lower than of Day 15 and Day 22 but still the majority of vaccinated subjects achieved titres over the threshold. Difference in SRRs on Day 8 was 46.1% in the IEP.

In study EBSI-CV-317-005 in elderly subjects (≥65 yoa), the primary endpoint was met as the difference in SRRs on Day in the IEP was 86.2% and achieved superiority over placebo. The lower bound of the 95% CI of the difference in SRRs on Day 22 of subjects ≥75 years was below 70 but this is likely due to low sample size in this group (n=40 for CHIKV VLP vaccine and placebo) and the differences in SRRs were quite high (≥65 to <75 years: 87.2%, ≥75 years: 82.5%) and within expectations for elderly immune systems. Day 15 difference in SRR was 79.5% in the IEP and thus lower than on Day 22. The difference in SRRs on Day 183 was 74.4% in the IEP and therefore the majority of vaccinees still above the threshold.

Results of other endpoints (GMTs, GMFI, numbers and percentages of participants with an anti-CHIKV SNA titre ≥15 [≥LLOQ] (and thus considered seropositive) and 4-fold rise over baseline), analysis of the few subjects seropositive at baseline, and supportive studies, corroborate the SRR findings of both studies and indicated that CHIKV VLP is indeed immunogenic.

The CHIKV VLP vaccine is based on the chikungunya virus (CHIKV) Senegal strain 37997 from the West African (WA) lineage. Indeed, in study EBSI-CV-317-002 (60 participants aged 18 to 65 years vaccinated with CHIKV VLP vaccine), the 3 major lineages Asian, WA, and ECSA were tested for CHIKV VLP vaccine cross-neutralization using the following 4 strains: 15561 (Asian – Thailand 1962), 181/25 (Asian – attenuated strain of 15561), LR (ECSA/IOL – La Reunion 2006), and PM2951 (WA – Senegal 1966). At Day 22, GMTs for strains 181/25, 15561, LR, and PM2951 were 584, 551, 905, and 2584, respectively, and seroconversion (titre ≥20) was seen in 100% of participants. At Day 182, GMTs for strains 181/25, 15561, LR, and PM2951 were 204, 184, 148, and 686, respectively, and seroconversion remained high (100% for strains 181/25 and PM2951 and 98% and 95% for strains 15561 and LR, respectively).

3.3. Uncertainties and limitations about favourable effects

No efficacy data are available as a field efficacy trial in a chikungunya-endemic area is considered a formidable challenge and was indeed agreed to be unfeasible in the scientific advice.

Moreover, there is no established immunological correlate of protection against Chikungunya disease.

Hence, clinical efficacy is inferred by use of a surrogate immunogenicity parameter in form of an anti-CHIKV SNA titre of at least 100 (using the CHIKV-Luc NT80 assay) that can be considered reasonably likely to predict protection from CHIKV disease. The evidence to support this titre threshold stems from a prospective sero-epidemiologic study (published by Yoon et al., PLoS Negl Trop Dis. 2015) in subjects with prior exposure to CHIKV and from a non-human primate (NHP) passive transfer/challenge study (PAS-NHP-CHIK-003) using pooled sera from humans vaccinated with the CHIKV VLP vaccine. During several scientific advices, that threshold titre was intensely discussed and finally agreed to be at least 100 to account for uncertainties. Nonetheless, this threshold's robustness relies on the aforementioned analyses and its actual validity and clinical relevance remains elusive in absence of actual efficacy data. The approach is aligned with the Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1).

Immunogenicity data are limited to anti-CHIKV SNA titres, or analyses thereof. Analyses of other parameters of an immune response, for instance, binding antibodies or cell-mediated responses were not provided. However, considering that results of such additional immune response analyses would be – in absence of a suitable link to clinical relevance – hard to interpret from a clinical point of view, further analyses are not requested.

The Applicant proposes to conduct a post-approval effectiveness study in an CHIVK-endemic area. Evidence on feasibility and interactions with public health institutions have been provided. Based on the outlined plans it is anticipated that relevant data on CHIKV VLP vaccine effectiveness in case of a CHIKV outbreak can be generated. Further assessment of the protocol will be conducted in the post-authorization phase.

Long-term data to inform persistence of humoral immune responses beyond 6 months and booster administration are scarce and have their limitations. Pertinent data from study EBSI-CV-317-008, which serves as rollover study for the pivotal studies and plans also to investigate booster administrations, is expected to be provided by the Applicant post-approval.

