

**CBER CMC BLA Review Memorandum**

**BLA STN 125777**

**Chikungunya Vaccine, Live**

**Shufeng Liu, PhD, CBER/FDA**

**1. BLA#:** STN 125777

**2. APPLICANT NAME AND LICENSE NUMBER**

Valneva Austria GmbH

**3. PRODUCT NAME/PRODUCT TYPE**

Proper name: Chikungunya Vaccine, Live

Proprietary name: IXCHIQ

**4. GENERAL DESCRIPTION OF THE FINAL PRODUCT**

IXCHIQ is a live attenuated chikungunya vaccine that is based on the La Reunion strain (LR-CHIKV clone LR2006-OPY1) of (b) (4). The live attenuated chikungunya virus (CHIKV) was constructed by deleting (b) (4) amino acids in the nsP3 viral replicase complex gene. IXCHIQ vaccine is a sterile and lyophilized product to be reconstituted with the supplied prefilled syringe of diluent (sterile water for injection). A single dose is 0.5 mL after reconstitution. The vaccine is administered intramuscularly (IM) into the deltoid muscle as a single injection and is indicated for the prevention of disease caused by chikungunya virus in individuals 18 years and older.

The proprietary name, IXCHIQ, was approved during BLA review. In this memo, the vaccine is referred to as VLA1553 and IXCHIQ.

**5. MAJOR MILESTONES**

Filing Meeting: February 2, 2023

Advisory Committee Meeting: Not applicable

PeRC meeting: June 20, 2023

Major Amendment: August 11, 2023

Action Due Date: August 22, 2023

Revised Action Due Date: November 3, 2023

**6. CMC/QUALITY REVIEW TEAM**

Reviewer/Affiliation	Section/Subject Matter
Shufeng Liu, OVRD/DVP	Module 3 (except for facilities and equipment information), Modules 4 (non-clinical) and 5 (assays used to assess clinical endpoints), Module 1 (labeling)
Viviana Ramirez, OCBQ/DMPQ	Modules 3 (facilities and equipment information) and manufacturing records
Tao Pan, OCBQ/DBSQC	DS Release Assays and Method Validations: (b) (4) DP Release Assays and Method Validations: Appearance, (b) (4)

<b>Reviewer/Affiliation</b>	<b>Section/Subject Matter</b>
	Recombinant Human Albumin (rHA), Sucrose, D-Sorbitol, L-Methionine, Residual moisture
Karla Garcia, OCBQ/DBSQC	DS Release Assays and Method Validations: (b) (4) [REDACTED]
Alicia Howard, OCBQ/DBSQC	DS Release Assays and Method Validations: (b) (4) [REDACTED] DP Release Assays and Method Validations: Identity, Infectious Virus Concentration
Ho-Hsiang Wu, OBPV/DB	Statistical (quality/CMC related assays)
Maria Anderson, OCBQ/DBSQC	Lot Release Protocol

## 7. INTER-CENTER CONSULTS REQUESTED

None requested

## 8. SUBMISSION(S) REVIEWED

<b>Date Received</b>	<b>Submission</b>	<b>Comments/ Status</b>
August 17, 2022	STN 125777/0	Reviewed
September 22, 2022	STN 125777/0.1	Reviewed
October 28, 2022	STN 125777/0.2	Reviewed
December 22, 2022	STN 125777/0.3	Reviewed
January 20, 2023	STN 125777/0.5	Reviewed
February 17, 2023	STN 125777/0.11 (response to IR dated February 01, 2023)	Reviewed
March 09, 2023	STN 125777/0.16 (response to IR dated February 08, 2023)	Reviewed
March 10, 2023	STN 125777/0.18 (response to IR dated February 24, 2023)	Reviewed
March 14, 2023	STN 125777/0.19 (response to IR dated March 09, 2023)	Reviewed

<b>Date Received</b>	<b>Submission</b>	<b>Comments/ Status</b>
April 04, 2023	STN 125777/0.26 (response to IR dated March 23, 2023)	Reviewed
April 18, 2023	STN 125777/0.29 (response to IR dated April 07, 2023)	Reviewed
May 11, 2023	STN 125777/0.39 Shipment validation	Reviewed
May 17, 2023	STN 125777/0.41 (response to IR dated May 08, 2023)	Reviewed
May 30, 2023	STN 125777/0.45 (response to IR dated May 22, 2023)	Reviewed
June 06, 2023	STN 125777/0.47 (response to IR dated May 31, 2023)	Reviewed
June 21, 2023	STN 125777/0.54 (response to IR dated June 16, 2023)	Reviewed
June 28, 2023	STN 125777/0.56 (response to IR dated June 23, 2023)	Reviewed
July 05, 2023	STN 125777/0.60 Shipment validation	Reviewed
July 06, 2023	STN 125777/0.61 (response to IR dated June 29, 2023)	Reviewed
July 18, 2023	STN 125777/0.66 Shipment validation	Reviewed
August 10, 2023	STN 125777/0.80 Shipment validation	Reviewed
September 01, 2023	STN 125777/0.82 (response to IR dated August 24, 2023)	Reviewed
September 12, 2023	STN 125777/0.85 (response to IR dated September 07, 2023)	Reviewed
October 13, 2023	STN 125777/0.89 (response to IR dated October 06, 2023 and advice on October 11, 2023)	Reviewed

**9. Referenced REGULATORY SUBMISSIONS (e.g., IND BLA, 510K, Master File, etc.)**

<b>Submission Type &amp; #</b>	<b>Holder</b>	<b>Referenced Item</b>	<b>Letter of Cross-Reference</b>	<b>Comments/Status</b>
IND 17854	Valneva	Investigational New Drug (IND) submission	no	Contains information pertinent to the development of the vaccine.
DMF (b) (4)	(b) (4)	Recombinant Human Albumin (rHA)	yes	Information pertinent to control of materials and excipients was reviewed, assessed, and documented in Sections 3.2.S.2.3 and 3.2.P.4.
DMF (b) (4)	(b) (4)	Syringe	yes	Information pertinent to container closure was reviewed, assessed, and documented in this memo in Section 3.2.P.2.4.
DMF (b) (4)	(b) (4)	Rubber stopper	yes	Information pertinent to container closure was reviewed, assessed, and documented in this memo in Section 3.2.P.2.4.
DMF (b) (4)	(b) (4)	(b) (4)	yes	Information pertinent to container closure was reviewed, assessed, and documented in this memo in Section 3.2.P.2.4.

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
DMF (b) (4)	(b) (4)	(b) (4)	yes	Information pertinent to container closure was reviewed, assessed, and documented in this memo in Section 3.2.P.2.4.

## 10. REVIEWER SUMMARY AND RECOMMENDATION

### A. EXECUTIVE SUMMARY

Valneva Austria GmbH submitted the original Biologics License Application (STN #125777/0) as a rolling review with sections submitted on August 17, 2022, October 28, 2022, and December 22, 2022 (final roll), to seek approval of VLA1553, Chikungunya Vaccine, Live, for active immunization for the prevention of disease caused by chikungunya virus in individuals 18 years and older through the accelerated approval pathway. I reviewed the CMC section, preclinical studies, and assays used to assess immune response in the clinical studies.

#### ***Chemistry Manufacturing and Controls (CMC)***

The active substance of IXCHIQ is a liquid preparation of live attenuated chikungunya virus La Reunion strain (LR-CHIKV clone LR2006 OPY1). The attenuated virus (CHIKV Δ5nsP3) was constructed by deleting (b) (4) amino acids in nsP3 via (b) (4) technology. CHIKV Δ5nsP3 is propagated in Vero cells in media containing amino acids, vitamins, minerals and fetal bovine serum. The viral harvests are pooled, clarified and concentrated. The vaccine virus is further purified by chromatography and ultracentrifugation. The purified virus solution is (b) (4) and stored at (b) (4) for up to (b) (4) until formulation into drug product (DP).

IXCHIQ DP is a sterile and lyophilized powder to be reconstituted before injection with sterile water for a final 0.5 mL single dose presentation. The manufacturing of IXCHIQ DP consists of formulation, sterile filtration, filling, lyophilization, and packaging. During the formulation step, purified bulk DS is mixed with the formulation buffer to contain final concentrations of (b) (4) recombinant human albumin (rHA), (b) (4) sucrose, (b) (4) D-sorbitol, (b) (4) L-methionine, (b) (4) magnesium chloride, (b) (4) trisodium citrate di-hydrate and (b) (4) potassium phosphate. After filtration, the DP is filled into Type (b) (4) glass (b) (4) vials and lyophilized. Labeled vials are stored at 2-8°C for up to 24 months from the date of manufacture, which is defined as the date of unloading of the lyophilized vials from the (b) (4) unit. The vaccine contains no preservatives or adjuvants.

Testing is performed at multiple stages of the manufacturing process to ensure the product meets the pre-defined specifications.

- Release testing for DS includes: appearance, (b) (4)  
(b) (4)
- Release testing for final DP (lyophilized product) includes: appearance and solubility, (b) (4) identity, infectious virus concentration, rHA content, sucrose content, D-sorbitol content, L-methionine content, residual moisture, bacterial endotoxin, and sterility.
- Release testing for final DP (sWFI) includes: appearance of solution, appearance of solution / (b) (4)  
(b) (4)

The DP is formulated to a targeted concentration of (b) (4) TCID<sub>50</sub>/dose, which is (b) (4) than the release test titer of (b) (4) TCID<sub>50</sub>/dose. The upper limit specification of (b) (4) TCID<sub>50</sub>/dose is consistent with clinical studies that have shown safety of the product. Regarding the lower limit specification, the sponsor has set a lower limit specification of (b) (4) TCID<sub>50</sub>/dose to ensure a titer of 3.0 log<sub>10</sub> TCID<sub>50</sub>/dose at the end of the expiry period of 24 months. Data from clinical studies have shown that the vaccine is immunogenic when delivering (b) (4) TCID<sub>50</sub>/dose. Therefore, the end of shelf-life specification of 3.0 log<sub>10</sub> TCID<sub>50</sub>/dose is acceptable.

### ***Non-clinical Studies***

Data from one non-clinical pharmacological study (Report PHY1802-02) showed that vaccine virus RNA was detected in non-human primate (NHP) spleens (2/2 animals) and axillary lymph node (1/2 animals) at ~Day 90 and there was no detectable viral RNA from other tissues beyond day 60 or from plasma beyond day 9. Replicating VLA1553 virus was detected in spleen on day 2 but not on days 6 and 14.

Valneva performed a NHP passive transfer study (Report VAC1816-02) to establish a threshold titer for protection after vaccination with VLA1553. Based on the data, a surrogate of protection with a  $\mu$ PRNT<sub>50</sub> titer of >150 was considered to be reasonably likely to predict protection in humans and was applied to clinical evaluation of VLA1553 in Phase 3.

Results from passive transfer experiments in mice (Report RR-0065-01) demonstrated that the antibody quality in terms of protective neutralizing antibody titer induced by VLA1553 in individual human subjects is comparable at similar  $\mu$ NT<sub>50</sub> titers.

Data from a secondary non-clinical pharmacological study (Report RR-0066) suggested that the risk of transmission of VLA1553 to non-vaccinees is very low.

