

CBER CMC BLA Review Memorandum

BLA STN 125820

Chikungunya Vaccine, Recombinant

Shufeng Liu, PhD, CBER/FDA

1. **BLA#:** STN 125820

2. **APPLICANT NAME AND LICENSE NUMBER**

Bavarian Nordic, A/S

3. **PRODUCT NAME/PRODUCT TYPE**

Proper name: Chikungunya Vaccine, Recombinant

Proprietary name: VIMKUNYA

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

VIMKUNYA is a recombinant vaccine indicated for the prevention of disease caused by chikungunya virus (CHIKV) in individuals 12 years of age and older. VIMKUNYA contains non-infectious, purified virus-like particles (VLPs) consisting of CHIKV capsid protein (C) and envelope proteins E1 and E2, derived from CHIKV Senegal strain 37997. The purified VLPs are (b) (4) with formulation buffer and adsorbed on aluminum hydroxide [Al(OH)₃] as adjuvant. VIMKUNYA vaccine is presented as a sterile suspension, supplied in a single-dose pre-filled syringe, for intramuscular (IM) injection. Each 0.8-mL dose contains 40 mcg of CHIKV VLPs adsorbed on aluminum hydroxide (approximately 300 mcg aluminum per 0.8-mL dose).

The proprietary name, VIMKUNYA, was approved on September 10, 2024 during BLA review. In this memo, the vaccine is referred to as CHIKV VLP and VIMKUNYA.

5. **MAJOR MILESTONES**

Filing Meeting: July 29, 2024

Advisory Committee Meeting: Not applicable

PeRC meeting: December 17, 2024

Action Due Date: February 14, 2025

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)
Wei Wang, OCBQ/DMPQ	Module 3 (facilities and equipment information) and manufacturing records
Hsiaoling Wang, OCBQ/DBSQC	DS Release Assays and Method Validations: (b) (4) DP Release Assays and Method Validations: Appearance, pH, Aluminum content, (b) (4) Sub-visible particles
Karla Garcia, OCBQ/DBSQC	DS Release Assays and Method Validations: (b) (4)

Reviewer/Affiliation	Section/Subject Matter
	DP Release Assays and Method Validations: Bacterial Endotoxin, Sterility
Alicia Howard, OCBQ/DBSQC	DS Release Assays and Method Validations: (b) (4) DP Release Assays and Method Validations: Identity, (b) (4)
Yuan Hu 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

Date Received	Submission	Comments/ Status
April 29, 2024	STN 125820/0	Reviewed
June 10, 2024	STN 125820/0.1	Reviewed
June 17, 2024	STN 125820/0.2	Reviewed
July 11, 2024	STN 125820/0.4	Reviewed
August 19, 2024	STN 125820/0.12 (response to IR dated July 29, 2024)	Reviewed
September 12, 2024	STN 125820/0.18 Stability updates	Reviewed
September 20, 2024	STN 125820/0.20 (response to IR dated August 21, 2024)	Reviewed
October 4, 2024	STN 125820/0.25 (response to IR dated October 4, 2024)	Reviewed
October 28, 2024	STN 125820/0.33 (response to IR dated October 17, 2024)	Reviewed
November 12, 2024	STN 125820/0.37 (response to IR dated November 5, 2024)	Reviewed
November 13, 2024	STN 125820/0.38 Stability updates	Reviewed
November 18, 2024	STN 125820/0.41 (response to IR dated October 31, 2024)	Reviewed
December 13, 2024	STN 125820/0.49 (response to IR dated September 19, 2024)	Reviewed
January 2, 2025	STN 125820/0.57 (response to IR dated December 20, 2024)	Reviewed

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

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
IND 17998	Bavarian Nordic	Investigational New Drug (IND) submission	no	Contains information pertinent to the

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
(b) (4)				
DMF (b) (4)	(b) (4)	Syringe	yes	Primary packaging material syringes
BB-MF (b) (4)	(b) (4)	Syringe plunger	yes	(b) (4)
BB-MF (b) (4)	(b) (4)	Syringe plunger	yes	Pharmaceutical (b) (4) Sterilization Process
STN (b) (4)	(b) (4)	Syringe plunger	yes	Elastomeric Formulations, Coatings and Films

10. REVIEWER SUMMARY AND RECOMMENDATION

A. EXECUTIVE SUMMARY

Bavarian Nordic (BN) submitted the original Biologics License Application (STN 125820/0) as a rolling review application on April 29, 2024 (Part 1 – Modules 1, 2, and 4); June 10, 2024 (Part 2 – clinical); and June 17, 2024 (Part 3 – CMC), to seek

approval of Chikungunya Vaccine, Recombinant for active immunization for the prevention of disease caused by CHIKV infection in individuals 12 years of age and older through the accelerated approval pathway (21 CFR 601, subpart E). Bavarian Nordic proposed VIMKUNYA as the vaccine's proprietary name. A request for Priority review was granted on August 13, 2024. 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 VIMKUNYA contains VLPs formed by three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997: Capsid (C), Envelope 1 (E1), and Envelope 2 (E2). The Drug Substance (DS) is manufactured at the BN (b) (4) facility. (b) (4)

The formulated and adjuvanted BDP is then shipped to (b) (4) (b) (4) where it is filled as a single dose of 0.8 mL into 1-mL Type (b) (4) glass syringe barrels with a luer lock adapter and rigid cap. After filling, the syringe is sealed with a chlorobutyl rubber plunger stopper to create VIMKUNYA final Drug Product (DP). Each 0.8-mL dose contains approximately 40 µg CHIKV VLPs, 300 µg aluminum, 59.7 mg sucrose, 5.9 mg sodium citrate, 0.9 mg potassium phosphate dibasic, 0.4 mg potassium phosphate monobasic, and water for injection. The shelf life for VIMKUNYA is 36 months from the date of manufacture when stored at 2 - 8°C. The date of manufacture is defined as the date when the (b) (4)

VIMKUNYA does not contain any preservative or antibiotics.