Results from study EBSI-CV-317-002 (<65 yoa) demonstrated cross neutralization for the three major CHIKV lineages, these data were only based on a small sample size (n=60). Cross-neutralization data in individuals ≥65 yoa will be provided post-authorisation.

Immune responses in immunocompromised subjects have not been investigated. This uncertainty is reflected in section 4.4 of the SmPC.

Moreover, effects of concomitant vaccinations were not evaluated, as reflected in section 4.5 of the SmPC. It is recommended to investigate co-administration with other relevant vaccines (e.g., traveller vaccines) post-marketing.

Pregnant or lactating women were excluded from clinical studies. Eleven pregnancies occurred during the studies. No immunogenicity nor efficacy studies are planned in this population. This uncertainty is reflected in section 4.6 of the SmPC.

3.4. Unfavourable effects

A total of 3522 participants were exposed to at least one IM dose of CHIKV VLP vaccine (different antigen content +/- adjuvant). Overall, 3141 participants received a single dose of the clinically relevant 40/300 µg CHIKV VLP vaccine (n=2996 in Phase 3 studies mainly from EBSI-CV-317-004) of which approx. 200 individuals each were 12-17 years of age (subset in Phase 3 study EBSI-CV-317-004) or ≥65 years of age (entire Phase 3 study EBSI-CV-317-005).

Solicited AEs (Reactogenicity). A total of 37.1% of CHIKV VLP vaccinated individuals experienced any solicited AE (23.3% in the placebo group) that were mostly Grade 1 or 2 in severity. Grade ≥3 solicited AEs were reported as 1.6% and 0.5%, respectively.

- ***Local solicited AE*** rates were approx. 4-times higher in the CHIKV VLP group (23.4%) compared to placebo (8.0%) and were mainly due to injection site pain (very common), whereas injection site swelling or redness were uncommon (≤ 0.4% across groups).
- ***Systemic solicited AE*** rates were approx. 1.5-times higher in the CHIKV VLP group (30.7%) compared to placebo (21.6%) and were mainly due to the very common events of fatigue (18.7% vs. 13.6%), headache (17.0% vs. 13.9%) and myalgia (16.8% vs. 8.8%). Arthralgia, chills and nausea were commonly reported, whereas pyrexia was uncommon (≤0.8%) across groups.

All local and systemic solicited AEs are tabulated in section 4.8 of the SmPC.

Unsolicited AEs. Frequencies of unsolicited AEs were comparable between 40/300 µg CHIKV VLP vaccine recipients (492/3141; 15.7%) and placebo recipients (97/675; 14.4%). Frequencies of ***unsolicited AEs considered treatment-related*** were also comparable between participants receiving 40/300 µg CHIKV VLP vaccine (2.4%) or placebo (1.9%). By PT, the most frequently reported treatment-related unsolicited AEs in the CHIKV VLP vaccine group were headache (0.3%), arthralgia (0.3%), dizziness (0.2%), fatigue (0.2%) and rash (0.2%). In the placebo group fatigue (0.4%), arthralgia (0.3%), myalgia (0.3%) were most frequently reported.

AESIs (occurrence of new onset or worsening of arthralgia). A total of 8 AESI were reported by 8 participants (6 (0.2%) in the CHIKV VLP vaccine group and 2 (0.3%) in the placebo group). All AESI were non-serious, grade 1 or grade 2 in severity. Four AESI were assessed as related to study treatment (3 in the CHIKV VLP vaccine group and 1 in the placebo group).

SAEs. A total of 53 SAEs occurred in 41 participants: 1.1% (n=37) of all CHIKV VLP vaccine recipients (40/300 µg CHIKV VLP vaccine: n=31, other CHIKV VLP vaccine dose: n=6) and 0.6% (n=4) of placebo recipients reported at least 1 SAE. By SOC these SAEs were reported with highest frequency for Infections and Infestations (n=8 for all CHIKV VLP vaccine recipients vs. n= 2 for placebo). By PT no SAE was reported more than twice. No SAEs could be identified that could be attributed to CHIKV VLP vaccination.

No important identified nor important potential risk was identified in the available data.

3.5. Uncertainties and limitations about unfavourable effects

The following uncertainties and limitations have been identified and will be monitored via routine pharmacovigilance or with post-authorisation safety studies.