### ***Diagnostic Assay for Clinical Efficacy Endpoint Assessments***

The sponsor used a CHIKV Micro-Plaque Reduction Neutralization Test ( $\mu$ PRNT) assay to quantify the titer of neutralizing antibodies for CHIKV in human serum. The testing was performed by a contract lab, (b) (4). Information for the  $\mu$ PRNT assay to assess seroconversion and anti-CHIKV titers was submitted to IND 17854 and the BLA. Assay validation information was reviewed under the IND prior to testing of clinical samples and found to be appropriate.

Overall, the information provided in the BLA and amendments demonstrates that the manufacturing process is well-controlled with appropriate and adequate validations and in-process control testing. Moreover, adequate quality control testing has been conducted and stability data have been accrued with the (b) (4) DP. Therefore, I recommend approval of the product.

## **B. RECOMMENDATION**

### **I. APPROVAL**

#### **a. List of DS and DP Manufacturing Facilities**

- Manufacture of DS: Valneva Scotland Limited, Oakbank Park Road, Mid Calder, Livingston, West Lothian EH53 OTG, UK
- Manufacture of DP (lyophilized vaccine): (b) (4)
- Manufacture of DP (sWFI): (b) (4)

#### **b. List of approvable Comparability Protocols, if applicable**

- Comparability Protocol for Manufacture and Testing of Serum-Containing Vero (b) (4)
- Protocol for the Preparation and Qualification of CHIKV RNA Reference Standard Lots

#### **c. List of Post-Marketing Commitments (PMCs)/Post-Marketing Requirements (PMRs), if applicable.**

PMRs:

- An observational study with a test-negative, case-control design to assess the effectiveness of IXCHIQ vaccination in the prevention of symptomatic laboratory confirmed CHIKV cases after a single vaccination with IXCHIQ in the adolescent and adult population (12 years of age and older) in endemic areas of Brazil.



- A second adequate and well-controlled study to confirm effectiveness of IXCHIQ.
- Deferred pediatric study under PREA (VLA1553-321, double-blinded, multicenter, Phase 3 RCT) to evaluate safety and immunogenicity in children (12 to <17 years of age).
- Deferred pediatric study under PREA (VLA1553-221, double-blinded, multicenter, Phase 2 dose-finding RCT) to evaluate safety and immunogenicity in children (1 to <12 years of age).
- Deferred pediatric study under PREA (VLA1553-322, double-blinded, multicenter, Phase 3 dose-confirmation RCT) to evaluate safety and immunogenicity in children (1 to <12 years of age).
- Deferred pediatric study under PREA (VLA1553-222, double-blinded, multicenter, Phase 2 dose-finding RCT) to evaluate safety and immunogenicity in neonates and infants (<1 year of age).
- Deferred pediatric study under PREA (VLA1553-323, double-blinded, multicenter, Phase 3 dose-confirmation RCT) to evaluate safety and immunogenicity in neonates and infants (<1 year of age).

PMC:

- Observational study to evaluate the safety of live-attenuated chikungunya virus vaccine (IXCHIQ) in pregnant women aged 18-45 years exposed to the vaccine.

**d. Consideration for Inspectional Follow-up (e.g., flagging inspectional issues for future surveillance inspections)**

Inspection was waived. No specific CMC issues for inspectional follow up.

**e. Lot release requirements**

The Lot Release Protocol was reviewed by the DBSQC team and found to be acceptable.

**f. Established Conditions (ECs)**

Not applicable.

**g. List approvable ECs and associated reporting categories at the end of the Review Memo.**

Not applicable.

**II. COMPLETE RESPONSE (CR)**

None

**III. SIGNATURE BLOCK**

<b>Reviewer/Title/Affiliation</b>	<b>Concurrence</b>	<b>Signature and Date</b>
Shufeng Liu, CMC reviewer/DVP/OVRR	Concur	
Marian Major, Lab Chief/DVP/OVRR	Concur	
Robin Levis, Deputy Director/DVP/OVRR	Concur	

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**List of Abbreviations**

AET: Analytical Evaluation Threshold  
BSA: Bovine Serum Albumin  
BSE: Bovine Spongiform Encephalopathy  
CCI: Container Closure Integrity  
CHIKV: Chikungunya Virus  
CMA: Critical Material Attributes  
CPE: Cytopathogenic Effect  
CPP: Critical Process Parameters  
CPV: Continuous Process Verification  
CQA: Critical Quality Attribute  
CTM: Clinical Trial Material  
DP: Drug Product  
DS: Drug Substance  
EOSL: End of Shelf-Life  
EoPCB: End of Production Cell Bank  
FBS: Fetal Bovine Serum

(b) (4)

GCE: Genome Copy Equivalents  
GCV: Geometric Coefficient of Variation  
GMC: Geometric Mean Concentration  
GMP: Good Manufacturing Practice  
GMR: Geometric Mean Ratio  
GMT: Geometric Mean Titer  
HCP: Host Cell Protein  
IPC: In-Process Control  
IR: Information Request  
LLOQ: Lower Limit of Quantification  
LOD: Limit of Detection  
MCB: Master Cell Bank  
MEV: Minimum Extractable Volume  
MOI: Multiplicity of Infection  
NHP: Non-Human Primate  
PBS: Phosphate Buffered Saline

(b) (4)

PFS: Prefilled Syringe  
PP: Process Parameter  
PPQ: Process Performance Qualification  
rHA: Recombinant Human Albumin

(b) (4)







TCID<sub>50</sub>: 50% Tissue Culture Infectious Dose  
TAMC: Total Aerobic Microbial Count  
TSE: Transmissible Spongiform Encephalitis

TYMC: Total Yeast and Mold Count  
USP: United States Pharmacopeia  
vCJD: Variant Creutzfeldt–Jakob Disease  
WCB: Working Cell Bank  
WFI: Water for Injection  
WT: Wild Type  
WVSB: Working Virus Seed Bank

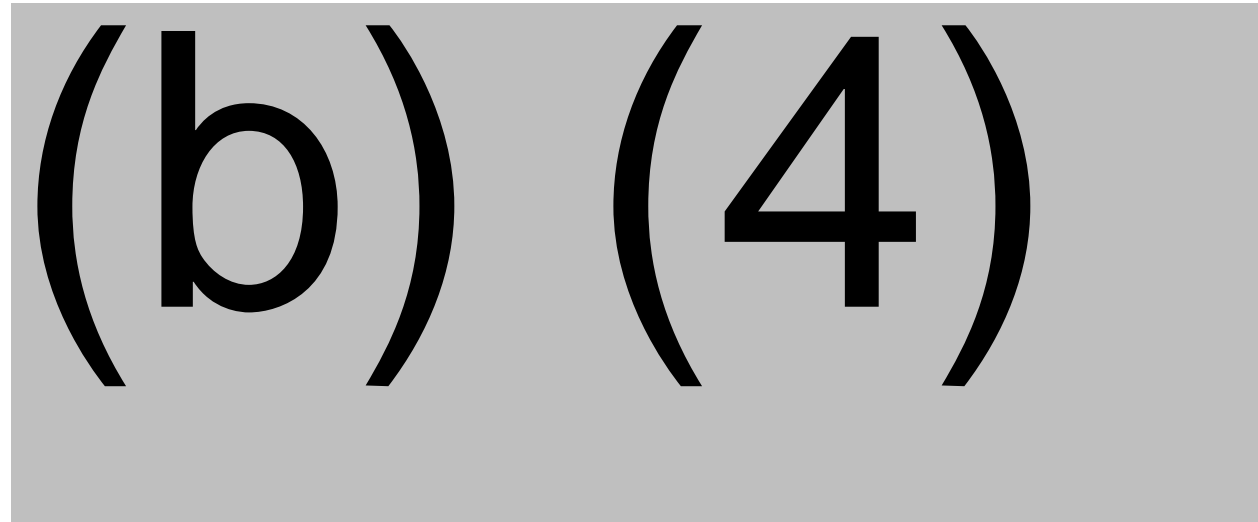
### Module 3

#### 3.2.S DRUG SUBSTANCE (DS)

(b) (4)



(b) (4)



34 pages determined to be not releasable: (b)(4)

**3.2.P DRUG PRODUCT****3.2.P.1 Description and Composition of the Drug Product**

The Drug Product (DP) is the live attenuated chikungunya virus vaccine to be administered by intramuscular route. The DP kit contains one vial of the lyophilized vaccine and one prefilled syringe of sterile water for injection for reconstitution. The lyophilized DP is supplied in a single dose format in Type <sup>(b) (4)</sup> glass <sup>(b) (4)</sup> vials. It is a white-to-creamy white homogeneous lyophilized cake. The solvent used for reconstitution is 0.5 mL sterile water for injection prefilled in a 1.5 mL syringe format. The reconstituted product is a colorless to amber suspension. The composition of the DP is described in Table 8 (adapted from Table 3.2.P.1-1 of the BLA).

**Table 8: DP Composition**

Component	Quantity per dose (0.5mL)	Function
Live-attenuated Chikungunya Virus	<sup>(b) (4)</sup> log <sub>10</sub> TCID <sub>50</sub>	<b>(b) (4)</b>
di-Potassium Hydrogen Phosphate	0.313 mg	
Potassium di- Hydrogen Phosphate	0.098 mg	
Trisodium citrate dihydrate	3.68 mg	
Sucrose	25 mg	
Magnesium Chloride hexahydrate	0.51 mg	
D-Sorbitol	2.5 mg	
L-Methionine	0.75 mg	
Recombinant Human Albumin (rHA)	0.05mg (equals 0.01%)	
Water for Injection	MEV: 0.50 mL	

**3.2.P.2 Pharmaceutical Development****3.2.P.2.1 Components of the Drug Product****3.2.P.2.1.1 Drug Substance**

The active component is a genetically modified live attenuated chikungunya virus harboring a <sup>(b) (4)</sup> amino acid deletion in nsP3 (non-structural Protein 3), one of the viral RNA replicase complex genes, leading to a reduced replication capability of the virus in vivo. The virus is propagated in Vero cells, purified by concentration/ultrafiltration, batch chromatography, sucrose gradient centrifugation, and formulated in a <sup>(b) (4)</sup>

to the current DP formulation buffer.

**3.2.P.2.1.2 Excipients**

The excipients include <sup>(b) (4)</sup> di-potassium hydrogen phosphate/ potassium di-hydrogen phosphate, <sup>(b) (4)</sup> trisodium citrate di-hydrate, <sup>(b) (4)</sup> sucrose, <sup>(b) (4)</sup> magnesium chloride hexahydrate, <sup>(b) (4)</sup> D-sorbitol, <sup>(b) (4)</sup> L-methionine, and 0.1 mg/mL rHA. Trisodium citrate dehydrate and di-potassium hydrogen phosphate/potassium di-



(b) (4)

### **3.2.P.2.2 Drug Product**

#### **3.2.P.2.2.1 Formulation Development**

The VLA1553 DP formulation used in the toxicology study and phase 1 trial was initially developed as a (b) (4) form to be stored (b) (4). The (b) (4) DP formulation buffer consisted of (b) (4) rHA, (b) (4) sucrose, (b) (4). However, the long-term stability data indicated a loss of (b) (4).