The VIMKUNYA DP is transported from the (b) (4) facility to (b) (4) (b) (4) in (b) (4) for assembly, labeling, and packaging to produce the Finished Drug Product (FDP).

Testing is performed at multiple stages of the manufacturing process to ensure the product meets the pre-defined quality specifications. DS release tests include (b) (4)

DP release tests consist of appearance, pH, aluminum content, osmolality, identity, total protein concentration, (b) (4) potency, bacterial

endotoxin, sub-visible particles, % adsorbed VLP, container content, container closure integrity, as well as syringe functionality for (b) (4) FDP release test is performed after assembly, labeling and packaging to confirm identity.

Non-clinical Studies

Bavarian Nordic performed a non-human primate (NHP) passive transfer efficacy study (Study PAS-NHP-CHIK-003) using serum from vaccinated human volunteers against CHIKV in Cynomolgus Macaques to establish a threshold serum neutralizing antibody titer that conferred protection following CHIKV challenge. Based on the data, a surrogate of protection with a Neutralizing Antibody Titer $NT_{80} \geq 100$ was deemed reasonably likely to predict protection in humans and was applied to clinical evaluation of VIMKUNYA in Phase 3 to support the accelerated approval.

Clinical Assays for Efficacy Endpoint Assessments

The applicant used a CHIKV luciferase (CHIKV-Luc) neutralization assay to quantify the titer of neutralizing antibodies for CHIKV in human serum. Information regarding the neutralization assay to assess seroconversion and anti-CHIKV titers was submitted to the IND 17988 and the BLA STN 125820. Assay validation information was reviewed under the IND prior to testing of the clinical samples and found to be appropriate.

Overall, the information provided in the BLA and amendments demonstrates that the manufacturing process is adequately controlled and validated with appropriate in-process control testing in-place to monitor the product quality. Moreover, adequate quality control testing has been conducted and sufficient stability data have been accrued with the (b) (4) DP lots to ensure the quality and safety of the vaccine. Therefore, I recommend approval of the product.

B. RECOMMENDATION

I. APPROVAL

a. List of DS and DP Manufacturing Facilities

- Manufacture of DS and BDP: Bavarian Nordic (b) (4)
(b) (4)
- Manufacture of DP: (b) (4)
(b) (4)
- Manufacture of FDP (b) (4)

b. List of approvable Comparability Protocols, if applicable

Not applicable

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

PMRs:

- To conduct a randomized, double-blind, placebo-controlled study to evaluate the efficacy, safety, and immunogenicity of VIMKUNYA, 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).
- Deferred pediatric study under PREA (EBSI-CV-317-006) to evaluate the safety and immunogenicity of VIMKUNYA in children 2 to <12 years of age.
- Deferred pediatric study under PREA (EBSI-CV-317-009) to evaluate the safety and immunogenicity of VIMKUNYA in neonates and infants 0 to <2 years of age.

PMCs:

- Study titled "VIMKUNYA Pregnancy Registry: An observational prospective study of the safety of VIMKUNYA vaccine exposure in pregnant individuals and their offspring." This prospective, observational registry study of pregnant individuals residing in the United States and European Union will evaluate maternal and infant outcomes (until one year of age) in at least 50 individuals exposed to VIMKUNYA up to 28 days prior to or at any time during pregnancy.
- To submit leachable data for unlabeled CHIKV VLP Drug Product syringes. Final Report Submission: October 31, 2028
- To conduct a study to quantify the residual (b) (4) plasmid DNA (pDNA) levels on the first (b) (4) consecutive CHIKV VLP (b) (4) lots, and to submit data from this study. Final Report Submission: June 30, 2026
- To conduct Finished Drug Product (FDP) transport validation studies (b) (4) as described in section 3.2.P.3.5 (2.5.3.3) of your BLA 125820/0. Final Report Submission: June 30, 2025
- To perform DP transportation validation using the DP PPQ lots. Final Report Submission: February 28, 2026

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

No specific CMC issues for inspectional follow up at Bavarian Nordic (b) (4)
(b) (4)

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

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Shufeng Liu, CMC Reviewer/DVP/OVRR	Concur	
Tony Wang, Lab Chief/DVP/OVRR	Concur	
Robin Levis, Deputy Director/DVP/OVRR	Concur	

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List of Abbreviations

AET: Analytical Evaluation Threshold

BDP: Bulk Drug Product

BN: Bavarian Nordic

BSE: Bovine Spongiform Encephalopathy

CCI: Container Closure Integrity

CHIKV: Chikungunya Virus

CMA: Critical Material Attributes

CoA: Certificate of Analysis

CPE: Cytopathogenic Effect

CPP: Critical Process Parameters

CPV: Continuous Process Verification

CQA: Critical Quality Attribute

(b) (4)