Seropositive individuals. The number of CHIKV seropositive individuals at baseline who received the 40/300 µg CHIKV VLP vaccine (n=72) was too low to draw any meaningful conclusions on the safety profile by serostatus. This will be monitored via routine pharmacovigilance.

Revaccination. It is unknown, if revaccination is needed to maintain efficacy and if so, how the safety profile might be impacted. Limited data on revaccination are available from Phase 2 studies. These data are mainly not derived using the clinically relevant formulation or potentially meaningful interval. Study **EBSI-CV-317-008** is a currently ongoing and will examinate a single booster dose at 3, 4 or 5 years post initial vaccination in feeder studies EBSI-CV-317-004 and EBSI-CV-317-005. This data is expected to be submitted post-authorisation.

Pregnant women were excluded from clinical trials, but some pregnancies were reported. No concern was identified in the data reported. However, the number of pregnant study participants is too limited to conclude on the safety of CHIKV VLP vaccine in pregnant individuals. Section 4.6 of the SmPC includes a careful wording on the benefits and risks in this population and 'Use during pregnancy and breastfeeding' is considered missing information in the RMP. A post-authorisation observational safety study will be conducted to further characterise this missing information.

3.6. Effects Table

Effects Table for Vimkunya to prevent disease caused by chikungunya virus infection in individuals 12 years of age and older.

Effect	Short Description	Unit	CHIKV VLP	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects (main, pivotal studies [40 µg CHIKV VLP/300 ug adjuvant])						
Immunogenicity (12-65yoa, IEP, Study EBSI-CV-317-004)	Seroresponse Rate on Day 22	n/N (%) [95% CI]	2503/2559 (97.8) [97.2, 98.3]	5/424 (1.2) [0.5, 2.7]	**Unc:** Clinical relevance of the anti-CHIKV SNA titre threshold (≥100) in inferring efficacy against CHIK or CHIKV disease elusive **SoE:** Difference in seroresponse rates (VLP minus Placebo) [95% CI]: 96.6% [95.0%, 97.5%]. Superiority met (defined as the lower bound of the 95% CI of the difference in seroresponse rates being above 70% [clinically significant] and statistical superiority to placebo (p<0.0001, chi-square test))	Summary of clinical efficacy

Effect	Short Description	Unit	CHIKV VLP	Placebo	Uncertainties/ Strength of evidence	References
Immunogenicity (≥65yoa, IEP, Study EBSI-CV-317-005)	Seroresponse Rate on Day 22	n/N (%) [95% CI]	165/189 (87.3) [81.8, 91.3]	2/183 (1.1) [0.3, 3.9]	**Unc:** clinical relevance of the anti-CHIKV SNA titre threshold (≥100) in inferring efficacy against CHIK or CHIKV disease elusive **SoE:** Difference in seroresponse rates (VLP minus Placebo) [95% CI]: 86.2 [80.0, 90.3]. Superiority met (defined as the lower bound of the 95% CI of the difference in seroresponse rates being above 70% [clinically significant] and statistical superiority to placebo (p<0.0001, chi-square test))	Summary of clinical efficacy
Solicited AEs (Reactogenicity)	Solicited administration site effects [a]	% of individuals	23.4	8.0	Transient effect, majority mild to moderate in severity	pooled data from ISS (mainly from study -004)
	Solicited systemic effects [b]	% of individuals	30.7	21.6		
Unsolicited AEs	all	% of individuals	15.7	14.4		
	related [c]	% of individuals	2.4	1.9		
SAEs	all	% of individuals	1.0	0.6		
	related	% of individuals	0	0		

Abbreviations: AE…adverse event, ISS = integrated summary of safety (pooled data from 3 Phase 2 and 2 Phase 3 studies), SAE = serious adverse event
Notes:
a Solicited administration-site effects include injection-site pain, redness and swelling

b Solicited systemic effects include fever, chills, fatigue, headache, myalgia, arthralgia, nausea
c by PT most frequent: CHIKV VLP: headache (0.3%), arthralgia (0.3%), dizziness (0.2%), fatigue (0.2%), rash (0.2%) vs. Placebo: fatigue (0.4%), arthralgia (0.3%), myalgia (0.3%)

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Although CHIKV infections and disease are currently of minor concern for the general European population and occur mainly in travellers to CHIKV-endemic areas, as a consequence of climate change, increased presence of CHIKV-harbouring arthropod vectors in continental Europe is considered an important threat for future public health.