For the subsequent clinical and commercial phases, the formulation of the DP was changed to a lyophilized form to improve product stability, facilitate storage and transport at 2-8°C and administration of the product. A (b) (4)

DP manufacturing and to avoid potential aggregation of CHIK virus. The final (b) (4)

The sponsor provided a report comparing immunogenicity of (b) (4) lyophilized product in mice. For a review of the comparability study, refer to Section 3.2.P.2.3 Manufacturing Process Development in this memo.

#### **3.2.P.2.2.2 Overages**

In order to compensate for expected manufacturing losses, the DP bulk target TCID<sub>50</sub> titer is adjusted to (b) (4) TCID<sub>50</sub>/dose, which is slightly higher than the release test titer of (b) (4) log<sub>10</sub> TCID<sub>50</sub>/dose.

There is no fill overage.

#### **3.2.P.2.2.3 Physicochemical and Biological Properties**

The DP is in a lyophilized form. The physicochemical properties of the DP are physical appearance, (b) (4). The lyophilized product is a white to slightly yellowish homogeneous cake with no visible particles or significant cracks. The

reconstituted product is a clear colorless to slightly yellowish solution with a (b) (4) levels are determined at DP release with a specification of (b) (4) The biological activity of the DP is determined by a TCID<sub>50</sub> assay.

The methods used to ensure the quality, purity, and safety of the sWFI prefilled syringes (PFS) are based on (b) (4)

### 3.2.P.2.3 Manufacturing Process Development

Main manufacturing process changes from phase I to phase III process development are: (b) (4)

(b) (4)

set-up were verified by the process revalidation study (Section 3.2.P.3.5) and all specifications were met.

### 3.2.P.2.4 Container Closure System

#### Lyophilized product

The container closure system of VLA1553 DP consists of a 2-mL Type<sup>(b) (4)</sup> borosilicate glass vial<sup>(b) (4)</sup> vial), a 13 mm bromobutyl rubber stopper, and a 13 mm Aluminum cap with polypropylene closure. The glass vial meets (b) (4)

requirements. The vials are manufactured by (b) (4). Routine control tests for the vials are the following: (b) (4)

. The rubber stopper complies with (b) (4). The rubber stoppers are supplied by (b) (4)

Tests for the stoppers are the following: physicochemical, microbiological and functionality, visual inspections, and dimensional checks. The flip-off cap complies with (b) (4). The caps are supplied by (b) (4)

Tests for the caps are the following: visual inspections, and dimensional checks.


#### Extractables and Leachables Assessment

The<sup>(b) (4)</sup> glass vials are made from (b) (4) glass tubing. An extractables study on the glass tubing was performed by the manufacturer (b) (4) by (b) (4)

The sponsor explained that the high concentration of these inorganic elements is expected as the elements represent glass composition. The potential for the glass vials leaching inorganic elements is reduced due to the solid state of the DP and the (b) (4) temperature.

An extractables study on the rubber stopper was performed by (b) (4)

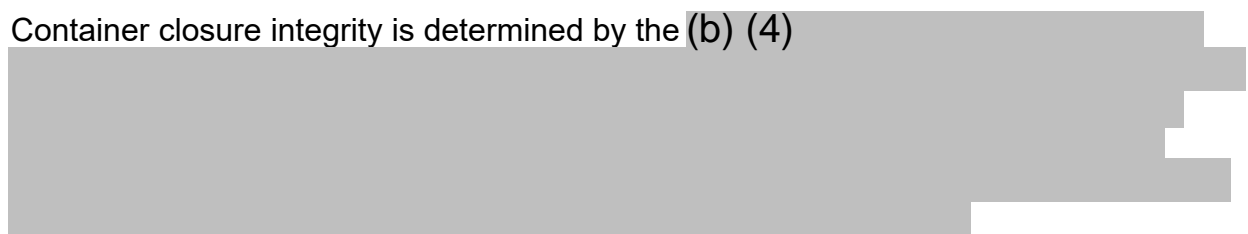
(b) (4)



A risk assessment demonstrated that none of the compounds would raise safety concerns at the measured concentrations.

#### Container Closure Integrity

Container closure integrity is determined by the (b) (4)



#### Container Closure System for Water for Injection

The primary container closure system of sWFI is comprised of a syringe barrel (1.5 mL Type<sup>(b) (4)</sup> borosilicate glass), a (b) (4) tamper-evident closure, and a rubber stopper (Bromobutyl rubber, type (b) (4)).

The glass (type<sup>(b) (4)</sup>) of the syringes complies with (b) (4). Syringes are manufactured by (b) (4). Tests performed on syringes are the following: certificate checks, visual inspections, and dimensional checks.


The rubber stopper meets (b) (4) compendial requirements. Tests on the latex free stopper are certificate checks, visual inspections, chemical identity, and dimensional checks.

The (b) (4) closure consists of a tamper-evident seal, a Luer lock, and a rubber tip cap. The bromobutyl rubber (type<sup>(b) (4)</sup>) tip cap complies with (b) (4). Tests for the cap are certificate checks, visual inspections, chemical identity, and dimensional checks. The tamper-evident seal and Luer lock are secondary packaging parts of the (b) (4) Closure, and do not make contact with the product.

#### Extractables and Leachables Assessment

The sponsor conducted an extractables and leachables risk assessment on the primary packaging components with direct product contact. Extractable screening testing was

first conducted on the components that directly contact product to identify and provide estimation of any potential leachable compounds/elements under exaggerated conditions. The following (b) (4)



#### Container Closure Integrity

Container closure integrity is determined by (b) (4) test method. The method was validated at (b) (4). Container closure integrity has been demonstrated through the PPQ and the stability testing program.

#### 3.2.P.2.5 Microbiological Attributes

The chikungunya lyophilized DP is supplied as a sterile product, which is confirmed by results of sterility, bacterial endotoxins, and package integrity testing. The DP (b) (4) is sterile filtered prior to filling. The aseptic filling process and the final sterile filtration were validated. The integrity of the container/closure system was verified on the (b) (4) PPQ batches and (b) (4) clinical lots 2005040029 (CTM3) and 2106090052 (CTM5).

Manufacturing of the sWFI PFS consists of (b) (4) steps prior to filling of the WFI, followed by (b) (4) step. Sterility and bacterial endotoxins testing are performed at release and during shelf life of the sWFI PFS in accordance with (b) (4). Container closure integrity was demonstrated through the process validation study and the stability programs.

#### 3.2.P.2.6 Compatibility

The DP is a lyophilized product supplied together with a solvent for reconstitution. The solvent is 0.5 mL of sterile WFI. The lyophilized product should be soluble within 1 minute in water for injection. The compatibility between the DP and its corresponding solvent for reconstitution is also monitored as part of the stability program. The

compatibility of the DP with its container closure system was demonstrated through the evaluation of stability studies and extractables and leachables studies.

#### **Overall Reviewer's Assessment of Section 3.2.P.2:**

- On February 01, 2023, the following IR was sent to the sponsor:

In the description and composition of Drug Product section 3.2.P.1, you state that the pharmaceutical form is Powder and Solvent for Solution for Injection (0.5mL). In the Description and Composition of the DP, you state that sterile water for injection (sWFI) is filled in a (b) (4) syringe format (page 4/4 in Section 3.2.P.1 sWFI). However, the minimum extractable volume is (b) (4) for the (b) (4) syringe and (b) (4) for the 1.5 mL syringe (Section 3.2.P.5.1). Please confirm if the sterile water for injection (sWFI) is filled in a (b) (4) syringe format or a 1.5 mL syringe format.

On February 17, 2023, the sponsor confirmed that the 1.5 mL syringe format with an extractable volume of 0.5 mL is used for the reconstitution and subsequent administration of the vaccine. The response is acceptable.

- The information provided is acceptable. Please also refer to the review memo from the device reviewer, Andrea Gray.

### **3.2.P.3 Manufacture**

#### **3.2.P.3.1 Manufacturer(s)**

The chikungunya VLA1553 DP is manufactured, filled, packaged, inspected, and tested at the sites indicated in Table 9 (derived from Table 3.2.P.3.1-1, Section 3.2.P.3.1 Manufacturer(s)).

**Table 9: DP Manufacturing and Testing Sites**

Site Name	Site Address	Specific Manufacturing Responsibilities or Type of Testing
(b) (4)	(b) (4)	<b>Manufacture</b> DP (Formulation, Aseptic Filling and Lyophilization) <b>DP Bulk</b> In-process testing: Bioburden and Sterility, <b>DP Lyophilized</b> In process testing: Visual inspection Release testing: Residual Moisture and Sterility
Valneva (b) (4)	(b) (4)	<b>DP Bulk (In-process Testing)</b> TCID <sub>50</sub> <b>DP Lyophilized Release Testing</b> Appearance; (b) (4) Identity; TCID <sub>50</sub> ; Recombinant Human Serum Albumin content (rHA); Sucrose content; D-Sorbitol content; L-Methionine content; Residual Moisture (stability only); Bacterial Endotoxin
(b) (4)	(b) (4)	<b>DP Lyophilized (Release Testing)</b> (b) (4)

Site Name	Site Address	Specific Manufacturing Responsibilities or Type of Testing
(b) (4)	(b) (4)	
(b) (4)	(b) (4)	<b>Final Packaged Lot</b> Final assembly of components and Secondary Packaging
(b) (4)	(b) (4)	<b>Storage</b> DP Lyophilized, Final Packaged Lot, Sterile Water for Injection
(b) (4)	(b) (4)	<b>Storage</b> DP Lyophilized, Final Packaged Lot, Sterile Water for Injection

The sWFI prefilled syringes (PFS) are manufactured and tested at (b) (4). The address, responsibilities and registration reference of each site are listed in Table 10 (derived from Table 3.2.P.3.1-1, Section 3.2.P.3.1 Manufacturer of the BLA).