CTM: Clinical Trial Material

DP: Drug Product

DS: Drug Substance

DSP: Downstream Process

EOSL: End of Shelf-Life

(b) (4)

(b) (4)

FDP: Finished Drug Product

(b) (4)

(b) (4)

GCV: Geometric Coefficient of Variation

GMP: Good Manufacturing Practice

GMR: Geometric Mean Ratio

GMT: Geometric Mean Titer

HCP: Host Cell Protein

HEK293: Human Embryonic Kidney 293

IPC: In-Process Control

IPT: In Process Test

IR: Information Request

IV: Intravenous

KPP: Key Process Parameter

LLOQ: Lower Limit of Quantification

LOD: Limit of Detection

MCB: Master Cell Bank

MEV: Minimum Extractable Volume

(b) (4)

(b) (4)

NHP: Non-Human Primate

NIH: National Institutes of Health

NOR: Normal Operating Range

PAR: Proven Acceptable Range

(b) (4)

(b) (4)

(b) (4)

PFS: Prefilled Syringe

(b) (4)

PMC: Post-Marketing Commitment

PP: Process Parameter

PPQ: Process Performance Qualification

(b) (4)

(b) (4)

SNA: Serum Neutralizing Antibody

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

TSE: Transmissible Spongiform Encephalitis

(b) (4)

(b) (4)

(b) (4)

(b) (4)

VLP: Virus-like Particle

VRC: Vaccine Research Center

WCB: Working Cell Bank

WFI: Water for Injection

WT: Wild Type

Module 3

3.2.S DRUG SUBSTANCE

3.2.S.1.1 - 1.3 Nomenclature, Structure and General Properties

(b) (4)

41 pages have been determined to be not releasable: (b)(4)

(b) (4)

3.2.P DRUG PRODUCT

3.2.P.1 Description and Composition of the Drug Product

The CHIKV VLP DP is a sterile suspension comprising 40 µg CHIKV VLP adsorbed on 300 µg aluminum hydroxide adjuvant and stabilized with formulation buffer. It is supplied as single dose 1-mL pre-filled glass syringes, containing a deliverable dose volume of 0.8 mL of CHIKV VLP vaccine for intramuscular administration. The composition of the DP is described in Table 9 (adapted from Table 3.2.P.1-1 of the BLA).

Table 9. DP Composition

Component	Function	Quality Standard	Amounts per dose (0.8 mL)
CHIKV VLP	Active Ingredient	In house	40 µg
Aluminum hydroxide (b) (4)	Adjuvant and stabilizer	(b) (4)	300 µg ^a
(b) (4)	Stabilizer		59.7 mg
Sucrose	Buffering agent		0.4 mg
Potassium phosphate, monobasic	Buffering agent		0.9 mg
Potassium phosphate, dibasic	Stabilizer		5.9 mg
Sodium citrate, dihydrate	Solvent		qs to 0.8 mL
Water for Injection			

^a Amount is based on aluminum content

qs = quantum satis

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 DS contains three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997: C, E1, and E2, which self-assemble to form the VLP. The

DS (b) (4)

(b) (4)

(b) (4)

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

The initial formulation of the DP, developed by the VRC and used in the VRC Phase 1/2 clinical studies, was similar to the current DP formulation except that it was non-
adjuvanted and had a VLP concentration of (b) (4). In the Emergent Phase 2 studies, the excipient formulation remained the same as in the VRC Phase 1/2, except that it included 300 µg Aluminum/dose as an adjuvant. The Emergent Phase 2 vaccine was a field-formulated (bedside mix) vaccine with 3 components: CHIKV VLP DP, diluent, and aluminum hydroxide. The formulation buffer, adjuvant, and target adjuvant amount (300 µg) are identical between the Phase 2 and Phase 3 clinical products except that for Phase 3 DP the adjuvant is added during the BDP manufacturing, and the vaccine is provided in a pre-filled syringe. The formulation of commercial DP remains unchanged.

3.2.P.2.2.2 Overages

No overages are required. The vials are over-filled with a filling volume of (b) (4) mL to ensure a withdrawal volume of 0.8 mL.

3.2.P.2.2.3 Physicochemical and Biological Properties

The DP is a sterile aqueous buffered suspension containing CHIKV VLP adsorbed on aluminum hydroxide. The CHIKV VLPs are non-infectious and non-replicating, but structurally resemble the native CHIKV with respect to antigen presentation to the immune system. The pH, (b) (4), and aluminum content of the DP have been measured to characterize the physicochemical properties relevant to the safety and performance of the DP. The biological activity of the DP is determined by an (b) (4) assay. The (b) (4) potency value for the test sample is calculated as

the (b) (4) This
value provides a specific potency measurement of (b) (4)
(b) (4) and should be (b) (4) The
physicochemical and biological properties are determined by the control tests on the
(b) (4) filled DP.

3.2.P.2.3 Manufacturing Process Development

(b) (4)

(b) (4)

3.2.P.2.4 Container Closure System

(b) (4)

Container Closure System for Finished Drug Product

The primary container closure system of Finished Drug Product is composed of the Type (b) (4) glass barrel, Luer lock adapter, rigid cap, and rubber closure (b) (4) (b) (4) a rubber plunger stopper, a plastic plunger rod attached to the rubber plunger, and plastic finger flange.