To date, treatment of CHIKV disease is symptomatic, including non-salicylate analgesics and non-steroid anti-inflammatory therapy but no specific antiviral drugs against CHIKV exist. Moreover, only one prophylactic CHIKV vaccine (IXCHIQ, a LAV indicated for active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 18 years and older) is currently authorized. Hence, CHIKV VLP might pose an option and address an unmet medical need for a population that is either 12 to 18 yoa or for which a non-LAV vaccine is preferred or better suited, for instance immunocompromised individuals or pregnant or lactating women. Notably, neither immune responses in immunocompromised subjects, pregnant or lactating women, nor effects of concomitant vaccinations were evaluated.

Since there is no established immunological correlate of protection against chikungunya disease and an efficacy trial is challenging, clinical efficacy is inferred by use of a surrogate immunogenicity parameter in form of an anti-CHIKV SNA threshold titre of at least 100 that can be considered reasonably likely to predict protection from CHIKV disease. This approach is aligned with the Guideline on clinical evaluation of vaccines (EMEA/CHMP/VWP/164653/05 Rev. 1).

Although, based on that threshold, CHIKV VLP elicited humoral immune responses indicative of a considerable percentage of vaccinees being protected from CHIKV disease for at least 6 months, robustness of those data and clinical relevance thereof remains elusive in absence of actual efficacy data. The Applicant proposes to conduct a post-approval effectiveness study in an CHIVK-endemic area. Evidence on feasibility and interactions with public health institutions have been provided. Based on the outlined plans it is anticipated that relevant data on CHIKV VLP vaccine effectiveness in case of a CHIKV outbreak can be generated in the post-authorisation phase.

Data on persistence of humoral immune responses beyond 6 months and booster administration are scarce, and, in addition, uncertainties pertain to the limited amount of data in seropositive individuals; however, these data are not considered critical for the initial MAA and can be collected post-approval.

Cross-neutralization of CHIKV strains was only examined in a limited number of individuals <65 yoa (n=60) and cross-neutralization data in the elderly will become available post-authorisation. This approach is acceptable in the context of the overall immunogenicity results across different age groups.

The documented safety exposure is sufficient for an adequate assessment of the safety profile of CHIKV VLP. The safety profile is mainly characterised by reactogenicity events, most frequently injection site pain, fatigue, headache and myalgia. Severity was mostly mild to moderate and the majority of reactogenicity events resolved within a few days. Unsolicited events considered treatment related do not raise concerns. SAEs were infrequently reported for both CHIKV VLP and placebo. No related SAEs were identified. In summary, the CHIKV VLP safety profile is considered acceptable.

3.7.2. Balance of benefits and risks

The available clinical data demonstrated that Vimkunya induces CHIKV-specific neutralizing antibody titres above the threshold considered reasonably likely to predict protection from CHIKV disease in the majority of vaccinees for at least 6 months. However, in absence of efficacy data, uncertainties as regards the actual effective protection against CHIKV disease remain. A post-authorisation efficacy study is currently planned as a condition to the marketing authorisation to confirm and characterize the clinical protection offered by the vaccine.

In addition, the safety profile of the proposed dose and regimen of Vimkunya in individuals 12 years of age and older is considered acceptable.

3.8. Conclusions

The overall benefit/risk balance of Vimkunya is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Vimkunya is favourable in the following indication:

Vimkunya is indicated for active immunisation for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 12 years and older.

The use of this vaccine should be in accordance with official recommendations.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and

interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.
- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Description	**Due date**
Post authorisation efficacy study (PAES): In order to confirm the efficacy of VIMKUNYA in individuals 12 years and older, the MAH should conduct and submit the results of a randomized, placebo-controlled, double-blind, event-driven study to analyse efficacy, safety, and immunogenicity of VIMKUNYA in the prevention of chikungunya disease in healthy adults and adolescents in CHIKV-endemic areas, according to an agreed protocol.	Final report due date: 31st August 2030

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that chikungunya virus (CHIKV) virus-like particle (VLP) proteins (capsid protein (C) and envelope proteins E1 and E2) derived from CHIKV Senegal strain 37997 produced in human embryonic kidney cells by recombinant DNA technology and which are adsorbed on aluminium hydroxide, hydrated, is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

Refer to Appendix on new active substance (NAS).

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0159/2021 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.