**Table 10: Manufacturing Sites of sWFI PFS**

Site name and address	Responsibilities
(b) (4)	Manufacture Visual inspection Quality control testing
(b) (4)	Quality control testing
(b) (4)	Visual inspection Quality control testing
(b) (4)	Visual inspection Quality control testing Storage

### 3.2.P.3.2 Batch Formula

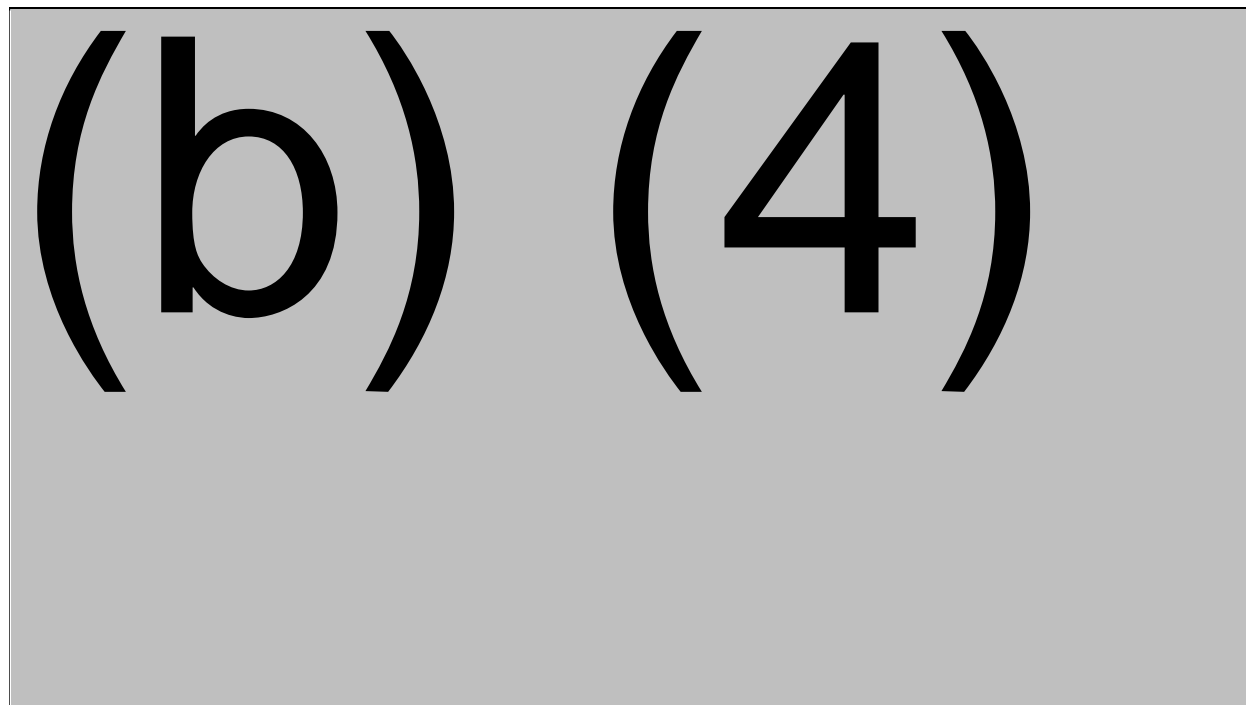
(b) (4)

(b) (4)

(b) (4)







**3.2.P.4 Control of Excipients**  
**3.2.P.4.1 Specifications**

The excipients used in the manufacture of VLA1553 DP are listed in Section 3.2.P.3.1.2 of this memo. The excipients are accepted by Valneva based on a certificate of analysis from a qualified supplier. All materials are tested by Valneva and/or qualified laboratories as part of VLA1553's release process.

The specifications for rHA, di-potassium hydrogen phosphate, potassium di-hydrogen phosphate, trisodium citrate dihydrate, sucrose (saccharose), magnesium chloride hexahydrate, D-sorbitol and L-methionine are based on (b) (4)

[Redacted]

(b) (4)

[Redacted]

[Redacted]

**3.2.P.4.4 Justification of Specifications**

All excipients are tested in compliance with the current (b) (4) monographs.

**3.2.P.4.5 Excipients of Human or Animal Origin**

VLA1553 DP does not contain excipients of human or animal origin.

**3.2.P.4.6 Novel Excipient**

No novel excipients are used for the formulation of VLA1553 vaccine.

**Overall Reviewer's Assessment of Section 3.2.P.4:**

- ☐ The information provided is acceptable.

**3.2.P.5 Control of Drug Product****3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)**

The release and stability tests and specifications for the lyophilized DP are summarized in Table 12 (derived from Table 3.2.P.5.1-1 of the BLA).

**Table 12: Release Test Methods and Specification for Lyophilized DP**

Test	Method	Release Specification	Stability Specification
Appearance and Solubility	Visual control (Lyophilized product)	White to slightly yellowish homogeneous cake with no visible particles or significant cracks	White to slightly yellowish homogeneous cake with no visible particles or significant cracks
Appearance and Solubility	Solubility (Lyophilized product)	Soluble within 1 min upon reconstitution	Soluble within 1 min upon reconstitution
Appearance and Solubility	Visual control (Dissolved product)	Clear colorless to slightly yellowish solution	Clear colorless to slightly yellowish solution
(b) (4)	(b) (4)	(b) (4)	(b) (4)
Extractable volume	(b) (4)	(b) (4)	N/A
(b) (4)	(b) (4)	(b) (4)	N/A
Identity	(b) (4)	(b) (4)	N/A
Infectious virus concentration	TCID <sub>50</sub>	(b) (4)	≥ 3.0 log <sub>10</sub> TCID <sub>50</sub> /0.5 mL (End of shelf-life)
Recombinant Human Albumin (rHA) Content	(b) (4)	(b) (4)	N/A
Sucrose Content	(b) (4)	(b) (4)	N/A
D-Sorbitol Content	(b) (4)	(b) (4)	N/A
L-Methionine Content	(b) (4)	(b) (4)	N/A

Test	Method	Release Specification	Stability Specification
Residual Moisture	(b) (4)	(b) (4)	(b) (4)
Bacterial Endotoxins	(b) (4)	(b) (4)	(b) (4)
Sterility	(b) (4)	Conforms, no microbial growth	Conforms, no microbial growth
Container Closure Integrity Testing (CCIT)	(b) (4)	N/A	Container closure integrity maintained

### Justification of Specifications

All specifications were appropriately justified.

Infectious Virus Concentration (Potency) is determined by a TCID<sub>50</sub> assay. (b) (4)

[REDACTED]

The tests and specifications applied for release and stability testing of the Sterile Water for Injection are provided in Table 13 (derived from Table 3.2.P.5.1-1 of the BLA). All tests were (b) (4) except for the CCIT, which was by the (b) (4) SOP.

**Table 13: Product Specifications for Release and Stability Testing**

Test Parameter	Specification
Appearance of solution	Clear, colorless, odorless liquid, essentially free from visible particulates
	Clear and colorless solution, practically free from visible particles

(b) (4)

#### Justification of Specifications for sWFI

The specifications and the acceptance criteria are in accordance with (b) (4)

#### Overall Reviewer's Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6:

- The following IR was sent to the sponsor on February 01, 2023:

Regarding drug product stability, in Section 3.2.P.8.1, we note that the proposed stability acceptance criterion for potency (Infectious virus concentration) is  $\geq 3.0 \log_{10} \text{TCID}_{50}/0.5 \text{ mL}$ , which is based on the End of shelf-life (EOSL) limit.

Please provide an explanation and justification on how the EOSL was established and submit data to support your requested specification. The data currently submitted supports a shelf life of 12 months with a stability acceptance criterion of (b) (4) /0.5 mL.

On February 17, the sponsor submitted responses in STN 125777/0.11. According to the sponsor, their clinical data demonstrated that ranges of (b) (4)  $\text{TCID}_{50}$  at timepoint of injection (Day 0) induced comparable immune responses. The minimal clinical dose proven to be effective is  $3.0 \log_{10} \text{TCID}_{50}$ , which is set as the end of shelf-life specification. Thus, the proposed end of shelf-life specification for potency is  $3.0 \log_{10} \text{TCID}_{50}$ .

However, in the Phase 1 CSR, the sponsor stated that the low dose used in Phase 1 is (b) (4)  $\text{TCID}_{50}$ , medium dose is (b) (4)  $\text{TCID}_{50}$ , and high dose is (b) (4)  $\text{TCID}_{50}$ . The following IR was sent to the sponsor on February 24, 2023:

Regarding your response to CBER Comment 1, in Section 3.2.P.5.6.6, you state that in the Phase 1 study, the applied dose at timepoint of injection was (b) (4)  $\text{TCID}_{50}/\text{dose}$  for the low dose group. However, in the Phase 1 Clinical Study Report in Section 5.3.5.1, you state that the dose used in the low dose arm is (b) (4)  $\text{TCID}_{50}/\text{dose}$ , which is equivalent to (b) (4)  $\text{TCID}_{50}/\text{dose}$ . Please explain this discrepancy.

On March 10, 2023, the sponsor provided the response in STN 125777/0.18. The sponsor clarified that the doses stated in the Phase 1 Clinical Study Protocol and Clinical Study Report represent the target values for the target values for Drug Product Bulk Formulation. In the Phase 1 study, batch (b) (4) was used for the high dose group, batch (b) (4) was used for the low dose and medium dose groups. The first vaccination in the Phase 1 (Day 0, all groups) occurred in March / April 2018 (20 sentinels) and June 2018 (100 subjects). Based on the stability data summarized in Table 14 (derived from Table 1, Section 1.11.1 under 125777/0.18), the actual dose at the time of vaccination was (b) (4)  $\text{TCID}_{50}$  for the low dose group, (b) (4)  $\text{TCID}_{50}$  for the medium dose group, and (b) (4)  $\text{TCID}_{50}$  for the high dose group.

(b) (4)

The response is acceptable.  
☐ The information provided is acceptable.

(b) (4)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

(b) (4)

(b) (4)

[REDACTED]

1 page determined to be not releasable: (b)(4)

### 3.2.P.8 Stability

#### 3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data

The proposed shelf-life and storage conditions for the DP are 24 months at  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ . In support of this claim, (b) (4) lyophilized clinical lots: Lot 2005040029 used in the Phase 3 Clinical Trial VLA1553-301, and the (b) (4) PPQ Lots (b) (4) used in the lot-to-lot Consistency Trial VLA1553-302 were placed on primary stability studies.

Primary stability studies include (b) (4) studies: (b) (4)

Stability Results at  $5 \pm 3^{\circ}\text{C}$ : For the long-term storage condition study, the following characteristics were determined at regular intervals over the course of (b) (4) months: appearance of the freeze-dried product, appearance after dissolution, dissolution time, (b) (4) infectious virus concentration ( $\text{TCID}_{50}$ ), residual moisture, bacterial endotoxins, and sterility. Data up to (b) (4) months were provided. All results met the pre-defined acceptance criteria in place at the time of testing, supporting the proposed shelf life of 24 months at  $5 \pm 3^{\circ}\text{C}$ . Although potency trended downwards over (b) (4) months, the values were within the acceptance criteria.

Stability Results at (b) (4) For this study, the following characteristics were determined over the course of (b) (4) months: appearance of the freeze-dried product, appearance after dissolution, dissolution time, (b) (4) infectious virus concentration ( $\text{TCID}_{50}$ ), residual moisture, bacterial endotoxins, and sterility. Under accelerated conditions of (b) (4), the  $\text{TCID}_{50}$  titers decreased significantly within (b) (4), remaining within specification for only the first month.

Stability Results at (b) (4) For this study, the following characteristics were determined over the course of (b) (4) weeks: appearance of the freeze-dried product, appearance after dissolution, dissolution time, (b) (4) infectious virus concentration ( $\text{TCID}_{50}$ ), residual moisture, bacterial endotoxins, and sterility. When stored at (b) (4) infectious virus concentration has shown a significant decreasing trend, being out of specification after (b) (4).

Stability data for Cumulative Stability are being generated. In this study, Lots (b) (4) were stored at (b) (4) prior to the start of the stability study described above for the primary stability data. Data up to (b) (4) months were provided for Lot (b) (4) and up to (b) (4) months for Lot (b) (4) of storage at  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ . All results met the pre-defined acceptance criteria.