The glass barrel, rubber closure, and rubber plunger stopper are product contact components. The Type (b) (4) borosilicate glass of the syringes complies with (b) (4)

(b) (4) Syringes are manufactured by (b) (4)

(b) (4) Release testing for the syringe includes (b) (4)

(b) (4)

The rubber closure and rubber plunger are manufactured by (b) (4) and comply with (b) (4) Release testing for the rubber closure includes

(b) (4) A (b) (4)

(b) (4)

(b) (4) is used as a syringe lubricant.

The Luer lock adapter and rigid cap meet (b) (4) requirements and do not contact the product.

Extractables and Leachables Assessment

An elemental extractables study was performed on the syringe, syringe tip cap, and rubber plunger. The compounds identified with quantities higher than the (b) (4)

(b) (4) are as follows: (b) (4)

(b) (4)

were present in the test article at levels greater than the Permissible Daily Exposure (PDE). All (b) (4) compounds originate from the rubber plunger. However, due to the (b) (4) coating of the rubber plunger, leaching of these compounds is not expected to reach levels that could impact product safety. This is being assessed by the ongoing leachables study.

A leachables study on the DP syringes is ongoing. This leachable study included PPQ batch 2 (b) (4) which will be stored at 2 - 8°C for 36 months (end of shelf life) and tested for leachables periodically per (b) (4) Samples are analyzed by (b) (4)

(b) (4) for (b) (4) by (b) (4) for (b) (4)
(b) (4) and by (b) (4) for (b) (4)
(b) (4) The (b) (4) screening methods applied were confirmed to reliably detect the (b) (4) extractables with concentrations exceeding the PDE. Results from the initial time point (Time = 0 months) have not shown any leachable compounds at or above the reporting threshold of (b) (4). This ongoing study will be listed as a PMC. The final leachable study report will be provided by October 31, 2028, as a “PMC Submission – Final Study Report.”

Container Closure Integrity

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

3.2.P.2.5 Microbiological Attributes

The CHIKV VLP DP is supplied as a sterile product, which is confirmed by testing of sterility, bacterial endotoxins, and container closure integrity. The (b) (4)

The DP manufacturing process is performed as an aseptic fill-finish process. Package integrity testing is performed as part of the stability program.

3.2.P.2.6 Compatibility

Compatibility of CHIKV VLP vaccine with the primary packaging components has been assessed across the product shelf life in multiple stability studies.

In addition, an in-use stability study was performed to assess the CHIKV VLP compatibility in the syringe and any effects on product quality when stored at room temperature in the syringe. Prefilled syringes from (b) (4) Phase 3 DP Lots (b) (4) (b) (4) were removed from storage at 2 - 8°C. The PFSs were tested for appearance, pH, (b) (4) aluminum content, container content, total protein concentration, (b) (4) potency after being held at room temperature for 0, 2, (b) (4) hours. All results met the predefined acceptance criteria, except that (b) (4) aluminum content result from Lot (b) (4) was OOS. This study demonstrated that the PFSs are stable for up to (b) (4) at room temperature. In the BLA, the applicant requested an in-use shelf-life duration of 2 hours, which is acceptable.

Overall Reviewer’s Assessment of Section 3.2.P.2:

- On July 29, 2024, the following IR was sent to the applicant:

On page 8 of Section 3.2.P.2.2 Formulation Development, you describe that the vaccine used in Emergent Phase 2 studies contained (b) (4) of CHIKV VLP. However, Table 2 of the same section indicates that the concentration of CHIKV VLP in the Phase 2 Drug Product is (b) (4). Please clarify this discrepancy.

On August 19, 2024, the applicant confirmed that the concentration of (b) (4) in Table 2 of Section 3.2.P.2.2 Formulation Development is a typographical error. This table has been updated with this submission to reflect the correct concentration of (b) (4). 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 CHIKV VLP DP is manufactured, filled, packaged, inspected, and tested at the sites indicated in Table 10 (derived from Table 3.2.P.3.1-1, Section 3.2.P.3.1 Manufacturer(s)).

Table 10. DP Manufacturing and Testing Sites

Site Name	Site Address	Specific Manufacturing Responsibilities or Type of Testing
Bavarian Nordic (b) (4) (b) (4)	(b) (4)	Manufacture and release of BDP Release and stability testing of BDP, DP and FDP
Bavarian Nordic A/S	(b) (4)	Release of DP and FDP
(b) (4)		Manufacture of DP
		Release testing of DP: bacterial endotoxins
		Visual Inspection of DP
		FDP final assembly, labeling and packaging
		PPQ sterility testing
		Sterility for DP release
		CCIT
		Syringe functionality for PPQ stability

Site Name	Site Address	Specific Manufacturing Responsibilities or Type of Testing
(b) (4)		Phase 3 clinical trial material stability testing: appearance, pH, container content, aluminum content, total protein concentration, (b) (4) potency, sterility

3.2.P.3.2 Batch Formula

The validated DP batch size is between (b) (4) to produce (b) (4) syringes. The (b) (4) of the DP is (b) (4). The quantities of each component are presented in Table 11 (derived from Table 1, Section 3.2.P.3 Manufacture).