Supportive stability studies include stability data of the VLA1553 (b) (4) DP used in the Phase 1 clinical study (Lot H17-4001 High virus titer, Lot H17-4002 Medium virus titer and Lot H18-9000 High virus titer) under normal (b) (4) and accelerated ( $+5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ ) conditions. Data was provided for storage at (b) (4) for Lots H17-4001, H17-4002, H18-9000 for up to (b) (4) months, up to (b) (4) months, and up to 18 months, respectively. All test results met specifications. The accelerated stability data at





below the accepted titer if it is stored at (b) (4) after reconstitution. We issued two IRs to the sponsor regarding the deficiency. This issue was resolved. Please see Reviewer's Assessment of the Labeling Review section for details.

- On June 16, 2023, the following IR was sent to the sponsor:

We note in Section 3.2.P.8.2.2 Stability Commitment, it was mentioned that "Additionally, a commitment is made to promptly report any stability data outside of the approved specifications at the recommended storage condition within the proposed shelf life of 24 months, which may raise issues on the safety and efficacy of the Drug Product." Please note that according to 21 CFR 600.14, a Biologic Product Deviation Report (BPDR) must be submitted to the Agency for any stability test results that are outside of the approved specifications.

On June 21, 2023, the sponsor provided the response in STN 125777/0.54. The sponsor confirmed that a BPDR will be submitted to the agency for any stability test results that are outside of the approved specifications at the recommended storage condition, within the proposed shelf life of 24 months, for product distributed in the US. The response is acceptable.

- On September 07, 2023, the following IR was sent to the sponsor:

Please define the "date of manufacture" of the Lyophilized Antigen Component of Chikungunya Vaccine, Live.

The sponsor responded on September 12, 2023 under STN 125777/0.85. The "date of manufacture" of the Lyophilized Antigen Component of Chikungunya Vaccine, Live is defined as the date of unloading of the lyophilized vials from the freeze-dryer unit. The response is acceptable.

- The information provided is acceptable. Deficiencies were identified and were resolved.

### 3.2.A APPENDICES

#### 3.2.A.1 Facilities and Equipment

Please refer to DMPQ reviewer's memo for review information pertaining to this section.


#### Overall Reviewer's Assessment of Section 3.2.A.1:

- Facilities and Equipment was reviewed by DMPQ. Onsite Inspection was waived.

#### 3.2.A.2 Adventitious Agents Safety Evaluation

Please refer to Section 3.2.S.2.3 of this memo for assessment of materials of biological origin in DS and for the adventitious agent's safety evaluation of the cell lines and virus seed lots used to produce the bulk DS. No excipients from human or animal origin are used for the formulation of the DP. No raw material of human origin has been used in any manufacturing stage.

(b) (4)



### **Viral Clearance Studies**

No viral clearance studies were performed as the vaccine is a live virus vaccine.

#### **Overall Reviewer's Assessment of Section 3.2.A.2:**

- ☐ The information provided is acceptable.

### **3.2.A.3 Novel Excipients**

No novel excipients are used for formulation of the vaccine.

### **3.2.R Regional Information (USA)**

#### **Executed Batch Records**

Executed batch records (BR) provided in the BLA submission include: Production Records for DS batch (b) (4), Production Records for DP batch (b) (4), and Production Records for sWFI batch (b) (4).

### **Method Validation Package**

Method validation protocols and validation reports were reviewed and discussed in Sections 3.2.S.4.2 and 3.2.S.4.3 for DS and Sections 3.2.P.5.2 and 3.2.P.5.3 for DP.


### **Combination Products**

The chikungunya vaccine candidate kit contains one single-use (b) (4) vial with the lyophilized DP and one prefilled syringe containing 0.5 mL sterile water for injection for reconstitution and administration of the reconstituted vaccine. Per FDA Guidance for Industry and FDA Staff "Current Good Manufacturing Practice Requirements for Combination Products", the kit is considered as a combination product. The pre-filled syringe is reviewed by Andrea Gray.

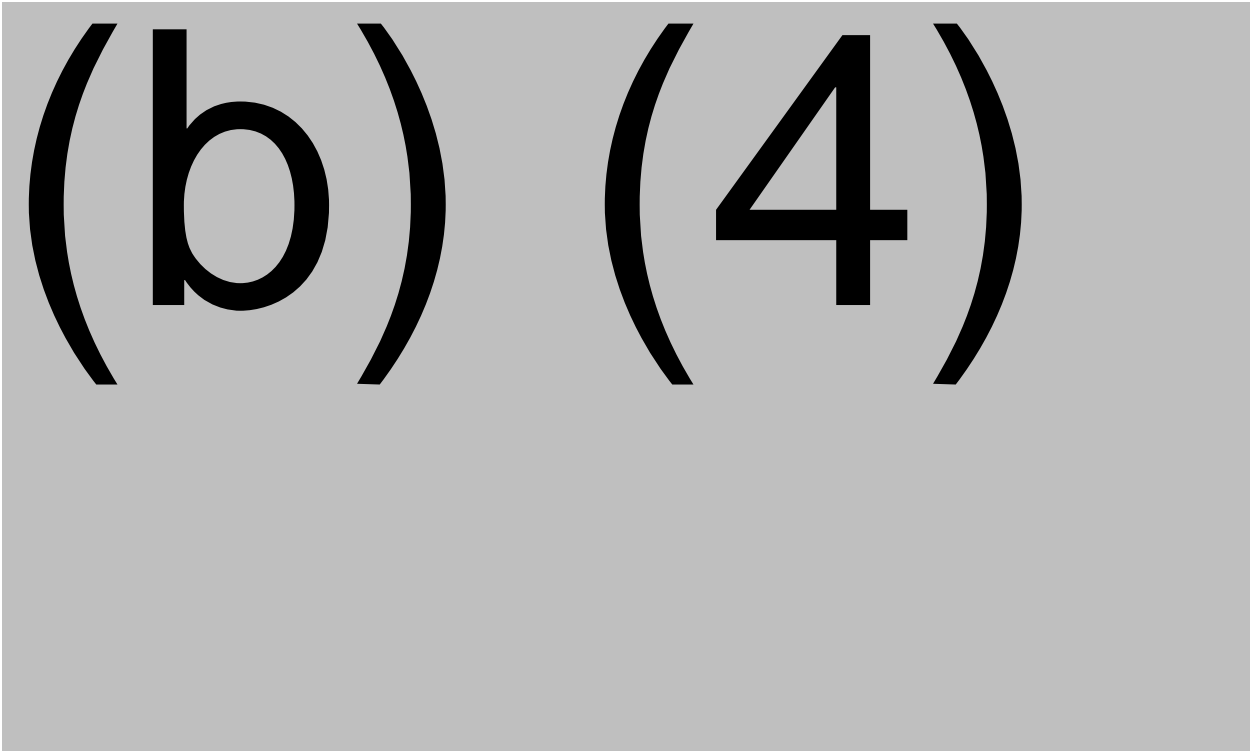
## Comparability Protocols

Valneva submitted (b) (4) comparability protocols:

(b) (4)



(b) (4)



## Module 1

### A. Environmental Assessment or Claim of Categorical Exclusion

Valneva claimed a categorical exclusion from the requirement to prepare an environmental assessment based on 21 CFR 25.31(c). To the applicant's knowledge, no extraordinary circumstances exist that would require the preparation of an environmental assessment. The claim for categorical exclusion is acceptable.

## **B. Reference Product Designation Request**

## **C. Labeling Review**

### **Full Prescribing Information (PI):**

We reviewed and commented on the product-related sections of the PI listed below. Please see the approved PI for information on the following sections:

- Section 2: Dosage and Administration
- Section 3: Dosage Forms and Strengths
- Section 11: Description
- Section 12: Clinical Pharmacology
- Section 13: Nonclinical Toxicology
- Section 16: How Supplied/Storage and Handling

### **Reviewer's Assessment:**

On May 08, 2023, the following IR was sent to the sponsor:

In Section 16.2 (Storage and Handling) of the Prescribing Information (Section 1.14 Labeling), you state that reconstituted IXCHIQ, if not used immediately, (b) (4)

- (b) (4)
- a. Please provide supporting data showing reconstituted IXCHIQ can be (b) (4)
  - b. We note that the potency for lots (b) (4)  
(b) (4) (Section 3.2.P.8.3.6 In-use Stability Data). The proposed end-of-shelf life potency for DP is 3.0 log<sub>10</sub> TCID<sub>50</sub>. If a lot with a potency of 3.0 log<sub>10</sub> TCID<sub>50</sub> is reconstituted and (b) (4), the potency may decline beyond the acceptable specification. Please justify the storage conditions and hold times of reconstituted DP specified in the PI.

On May 17, 2023, the sponsor provided a justification for the storage conditions and hold times of reconstituted DP to Amendment 125777/0.41. We did not agree with Valneva's assessment, a follow-up IR was sent to the sponsor on May 31, 2023:

We have reviewed your responses to our May 8, 2023 Information Request submitted under amendment 41 to BLA 125777/0 on May 17, 2023, and have the following comments:

Regarding your response to CBER Comment 3, please note that the storage conditions and hold times of reconstituted Drug Product (DP) specified in the PI should be supported by in-use stability data. Therefore,

- a. If product will be stored at 2°C to 8°C (b) (4), please perform in-use stability studies to support product quality under these conditions.
- b. Based on review of the submitted data related to product storage at (b) (4), we still have concerns regarding the stability of your vaccine. Please consider changing the in use instructions to administer the vaccine immediately after reconstitution.

On June 6, 2023, the sponsor responded (Amendment 125777/0.47) that they will revise the PI sections 2.3 and 16.2 to state that the reconstituted vaccine must be used immediately upon reconstitution. The response is acceptable.

## Modules 4 and 5

### Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints

#### Module 4 Nonclinical Studies

##### 4.2.1 Pharmacology

##### 4.2.1.1 Primary Pharmacodynamics

Many aspects of the preclinical evaluation of the vaccine virus have been studied in academic and industry laboratories in a variety of animal models. Of note, a VLA1553 vaccine challenge study in NHPs was published in Roques et al., JCI Insight, 2017, 2(6): e83527, and was not submitted to the BLA. Table 15 summarizes the nonclinical studies submitted to the BLA.

**Table 15 Nonclinical Studies**

Report Number	Animal Used	Study Objective
PHY1802-02	NHPs	To evaluate the risk of viral persistence after vaccination with VLA1553
VAC1816-02	NHPs	To establish a threshold titer for protection after vaccination with VLA1553
RR-0065-01	Mice	To assess the antibody quality in terms of protective neutralizing antibody titer induced by VLA1553 in individual human subjects
RR-0066-01	Mosquitoes, Mice	To evaluate the risk of transmission of VLA1553 to non-vaccinees by mosquitoes
RR-0048-02	Humans	To demonstrate the genetic stability of the attenuated virus in vivo following administration to humans

**Report PHY1802-02: Study of persistence of CHIKV attenuated vaccine VLA1553 infection in NHPs (“Valneva II”)**

Report PHY1802-02 contains a summary of the non-clinical study to assess the risk of persistent infection in non-human primates (NHPs) after immunization with VLA1553. There is mounting evidence for persistent CHIKV infection in humans and non-human primates. Since VLA1553 contains live attenuated CHIKV with reduced replication capacity, there is a theoretical risk that the vaccine causes persistent infection in the vaccinees. In the pre-IND meeting (PS 003183) of March 15, 2017 and after reviewing of IND 17854 Am 0, CBER issued IRs to the sponsor requesting assessment of the absence of persistent infection after immunization with VLA1553.

In this study, Valneva evaluated the risk for viral persistence after vaccination with VLA1553 in comparison to WT CHIKV over a period of 90 days. This work was carried out at (b) (4). The study was performed based on the study protocol PHY1802-SP submitted in IND 17854 Amendment 6. This protocol was reviewed by the review team and was found to be acceptable.

**Study Approach***Animal and Groups*

Sixteen CHIKV-naive young-adult female (b) (4) macaques were selected based on age and weight. Twelve animals received  $3.2 \times 10^6$  TCID<sub>50</sub> VLA1553 in 0.1 mL intramuscularly (i.m.) (Group A), and four received wild type (WT) CHIKV LR2006-OPY1 at the same dose and via the same route (Group B). The dose of VLA1553 is 1 log higher than the highest dose ( $3.2 \times 10^5$  TCID<sub>50</sub>) used in the Phase 1 clinical trial and 2 logs higher than the dose ( $3.2 \times 10^4$  TCID<sub>50</sub>) used in the Phase 3 study and proposed for licensure. VLA1553 exposed animals (2 per time point) were euthanized on days 2, 6, 14, 28, 60 and 90 post-exposure, and WT CHIKV exposed animals (2 per time point) were euthanized on days 6 and 90.

*CHIKV Quantification in Plasma and other NHP Body Fluids*

Total CHIKV RNA in NHP plasma, saliva, vaginal fluid, cerebrospinal fluid (CSF), synovial fluid and urine was quantified using two CHIKV-specific (b) (4) assays: (b) (4) RNA extraction and (b) (4) (lower limit of quantification (LLOQ) = 5000 copies/mL), and (b) (4) RNA extraction and (b) (4) (LLOQ = 500 copies/mL). The Limit of Detection (LOD) was calculated as 720 copies/mL for the (b) (4) RNA extraction and (b) (4) and 60 copies/mL for the (b) (4) extraction and (b) (4). The same assays were used for detection of both WT CHIKV and VLA1553 RNA. Both (b) (4) methods were validated, and the validation reports were submitted in Section 4.2.2.1 of the BLA and reviewed below.

*CHIKV Quantification in Tissue Samples*

A relative (b) (4) assay was used to determine the viral levels in selected tissues. The CHIKV RNA was quantified by the (b) (4) assay describe above. GAPDH was

used as an internal control. The assay values were reported as the ratio of CHIKV RNA to cellular GAPDH RNA and were calculated using the following equation:

(b) (4)

LOD depends on the amount of GAPDH RNA within the sample and on the quantity of CHIKV RNA (b) (4). Therefore, the LOD varies from one tissue to another, from  $1 \times 10^{-6}$  for liver to  $6 \times 10^{-5}$  for joints.

The quantification of replicating WT CHIKV and VLA1553 was performed on selected tissues using a (b) (4)

## Results

Plasma viremia (viral RNA) in NHPs receiving VLA1553 was observed from 6 hours to 9 days post exposure (dpe). At day 9, VLA1553 RNA was detected in 8 samples using the (b) (4) RNA extraction and (b) (4) – viral titers for animals CFF008, CFF013 were 450 copies/mL and 321 copies/mL, respectively, which were below the LLOQ (500 copies/mL) but above the LOD (60 copies/mL). Another 6 samples tested at day 9 were below the LOD. The peak viremia RNA titers in NHPs receiving VLA1553 ( $2.7 \times 10^4$ – $6.8 \times 10^7$  copies/mL) were approximately 3 logs lower than for NHPs receiving the WT CHIKV ( $9.2 \times 10^7$ – $4.5 \times 10^9$  copies/mL).

VLA1553 RNA was detected in saliva at days 2 and 4, as well as in vaginal fluid at 2 and 6 dpe. No CHIKV RNA was detected in cerebrospinal fluid, synovial fluid, and urine of the animals, regardless of whether they were exposed to wild type CHIKV or VLA1553.

The sponsor assessed the dissemination of CHIKV in spleen, liver, lymph nodes, arm and hand/finger muscles, brain, knee and elbow capsule, skin, cervix and vagina. VLA1553 RNA was detected at 90 dpe in spleen (2/2 animals) and axillary lymph node (1/2 animals); up to 60 dpe from inguinal lymph node; 28 dpe in liver, knee capsule and skin contra-lateral site; up to 14 dpe in nasopharyngeal lymph node, digiti abductor, skin injection site and cervix; and 6 dpe in extensor digitalis communis. In most examined tissues, viral RNA titers of VLA1553 were approximately 2-3 logs lower than for wild-type CHIKV. No VLA1553 RNA was found among the vaccinees in biceps brachii, flexor carpi ulnaris, elbow capsule and vagina, whereas among the WT CHIKV animals RNA was detected in these tissues. Importantly, VLA1553 RNA was not detected in any brain tissue (encephalon, mesencephalon, cerebellum, plexus choroid), whereas WT CHIKV RNA was detected in the choroid plexus at 6 dpe at low titers.

In two animals, a TCID<sub>50</sub> assay in selected tissues showed replicating WT CHIKV at 6 dpe in the spleen and digiti abductor, and in the axillary lymph node of one animal. In contrast, replicating VLA1553 was detected in spleen of one animal on day 2 but not on days 6 and 14. Tests for replicating virus were not performed on any tissue past day 14.



**Reviewer comment:** We had multiple discussions within DVP regarding this study. On September 08, 2022, DVP shared the following information with the review team:

“We would like to share some data presented in Valneva's study of persistence of CHIKV attenuated vaccine, VLA1553, infection in NHPs. Please note:

- Data reported show that vaccine virus RNA was detected in spleens (2/2 animals) and axillary lymph node (1/2 animals) at ~Day 90.
- Samples were not available at this time point to assess whether there is replicating virus in these tissues at day 90.
- Available study data show that there is no detectable viral RNA from other tissues beyond day 60 or from plasma beyond day 9.
- Replicating VLA1553 virus was detected in spleen on day 2 but not on days 6 and 14.
- Tests for replicating virus were not performed on any tissue past day 14.

We wanted the clinical team to be aware of these data and to see whether there have been any safety signals potentially associated with persistence of the vaccine virus.”

The clinical team did not report any safety signals associated with persistence of the vaccine virus.

#### **Report VAC1816-02: Passive Transfer in Non-Human Primates study report of VLA1553 vaccine virus**

While there are ongoing epidemic outbreaks of CHIKV in the Americas and India, case numbers in inter-epidemic periods remain too small for a reasonably sized efficacy trial to be designed and conducted. Consequently, for an unlicensed vaccine the establishment of a serological correlate of protection based on data derived from epidemiological studies and animal challenge studies is necessary to allow evaluation of vaccine effectiveness against CHIKV. To address this, Valneva passively transferred human sera obtained from subjects in the Phase 1 study to NHPs to protect against CHIKV challenge. The ability of human sera to prevent viral disease endpoints in NHPs was established as a correlate and provided evidence of vaccine efficacy.

Report VAC1816-02 contains a summary of the non-human primate passive transfer study to establish a threshold titer for protection after vaccination with VLA1553. The study was performed based on the study protocol submitted in IND 17854 Amendment 16. This protocol was reviewed and found to be acceptable.

#### **Study Approach**

Human sera obtained from the clinical study VLA1553-101 were transferred to NHPs (b) (4) prior to challenge with the wild type CHIKV strain LR2006-OPY1.

The following serum pools were prepared using: negative control VLA1553-101 day 0, high titer pool day 28 (HS d28), medium titer pool day 28 (MS d28), low titer pool day 28 (LS d28), ultra-low titer serum pool day 28 ( ULS d28), high titer pool day 14 (HS d14), medium high titer pool day 84 (MHS d84), medium titer pool day 180 (MS d180), and high titer pool day 180 (HS d180).

Forty-six CHIKV-naïve NHPs were used in 4 rounds of experiments. Rounds 1 and 2 consisted of two groups of five animals receiving VLA1553-specific serum and 2 animals receiving non-immune serum. Rounds 3 and 4 consisted of two groups of five animals receiving VLA1553-specific serum and 1 animal receiving non-immune serum.

The first two rounds were performed with day 28 serum (groups 2, 3, 5b and 6b), whereas the third round was performed with serum derived from day 14, and day 84 (groups 8 and 9, respectively) due to limited availability of day 28 serum. The fourth round of experiments was done using day 180 post-vaccination serum (groups 11 and 12) where the titer for transfer was defined based on the outcome of rounds 1 and 2.

NHPs received 3 mL of human serum per kg of weight the day before CHIKV challenge. All injections were performed in the saphenous vein.

Just prior to challenge, a blood sample was drawn from each animal for the determination of neutralizing anti-CHIKV antibody titers analyzed by a micro-plaque reduction neutralization test (μPRNT). This measured titer was used to determine the neutralizing antibody titer required to protect NHPs from CHIKV diseases. The μPRNT assay was validated and was carried out by (b) (4). The validation report was provided in Section 5.3.1.4 and is reviewed below.

NHPs were challenged with WT CHIKV LR2006-OPY1 subcutaneously with 100 AID50 (50% Animal Infectious Doses) corresponding to 7,000 - 10,000 PFU (plaque-forming units).

After challenge, the sponsor assessed safety parameters (clinical assessment), plasma viremia, signs of CHIKV infection and relevant hematological parameters. Plasma viremia (viral RNA) was quantified by two (b) (4) methods. Both methods were validated, and the validation reports were provided in Section 4.2.2.1 of this BLA and are reviewed below.

At the end of the study, NHPs were euthanized and blood as well as various organs were collected from all animals.

### *Definition of Protection*

The main readout for protection was defined as the measurement of viremia by (b) (4) in plasma. The sponsor proposed a viremia of ≤150 TCID<sub>50</sub>/mL as protected, which is equivalent to 30,000 CHIKV genome copies/mL using a conversion factor (RNA copies per mL to TCID<sub>50</sub>/mL) of 200 (see review of Report RR-0089-02:

“Determination of the conversion factor for the TCID<sub>50</sub>/mL to GCE/mL ratio for CHIKV LR2006-OPY1 used in the NHP passive transfer study VAC1816 Report” below).

## Results

### Neutralization Titer in Serum of NHPs Prior to Challenge

The µPRNT<sub>50</sub> titers were measured for each individual animal on day -1 or day -6 prior to administration of human serum, as well as after administration of human serum and prior to wild type CHIKV challenge on day 0. The results were presented in Table 5 of the report (page 32/73).

### Plasma Viremia

Following CHIKV LR2006-OPY1 challenge, viral titers in NHPs were measured using (b) (4) giving a readout in RNA copies/mL. The data generated were presented in Figure 3 and the raw data were included in Annex 5.

#### *In control animals:*

For all rounds 1-4, CHIKV RNA was detected as early as day 1 post-exposure, the peak of viral production was observed on day 2-3 post exposure.