Table 11. Batch Formula for DP

Component	Quality Standard	(b) (4) Batch Size	(b) (4) Batch Size
CHIKV VLP	(b) (4)	(b) (4)	(b) (4)
Aluminum hydroxide			
(b) (4)			
Sucrose			
Potassium phosphate monobasic			
Potassium phosphate dibasic			
Sodium citrate dihydrate			
Water for Injection			

3.2.P.3.3 Description of Manufacturing Process

The manufacturing CHIKV VLP is divided in (b) (4) manufacturing processes:
(b) (4)

Bulk Drug Product Manufacturing

The manufacturing process for the BDP includes the following major steps:

(b) (4)

(b) (4)

Drug Product Manufacturing

Upon receipt at (b) (4) the BDP (b) (4) are stored at (b) (4) until use for DP production. The manufacturing process for the DP includes the following major steps:

(b) (4)

Finished Drug Product Manufacturing

The assembly (automated addition of plunger rod, application of label and manual addition of finger flange) and labeling of the DP syringes is performed on an automated machine. After labeling, the syringe trays are packaged in pre-labeled cartons (secondary) and shipper boxes (tertiary).

FDP lot numbers are assigned by the (b) (4) system at (b) (4). The lot numbers are in unique, non-repeating (b) (4) digits.

3.2.P.3.4 Controls of Critical Steps and Intermediates

To ensure manufacturing process consistency and the quality of the CHIKV VLP, CPPs, KPPs, IPCs, IPTs, and release control tests have been implemented.

The CPPs were identified using a risk-based approach based on impact to the product critical quality attributes. The CPPs and their target values and ranges for the BDP, DP, and FDP manufacturing processes are listed in table 12 (adapted from Tables 2, 3, and 5 of 3.2.P.3.4 of the BLA).

(b) (4)

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

(b) (4)

3.2.P.3.5 Process Validation and/or Evaluation

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

DP Process Performance Qualification

The three consecutive DP PPQ batches (b) (4) were produced from BDP batches (b) (4) at the (b) (4) facility. Process validation lots were manufactured at a scale size range of (b) (4)

The process validation covering the manufacturing steps and parameters studied for CHIKV VLP DP includes:

(b) (4)

Results for the process parameters of the PPQ runs were provided in Table 9 under Section 3.2.P.3.5 of the BLA. All CPPs remained within their designated normal operating ranges, except that the (b) (4)

(b) (4)

(b) (4) FDP lots (b) (4) were manufactured at (b) (4) at a batch size of (b) (4) PFS and then used for the PPQ assessment. The PPQ of the FDP manufacturing process covers (b) (4) main steps: (b) (4)

The packaging process was not included in the PPQ study. The PPQ study results were presented in Table 21 under Section 3.2.P.3.5 of the BLA. All results met the acceptance criteria. Table 22 in Section 3.2.P.3.5 of the BLA provided the release assays, specifications, and results for each PPQ lot. All the release tests complied with the acceptance criteria.

In addition, validation of container closure integrity (CCI), manufacturing yield calculation, and visual inspection were conducted.

Shipping Validation

The (b) (4) is transported from (b) (4)

A shipping validation has been conducted by monitoring (b) (4) shipments from (b) (4). The (b) (4) runs consisted of (b) (4) filled with (b) (4) PPQ batch and were shipped (b) (4) from (b) (4). The (b) (4) shipment consisted of (b) (4) filled with (b) (4) (b) (4) (b) (4) and (b) (4) filled with (b) (4) (b) (4). The (b) (4) run was shipped round trip from (b) (4) and back to (b) (4) and the (b) (4) (b) (4) were visually examined for sterility and tested for (b) (4). For each shipment, temperature (b) (4) were placed. The shipping performance validation demonstrated that the shipping container and packaging configuration can maintain shipping temperatures and product quality within the pre-determined acceptance criteria.

The DP is transported in a qualified refrigerated (b) (4) from (b) (4) for final assembly, labeling and packaging. As part of DP transport validation, a simulated shipping study was performed using filled DP PFS (b) (4) to evaluate the impact of (b) (4) on product during transportation. In this study, a pallet of DP was shipped to (b) (4) (b) (4) for visual inspection, CCIT, as well as (b) (4) testing. After transport testing at (b) (4) the PFS samples were shipped to BN (b) (4) for analytical testing to evaluate the drug product stability after the shipping stress. Test results for appearance, container content, pH, (b) (4) potency, total protein content, aluminum content, subvisible particles, (b) (4) were provided in Table 35 of Section 3.2.P.3.5. of the BLA. All results met the pre-defined specifications. In addition, the applicant proposed to conduct a performance qualification study to cover (b) (4) shipments of DP from (b) (4) to (b) (4) (b) (4). The study report will be submitted by February 28, 2026, as a "PMC Submission – Final Study Report."

Bavarian Nordic submitted a validation protocol for the commercial FDP transport from (b) (4) for finished product distribution. The final study report will be submitted by June 30, 2025, as a “PMC Submission – Final Study Report.”

The results of the shipping validation studies will be reviewed by Wei Wang from DMPQ and the device reviewer Andrea Gray.

Overall Reviewer’s Assessment of Section 3.2.P.3.5:

- The following IR was sent to the applicant on September 19, 2025:

On page 4 of Section 3.2.P.3.3., the batch numbers for BDP are assigned in ascending order. However, Table 8 of 3.2.P.3.5. Process Validation and/or Evaluation shows that the expiration date of BDP batch (b) (4) comes before that of (b) (4) presumably earlier BDP batches (b) (4). Please clarify.

On October 4, 2024, the applicant explained that at the time of manufacture, BDP batches (b) (4) had a shelf life of (b) (4) from the date of manufacture. Subsequently, additional BDP long term stability data at (b) (4) from (b) (4) phase 3 clinical trial material batches were gathered and the shelf life for BDP was extended to (b) (4). Change controls were initiated to extend the shelf-life from (b) (4) for batches (b) (4). Batch (b) (4) was planned to be filled prior to the initial (b) (4) expiration date so a change control was not issued to extend the expiration date to (b) (4). The response is acceptable.

- On October 17, 2024, an IR was sent to the applicant:

As we communicated to you on January 5, 2024 (pre-BLA CMC written response), your original BLA submission should include description of the procedure(s) for the shipment of DP to distribution sites and provide a summary of results from shipping validation studies. In Sub-section 2.5.3.3 of Section 3.2.P.3.5. Process Validation or Evaluation, you submitted “Planned Finished Drug Product Transport Validation Studies.” Please indicate when you plan to submit the final validation study report for these ongoing validation studies.

On October 28, 2024, Bavarian Nordic submitted responses to STN 125820/0.33. Bavarian Nordic would like to request that the final shipping validation study report be provided to the BLA as a PMC. Bavarian Nordic proposed that this PMC be submitted to the CBER by June 2025.

- The information provided is acceptable. Please also refer to review memos from Wei Wang (DMPQ) and Andrea Gray.

3.2.P.4 Control of Excipients

3.2.P.4.1 Specifications

The excipients used in the manufacture of CHIKV VLP DP are listed in Section 3.2.P.2.1.2 of this memo. There are no (b) (4) or novel excipients used in the manufacture of CHIKV VLP DP. The excipients are accepted by the applicant based on a Certificate of Analysis from the qualified suppliers. Water for injection (WFI) is supplied through an in-house water loop.

The specifications for aluminum hydroxide (b) (4) sucrose, potassium phosphate monobasic, potassium phosphate dibasic, sodium citrate, and WFI are based on current (b) (4). All excipients are tested according to the pre-specified specifications upon receipt.

3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures

Aluminum content in aluminum hydroxide (b) (4) is determined by a (b) (4) (b) (4) method. The validation assessments include (b) (4) (b) (4)

(b) (4)

All other analytical methods used for excipients are (b) (4) methods and comply with (b) (4) requirements.

3.2.P.4.4 Justification of Specifications

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

To verify the aluminum content claimed by the supplier, the acceptance criterion for aluminum content in aluminum hydroxide (b) (4) was set as the reported value on vendor's CoA (b) (4)

3.2.P.4.5 Excipients of Human or Animal Origin

CHIKV VLP 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 CHIKV VLP vaccine.

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 test specifications for the BDP are summarized in Table 14. (derived from Table 1 in 3.2.P.5.1 of the BLA).

Table 14. BDP Specifications

Test	Method	Release Specification	Stability Specification
(b) (4)			

The methods and specifications applied for release and stability testing of the DP are provided in Table 15 (derived from Table 2 of 3.2.P.5.1 of the BLA).

Table 15. DP Specifications

Test	Method	Release Specification	Stability Specification
Appearance	Visual inspection (b) (4)	Transparency/Turbidity: Cloudy Color: White State: Liquid Particles: Free from visible extraneous particles	Transparency/Turbidity: Cloudy Color: White State: Liquid Particles: Free from visible extraneous particles
pH	(b) (4)		
Aluminum content			
(b) (4)			
Identity			
Total protein concentration			
(b) (4)			
(b) (4) potency			
Bacterial endotoxins			
Sterility			
Sub-visible particles			
% Adsorbed VLP			
Container content			
Container closure integrity			
Syringe functionality for: (b) (4)			

Release testing for FDP includes identity testing after labeling the PFS by (b) (4)
(b) (4)

Justification of Specifications

Specifications were based on historical data, clinical data or comply with (b) (4)


(b) (4) All specifications were justified.

- Aluminum content is determined by a quantitative (b) (4) method. A target of 300 µg aluminum/dose was chosen based on CHIKV adsorption studies to aluminum hydroxide. The aluminum content acceptance criterion was set at (b) (4) per dose for (b) (4) (b) (4) DP, corresponding to (b) (4) to center around the target and account for variability in the manufacturing process and testing.

- The protein concentration specification of (b) (4) for (b) (4) has been evaluated and is justified by the following: (b) (4)

- (b) (4)

(b) (4)



- (b) (4) potency is calculated as the (b) (4) (b) (4) and therefore is a measure of the specific potency per CHIKV VLP protein amount. The theoretical (b) (4) potency ratio should be close to (b) (4) at release. The release acceptance criterion is (b) (4) (b) (4) DP, which is also the stability acceptance criterion for (b) (4) The DP stability acceptance criterion is (b) (4) The lower limits of (b) (4) were established based on data from the (b) (4) potency correlation study RPT063218, which demonstrated that an (b) (4) potency ratio of (b) (4) was as (b) (4) as samples with ratios around (b) (4)
- The percentage of CHIKV VLP adsorbed to adjuvant is performed to determine the level and consistency of association of the antigen with the adjuvant, the integrity of the antigen in association with the adjuvant, and the extent of release of the antigen from the adjuvant over time. An acceptance criterion of (b) (4) was set for both release and stability based on data from (b) (4) development batches and (b) (4) PPQ batches.