#### *In human serum treated animals:*

In round 1, animals were treated with low (group 2) or medium titer sera (group 3). Viremia remained below 10,000 copies of RNA/mL and lasted only 2 to 3 days or less in both treated groups. The mean of produced virus (as quantified by the area under the curve) was more than 5 log<sub>10</sub> lower than in the control group. There was no significant difference between the two treated groups (Figure 3B, Round 1). Among all treated animals in all rounds, one single animal treated with low titer day 28 sera, #CD876, presented low level of viremia (4,085 copies/mL) on day 1 only. One single animal treated with medium titer day 28 serum, #BT914, showed no viremia at all.

In round 2, animals were treated with ultra-low (group 6b) or high titer sera (group 5b). Treatment with ultra-low titer serum (group 6b) resulted in a larger heterogeneity, longer duration (3 to 4 days) and in some animals, higher peaks (up to 3x10<sup>5</sup> RNA copies/mL) of plasma viremia. Treatment with high titer serum (group 5b) resulted in no virus detection at all in 4 out of 5 animals. The level of viremia observed in animal DB322, was 70 RNA copies/mL.

In rounds 3 and 4, animals received sera from day 14 (group 8, round 3) and day 84 (group 9, round 3) or day 180 post-vaccination (groups 11 and 12 in round 4) with targeted intermediate titer between the high and medium sera from d28. Viremia in all these animals lasted for 2-3 days with a highest peak viremia of 1.3x 10<sup>5</sup> RNA copies/mL observed. In all animals, viral RNA copies were largely decreased more than 4 logs compared to control animals.

The sponsor converted the peak viremia data for all animals as measured by (b) (4) (RNA copies /mL) to TCID<sub>50</sub> values (viral particles in TCID<sub>50</sub>/mL) using a conversion factor of 200, which was based on previously published observations (Roques et al., JCI Insight, 2017, 2(6): e83527). The converted values were listed in Table 5 of the report.

Based on the converted TCID<sub>50</sub> values and corresponding  $\mu$ PRNT<sub>50</sub> titers, a cut-off PRNT<sub>50</sub> titer of  $\geq 50$  was determined as the minimum titer at which  $\geq 90\%$  of the treated NHPs had a viremia of  $\leq 150$  TCID<sub>50</sub>/mL and were considered protected.

**Reviewer comment:** After reviewing the information submitted to IND 17854 in support of Valneva's proposed correlate of protection of a  $\mu$ PRNT<sub>50</sub> titer of 1:50, CBER provided comments to the sponsor on March 16, 2021. CBER did not agree with the proposed correlate of protection of  $\mu$ PRNT<sub>50</sub> titer of 1:50, as CBER did not agree that the proposed threshold of TCID<sub>50</sub>  $\leq 150$  represents non-meaningful viremia. CBER recommended the complete prevention of viremia (0 genome copies/mL) as an appropriate, clinically meaningful endpoint for assessing vaccine effectiveness. Based on GCE=0 (TCID<sub>50</sub>=0), CBER considered a surrogate of protection titer of  $>150$  to be reasonably likely to predict protection in humans. Therefore, a surrogate of protection titer of  $>150$  is an acceptable endpoint to support accelerated approval of the vaccine. Valneva agreed with the CBER comments. A surrogate of protection with a  $\mu$ PRNT<sub>50</sub> titer of  $>150$  was applied to clinical evaluation of VLA1553 in Phase 3.

#### **Report RR-0089-02: Determination of the conversion factor for the TCID<sub>50</sub>/mL to GCE/mL ratio for CHIKV LR2006-OPY1 used in the NHP passive transfer study VAC1816 Report**

In the above passive transfer study in NHPs (Report VAC1816-02), the main readout for protection was defined as the measurement of viremia by (b) (4) in plasma in Genome Copy Equivalents (GCE/mL). The GCE/mL values not only reflect live, replicating CHIKV, but also non-viable viral particles.

In the passive transfer study, the sponsor proposed a viremia of  $\leq 150$  TCID<sub>50</sub>/mL as protective, which is equivalent to 30,000 CHIKV genome copies/mL using a conversion factor of 200. This conversion factor was based on previous published observations (Roques et al., JCI Insight, 2017, 2(6): e83527).

In this study, the sponsor performed the following two experiments to confirm the conversion factor of 200 in the matrix of NHP plasma:

##### **Experiment 1:**

Analysis of the ratio between GCE/mL and TCID<sub>50</sub>/mL for plasma from healthy (b) (4) macaques spiked with WT CHIKV LR2006-OPY1.

CHIKV-negative plasma from healthy, (b) (4) macaques was spiked with WT CHIKV LR2006-OPY1 ( $5.7 \times 10^{10}$  GCE/mL) to obtain  $1 \times 10^9$ ,  $3.16 \times 10^8$ ,  $1 \times 10^8$ ;  $3.16 \times 10^7$ ,  $1 \times 10^7$ ,  $1 \times 10^6$ ,  $1 \times 10^5$ ;  $1 \times 10^4$ , and  $1 \times 10^3$  GCE/mL.

The amount of CHIKV RNA (GCE/mL) was quantified by classical RNA extraction and (b) (4). The TCID<sub>50</sub> titer was determined using a conventional Vero cell-based TCID<sub>50</sub> assay.

NHP plasma spiked with WT CHIKV LR2006-OPY1 showed a mean conversion factor of 323.

## Experiment 2:

In the 2<sup>nd</sup> set of experiments, the sponsor assessed the conversion factor for the NHP plasma samples from the passive transfer study VAC1816.

Twelve plasma samples derived from group 1 and group 2 control animals from study VAC1816 were selected for the determination of the TCID<sub>50</sub> titer. The GCE/mL titers of the samples were measured by (b) (4). The TCID<sub>50</sub> titer and GCE/mL titers were presented in Table 5 of the report, and 8 out of 12 samples returned TCID<sub>50</sub> values.

The conversion factors for the viral RNA copy number to TCID<sub>50</sub> were calculated as ranging from 183 to 1660, and only animal BT337 at day 2 showed a conversion factor of 183, which is less than 200. The sponsor calculated an average conversion factor of 436 for day 2 samples and 1172 for day 3 samples. A mean ratio of ~798 was calculated from all 8 conversion factors listed in Table 5.

**Reviewer comment:** Per the CBER comments of March 16, 2021 mentioned above, CBER did not agree that a threshold of TCID<sub>50</sub> < 150 represents non-meaningful viremia and recommended the complete prevention of viremia (0 genome copies/mL) as an appropriate, clinically meaningful endpoint for assessing vaccine effectiveness. Valneva agreed with the CBER request. Therefore, the conversion factor was not used for the final determination of the surrogate of protection.

## **Report RR-0065-01: Setup of a passive protection model in immunosuppressed (b) (4) mice and analyses of individual human sera from Phase I clinical study VLA1553-101**

In Valneva's passive transfer studies in NHPs (Report VAC1816-02), it was not possible to use individual human sera due to the high amount of serum required for transfer, but instead only pooled human sera from subjects from Phase 1 were used. This report (Report Number: RR-0065-01) summarized the passive transfer experiments in mice to assess person-to-person differences regarding antibody quality within a range of similar neutralizing antibody titers and their capacity to protect from CHIKV challenge.

This work was carried out by the PVD and QCIV departments of Valneva. This report contained the following two parts:

### **Part 1: Development of a passive transfer mouse model for CHIKV:**

Since Valneva's laboratories are not suitable for work with BSL3 pathogens such as wild type CHIKV, the sponsor developed a mouse model using the vaccine candidate VLA1553 as a challenge strain. The readout for protection from VLA1553 challenge after passive transfer of VLA1553-specific serum is survival.

For this passive transfer mouse model, wild type (b) (4) mice received 200  $\mu$ L human sera via intraperitoneal (i.p) route. One day later, 1 mg of mAb MAR1-5A3 for immunosuppression was administered also via i.p. and mice were then challenged 2 days after serum transfer with  $1 \times 10^4$  of VLA1553 (DS lot B3007460) subcutaneously (s.c). Mice were monitored for 14 days post-challenge for survival. In the control group, all animals succumbed within 14 days post-challenge.

## **Part 2: Passive transfer of individual human sera from VLA1553-101:**

Ten individual human sera obtained at 12 months post-VLA1553 vaccination were assessed in the passive protection mouse model. Two experiments were performed (5 serum samples per mouse experiment):

### **Experiment 1:**

Five individual human serum samples (VLA1553-101 1553(b) (6) ; VLA1553-101 1553(b) (6) ; VLA1553-101 1553(b) (6) ; VLA1553-101 1553(b) (6) and VLA1553-101 1553(b) (6) with a  $\mu$ NT<sub>50</sub> titer  $\sim$ 640 were selected. The samples were diluted 2-fold to reach a starting titer of 320 and tested in 2-fold serial dilutions with  $\mu$ NT<sub>50</sub> titers ranging from  $\sim$ 320 to  $\sim$ 10. Two days prior to challenge via the i.p route (b) (4) mice (5 animals per group) received 40  $\mu$ L of human immune serum with respective  $\mu$ NT<sub>50</sub> titers (plus 160  $\mu$ L non-immune serum to reach an injection volume of 200  $\mu$ L). One day prior to challenge, mice were immunosuppressed by administration of 1 mg MAR1-5A3 i.p. and on the next day they were challenged s.c. with  $1 \times 10^4$  TCID<sub>50</sub> of VLA1553. Mice were monitored for survival for 14 days after challenge. For the highest  $\mu$ NT<sub>50</sub> titer dose ( $\sim$ 320) transferred to mice, three out of five serum samples provided protection of all mice, while samples 1553(b) (6) and 1553(b) (6) conferred 80% and 60% protection from lethal challenge with VLA1553, respectively. The correlation between transferred  $\mu$ NT<sub>50</sub> and survival rate of mice were summarized in Figure 13. In all groups, decreasing  $\mu$ NT<sub>50</sub> titers correlated with decreased survival rates.

### **Experiment 2:**

As in the first experiment, mice receiving sera with a  $\mu$ NT<sub>50</sub> titer of 320 demonstrated 80% and 60% survival for 2 out of 5 serum samples. The second passive transfer experiment (5 mice per group) was performed with another 5 individual human sera with a  $\mu$ NT<sub>50</sub>  $\sim$ 1280. The 5 selected sera were 2-fold diluted prior to i.p. administration for the highest dose group ( $\mu$ NT<sub>50</sub> titers ranging from 640 to 10). All human sera with a  $\mu$ NT<sub>50</sub> of  $\sim$ 640 fully protected immunosuppressed mice from the lethal VLA1553 challenge.

The 50% Protective Titer (PT<sub>50</sub>) of ten serum samples was calculated and presented in Figure 17 of the report (page 29/34). No significant differences were observed between the individual sera in terms of PT<sub>50</sub> titer.

**Reviewer comment:** Overall, the results demonstrate that the antibody quality in terms of protective neutralizing antibody titer induced by VLA1553 in individual human subjects is comparable at similar µNT<sub>50</sub> titers.

#### **RR-0048-02: CHIKV sequencing from Phase 1 clinical study human sera**

The VLA1553 vaccine consists of a genetically modified live attenuated CHIKV characterized by a (b) (4) deletion in the nsP3 (non-structural Protein 3), leading to attenuation of the virus in vivo.