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

- ☐ The information provided is acceptable.

3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures

The (b) (4), DP, and FDP release methods are listed in Section 3.2.P.5.1 Specifications of this memo.

(b) (4) tests were validated in compliance with the requirements of the (b) (4)

(b) (4) DVP was responsible for reviewing (b) (4)

(b) (4)

(b) (4)

(b) (4)

% Adsorbed VLP

(b) (4)

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

Protein Concentration by (b) (4) for (b) (4) DP

The test method used for (b) (4) DP is the same as for (b) (4) (refer to Section 3.2.S.4.3 of this memo), except for (b) (4)

The method was validated by evaluating (b) (4)

(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.5.2 and 3.2.P.5.3:

- The following IR was sent to the applicant on September 19, 2024. The applicant provided their responses to STN 125820/0.25 on October 4, 2024.

Comment 4:

Regarding validation report BN0109249 – % Adsorbed VLP Assay for CHIKV VLP,

- a. You state that the extended assay range of (b) (4) was validated based on linearity and accuracy results. However, the concentration levels of the test samples that used for linearity and accuracy assessments ranged from (b) (4) which did not encompass the range you defined. Please explain.
- b. The assay range should be established based on both precision and linearity/accuracy results. You state that precision of the assay in the DP matrix has already been demonstrated in validation report BN0097227. However, in report BN0097227, precision was investigated at concentration levels of (b) (4) which did not cover the defined assay range of (b) (4).
- c. The stability acceptance criterion for (b) (4) for DP is (b) (4). The stability acceptance criterion for % Adsorbed VLP for DP is (b) (4) which is calculated by (b) (4). To reliably quantify (b) (4) of CHIKV VLP that are not adsorbed on the adjuvant, the assay should be able to detect (b) (4) of VLP. Given the issues stated above, we recommend you revalidate the assay range to ensure it can detect a lower limit of (b) (4).

The applicant agreed that the validated range of the method should be based on the assessment of linearity/accuracy and precision. Combining the results of BN0097227 and BN0109249, the range of the (b) (4) assay was established at (b) (4). Bavarian Nordic would perform precision at the low end of the range for the % adsorbed testing to ensure it can detect (b) (4) of VLP. The response is acceptable.

Comment 5:

Regarding validation report BN0096870 – Determination of total protein concentration total protein concentration with (b) (4) Test for CHIK VLP (b) (4) DP, and FDP,

- a. You state that based on the linearity, the analytical range of the method was determined to be (b) (4). However, on page 6 of report BN0096870, the measured total protein concentrations of (b) (4) samples that were used for linearity and accuracy assessments ranged from (b) (4). Please clarify the discrepancy and if needed correct the error in the relevant sections.
- b. Please note the assay range should be established based on both precision and linearity/accuracy results. You assessed precision by testing (b) (4) DP

material at concentration levels of (b) (4) which did not encompass the range you defined. Please reassess the assay range based on available precision and linearity results submitted in report BN0096870.

- c. Please indicate how many analysts conducted the (b) (4) runs for intermediate precision assessment and over how many days.

The firm acknowledged that the validated range of the method should be based on the assessment of linearity/accuracy and precision. Based on linearity and precision, the assay range was revised to (b) (4). The applicant responded that the assay runs were performed by (b) (4) analysts over (b) (4). The response is acceptable.

- ☐ The information provided is acceptable.

3.2.P.5.4 Batch Analyses

Batch analysis records of the following CHIKV VLP BDP lots were submitted for review:

(b) (4) Phase 3 batches (b) (4),
(b) (4) engineering batches (b) (4) between CTM Phase 3 and PPQ campaign, (b) (4) PPQ batches (b) (4) and (b) (4) post-PPQ batches (b) (4). All the batches were manufactured at BN (b) (4) facility. Batch analysis data were provided in Table 4 to Table 7 of Section 3.2.P.5.4 of the BLA. All results are within the pre-defined acceptance criteria.

Bavarian Nordic provided DP batch analysis data for (b) (4) Phase 3 batches (b) (4), (b) (4) engineering batch (b) (4) and (b) (4) PPQ batches (b) (4). The Phase 3 batches were manufactured at (b) (4). The engineering and PPQ batches were filled at (b) (4). All batches met the specifications at the time of release.

Regarding the FDP, batch analysis data were provided for (b) (4) Phase 3 clinical trial batches (b) (4) and (b) (4) PPQ batches (b) (4).
(b) (4) The Phase 3 batches were manufactured at (b) (4).
(b) (4) The PPQ batches were manufactured at (b) (4).
(b) (4) Release data for the above lots were included in Tables 11 and 12 in Section 3.2.P.5.4 of the BLA and complied with pre-defined specifications.