Report RR-0048-02 contains a summary of the sequencing study to demonstrate the genetic stability of the attenuated virus in vivo following administration to humans.

The sponsor first evaluated the genetic stability of the deletion in the nsP3 gene during in vivo replication. Twenty serum samples were selected for sequencing the region of the (b) (4) deletion in the nsP3 gene, fourteen (14) taken on day 3 and six (6) taken on day 7 post-vaccination in the Phase I clinical trial. For all analyzed samples, the 61 amino acid deletion remained unchanged.

The sponsor also sought to address the potential concern regarding reversion due to second site mutations in the genome that might complement the deletion in the nsP3 gene. Two serum samples, one from subject (b) (6) day 3 and one derived from subject (b) (6) at day 7, were chosen for full genome sequencing using the Sanger method. The following mutations were detected: L270F and V410F in nsP1, and A258T in capsid were found in subject (b) (6) day 7 sample; L270F and V410F in nsP1, as well as D247N and G641D in nsP2 were detected in subject (b) (6) day 3 sample. The sponsor stated that all sequence heterogeneities were detected at a very low percentage and none of them occurred in nsP3. The sponsor concluded that there is no evidence indicating that VLA1553 replicating in humans could cause accumulation of mutations that would ameliorate the attenuation caused by the deletion of nsP3 gene.

#### **4.2.1.2 Secondary Pharmacodynamics**

##### **Report RR-0066-01: Summary Report on experiments regarding transmission of live attenuated chikungunya virus Δ5nsP3 vaccine (VLA1553) by mosquitoes**

Report RR-0066-01 contains a summary of experiments to analyze the ability of Aedes mosquitos to transmit VLA1553 from a vaccinated to a non-vaccinated individual by mosquitoes. Since VLA1553 contains live CHIKV, there is a theoretical risk that transmission from vaccinated individuals to immunocompromised individuals or pregnant women by mosquitoes could cause CHIKV-related disease.

CHIKV can be transmitted to humans by *Aedes* species mosquitoes, mainly *Ae. aegypti* and *Ae. albopictus*. Human to human transmission of CHIKV by *Aedes* mosquitoes involves the following steps:

- the mosquito feeds on a CHIKV infected human and the virus is taken up with the blood meal and replicates in the midgut of the mosquito.
- CHIKV enters the salivary glands of the mosquito and replicates further.
- CHIKV is then transmitted by transfer of salivary gland fluid when the mosquito feeds on another human.

Two sets of experiments were performed:

- 1) Virus uptake of VLA1553 and CHIKV wild-type strain LR2006-OPY1 were compared in the bodies of *Aedes albopictus* mosquitoes after in vitro feeding on a blood meal containing a high virus titer of 7 log<sub>10</sub> CCID<sub>50</sub>/mL, virus dissemination to the salivary glands was measured. Three independent experiments were performed. Virus transmission was assessed after allowing the mosquitoes to feed on mice. Two independent experiments were performed.
- 2) A minimum threshold blood meal virus titer required for uptake, dissemination and detection of VLA1553 in mosquito salivary glands was determined. Three independent experiments were performed.

This work was carried out at (b) (4) by trained staff of the (b) (4) laboratories. (b) (4) has in place an in vitro membrane feeding system that allows assessment of whether CHIKV can be transmitted via membrane feeding to *Aedes albopictus* mosquitoes, whether it is able to replicate in mosquito bodies, and whether it is also able to disseminate to mosquito salivary glands.

## Results

### Experiment 1:

At high blood meal virus titers (>7 log<sub>10</sub> CCID<sub>50</sub>/mL) similar proportions of mosquitoes were body positive when fed on VLA1553 (40.5%, 65% and 90% for three experiments) compared to those fed on wildtype CHIKV (97.6%, 96.2% and 95%).

A lower proportion of mosquitoes were saliva positive when fed on VLA1553 (0%, 6.7% and 22% for three experiments) compared to those fed on wildtype CHIKV (41.4%, 21% and 30%). This difference was not significant.

At high blood meal virus titers, body titers of VLA1553 fed mosquitoes were approximately 1 log lower than for wild-type CHIKV fed mosquitoes. Titers for wildtype CHIKV-fed mosquitoes ranged from 2.5 log<sub>10</sub> CCID<sub>50</sub>/mosquito to 7 log<sub>10</sub>



CCID<sub>50</sub>/mosquito, and for VLA1553-fed mosquitoes the titers ranged from 2 log<sub>10</sub> CCID<sub>50</sub>/mosquito to 6.5 log<sub>10</sub> CCID<sub>50</sub>/mosquito.

The saliva titers for wildtype CHIKV and VLA1553 fed-mosquitoes in the same experiments showed no difference. Titers for both groups ranged from 5.5 log<sub>10</sub> CCID<sub>50</sub>/mL to 7.5 log<sub>10</sub> CCID<sub>50</sub>/mL.

Transmission studies in mice were performed twice. These studies assessed whether mosquitoes fed on high titer wild-type CHIKV or VLA1553 were able to transmit the virus to (b) (4) mice.

The wild-type CHIKV and VLA1553 fed mosquitoes were placed in 3 pots and allowed to feed on three (b) (4) mice (1 mouse per pot of mosquitoes). In the 1st experiment, all 3 mice exposed to wildtype CHIKV-fed mosquitoes developed wild-type CHIKV positive viremia and died. None of the 3 mice exposed to VLA1553-fed mosquitoes developed positive viremia.

In the repeat experiment none of the mice developed detectable viremia, regardless of whether they were exposed to wildtype CHIKV-fed mosquitoes or VLA1553-fed mosquitoes.

The sponsor assessed viremia in the fed mosquitoes although only in terms of percent of mosquitoes that tested positive, titers were not provided. For the first experiment the lack of transmission to the mice of VLA1553 is consistent with there being no detectable virus in the saliva of the mosquitoes. In the repeat study there were saliva positive mosquitoes in the VLA1553 fed group although no virus was transmitted to mice. However, no virus was transmitted to mice from the wildtype CHIKV-fed group despite positive saliva testing. Overall, these data are inconclusive in terms of transmission of VLA1553 to mice.

## **Experiment 2:**

In the 2<sup>nd</sup> set of experiments mosquitoes were fed on blood meals containing 6, 5 and 4 log<sub>10</sub> CCID<sub>50</sub>/mL of VLA1553. Decreasing blood meal virus titers correlated with decreased rates of body and saliva virus-positive mosquitoes for all 3 replicate experiments.

Mean body positive rates were 78.7%, 53.7% and 14.07% for 6, 5 and 4 log<sub>10</sub> CCID<sub>50</sub>/mL of VLA1553, respectively. Mean saliva positive rates were 1.7%, 1.9% and 0% for the same groups. A total of 4 mosquitoes with positive saliva were observed out of 300 tested. Two each from the groups fed on 6 and 5 log<sub>10</sub> CCID<sub>50</sub>/mL of VLA1553 were saliva positive, no mosquitoes were saliva positive from the group fed on 4 log<sub>10</sub> CCID<sub>50</sub>/mL of VLA1553.

Mean body titers were 3, 1.3 and 0.4 log<sub>10</sub> CCID<sub>50</sub>/mosquito for mosquitoes fed on 6, 5 and 4 log<sub>10</sub> CCID<sub>50</sub>/mL of VLA1553, respectively. Mean saliva titers were 7.1 and 5.8

log<sub>10</sub> CCID<sub>50</sub>/salivary gland for mosquitoes fed on 6 and 5 log<sub>10</sub> CCID<sub>50</sub>/mL of VLA1553, respectively.

From the data submitted, no mosquitoes were saliva positive when fed on 4 log<sub>10</sub> CCID<sub>50</sub>/mL blood meals. The sponsor proposed a titer of 3.875 log<sub>10</sub> CCID<sub>50</sub>/mL in blood as a threshold for VLA1553 transmission.

The sponsor used published data for VLA1553 viremia in non-human primates (NHPs) to predict titers in vaccinated humans. Roques et al. (2017 JCI Insight 2:e83527) showed peak titers of 7 log<sub>10</sub> GCE/mL for VLA1553 vaccine in NHPs, which converts to 4.6 log<sub>10</sub> CCID<sub>50</sub>/mL using a 250 conversion factor, and a mean of 5.5 log<sub>10</sub> GCE/mL viral load which converts to 3.1 log<sub>10</sub> CCID<sub>50</sub>/mL. The sponsor proposed that, assuming similar levels of viremia in NHPs and humans, vaccine recipients might provide maximum vaccine blood meal titers of 3.1 to 4.6 log<sub>10</sub> CCID<sub>50</sub>/mL for 1-2 days.

In the Phase I clinical trial performed by Valneva, VLA1553 was administered intramuscularly at 3 different doses,  $3.2 \times 10^3$ ,  $3.2 \times 10^4$ , and  $3.2 \times 10^5$  TCID<sub>50</sub>/dose. Viremia was determined at Days 0, 3, 7 and 14. Viremia data were provided in Clinical Study Report for VLA1553-101 (Section 5.3.5.1 of this BLA). Plasma viremia peaked at Day 3 in all groups with the highest mean GCE reached in the high dose group ( $2.3 \times 10^5$  GCE/mL). GCEs in the low and medium groups reached mean titers of  $7.4 \times 10^4$  and  $8.9 \times 10^4$  GCE/mL, respectively. Viremia resolved by Day 14 in all groups (below limit of detection). Thus, assuming the highest dose vaccine is used, the expected titers in vaccinees may be  $\sim 3 \times 10^5$  GCE/mL. The data submitted in the clinical study protocol did not provide all values therefore there may be some vaccinees with much higher titers. The GCE/mL titer of  $3 \times 10^5$  converts to 1200 CCID<sub>50</sub>/mL using the 250 conversion factor or 3.1 log<sub>10</sub> CCID<sub>50</sub>/mL. This value is the same as the lowest value proposed by Valneva from the NHP studies and lower than titers tested in the mosquito studies described in this submission.

**Reviewer comment:** Overall, the data suggest that the probability of mosquitoes being saliva positive when feeding on vaccinees is extremely low. Therefore, the risk of transmission of VLA1553 to non-vaccinees is very low.

## 4.2.2 Pharmacokinetics









### 4.2.2.1 Analytical Methods and Validation Reports

(b) (4)


## Assay Description



(b) (4)



(b) (4)



## Module 5 Clinical Study Reports

### 5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

**Report VIE-DR-0181 [01]: Chikungunya virus cross-neutralization testing of VLA1553-101 sera at (b) (4)**

CHIKV can be genetically classified into 3 major lineages: West African (W-African), East-Central and South African (ECSA), and Asian. Additionally, the Indian Ocean (IOL)

sublineage emerged within the ECSA clade. Valneva's vaccine strain candidate VLA1553 is based on the La Reunion (LR) strain belonging to the (b) (4) sublineage of the (b) (4) lineage.

The objective of the study was to evaluate potential cross-neutralization of the vaccine-induced antibodies against wild type CHIKV isolates from all three major lineages.

In this study, 47 serum samples were selected from the Phase 1 study (Days 0, 7, 14, 84, 180 and 210 post-vaccination) for titration by (b) (4) and  $\mu$ PRNT assays.

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