There were no discrepancies or trends observed indicating any manufacturing difficulties, and the data supported consistency of product manufacture.

3.2.P.5.5 Characterization of Impurities

Impurities in CHIKV VLP (b) (4) DP and FDP that potentially can be carried over from the (b) (4) have been described in Section 3.2.S.3.2 Impurities of this memo. Potential impurities that may arise at the DP steps are (b) (4)

The applicant performed (b) (4) (b) (4) risk assessments. The overall risk classification is “no risk”. An assessment of the potential (b) (4) impurities for CHIKV VLP was performed by the applicant, and no risks were identified. The specification for WFI includes (b) (4) testing with an acceptance criterion of (b) (4). A risk assessment for extractables from single-use systems for the (b) (4) DP manufacture was performed, and no risks were identified. The extractables and leachables from the container closure system are summarized in Section 3.2.P.2.4 of this memo. Particulate contamination is monitored through visual inspection after DP filling process and at DP release. To ensure CHIKV VLP DP is supplied as a sterile product, bacterial endotoxin and sterility testing are performed at (b) (4) DP release, and CCIT is performed for DP stability.

Overall Reviewer’s Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5:

- On September 19, 2024, the following IR was sent:

On page 4 of Section 3.2.P.3.3. Description of the Manufacturing Process and Process Controls, you state that BDP batch numbers are assigned a unique (b) (4) number ranging from (b) (4). However, the lot numbers for the (b) (4) PPQ batches (b) (4) and (b) (4) post-PPQ batches (b) (4) do not follow this format. Please clarify.

The applicant submitted a response on October 4, 2024 as a part of amendment STN 125820/0.25. The firm explained that the (b) (4) numbering was mistakenly entered incorrectly into the (b) (4) system at (b) (4). As a result, the PPQ/post-PPQ batches were assigned batch numbers in the range of (b) (4) instead of (b) (4). The (b) (4) system has since been updated to align with the SOP, and future (b) (4) batches will be numbered within the (b) (4) range. The response is acceptable.

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

3.2.P.6 Reference Standards or Materials

The commercial primary and secondary reference standards for DP are the same as those used for (b) (4). Please refer to review of Section 3.2.S.5.

3.2.P.7 Container Closure System

Please refer to review of Section 3.2.P.2.4.

3.2.P.8 Stability

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

(b) (4)

Drug Product

The proposed shelf-life and storage conditions for the DP are 36 months at 2 - 8°C. In support of this claim, (b) (4) Phase 3 batches and (b) (4) PPQ batches were enrolled in long-term stability studies at 2 - 8°C and accelerated stability studies at (b) (4)

Long term stability study: For this study, the following characteristics were determined at regular intervals over the course of (b) (4) (Phase 3 batches) or (b) (4) (PPQ batches) (b) (4) appearance, pH, (b) (4) (PPQ batches), total protein concentration, (b) (4) % Adsorbed VLP(PPQ batches), aluminum content, sub-visible particles (PPQ batches), sterility, CCIT, and syringe functionality (b) (4) (b) (4) Data up to (b) (4) for (b) (4) phase 3 CTM batches (2861- (b) (4) up to 24 months for phase 3 batch (b) (4) and up to 6 month for (b) (4) PPQ batches (b) (4) (b) (4) were provided. All results met the pre-defined acceptance criteria.

Accelerated stability study: The analytical methods and acceptance criteria are identical to the long-term stability study specifications. The (b) (4) month accelerated stability study at 25°C is complete. All results were within the pre-defined acceptance criteria in place at the time of testing, suggesting that DP is stable for at least (b) (4) months at 25°C.

Finished Drug Product

(b) (4) Phase 3 clinical batches and (b) (4) PPQ batches were placed on stability at long-term storage conditions of 2 - 8°C. The stability indicating attributes for Phase 3 batches included visual inspection of package and components, container content, CCIT, and sterility. The stability results were provided up to 36 months for (b) (4) Phase 3 batches (b) (4) up to three months for the (b) (4) PPQ batches (b) (4) All results met the pre-defined acceptance criteria.

Additional Stability Studies

The following additional stability studies were conducted over the course of CHIKV VLP development:

Cumulative Hold Study: To simulate worst-case scenario, the engineering DP Batch (b) (4) produced from (b) (4) has been placed in a cumulative stability study including: (b) (4)

(b) (4) Data up to (b) (4) were provided for long-term stability and up to six months for accelerated stability. All data generated to date remained within the acceptance criteria in place at the time of testing.

Temperature Cycling Studies: Temperature cycling studies included studies on room temperature cycling and temperature excursions at (b) (4) Room temperature cycling studies were conducted with DP Batch (b) (4) and consisted of (b) (4) groups with (b) (4) (b) (4) cycles of (b) (4) storage, (b) (4) storage, (b) (4)

(b) (4)

Photostability: The photostability study was conducted on Engineering DP Batch (b) (4) (b) (4) Conditions consisted of (b) (4)

Samples without secondary packaging showed decreased (b) (4) and a (b) (4) (b) (4) indicating that the samples are sensitive to light exposure. In contrast, results from samples enclosed in secondary packaging showed no significant degradation or change in product characteristics, suggesting that the secondary packaging is sufficient to protect the DP from light.

(b) (4)

3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment

Bavarian Nordic commits to placing (b) (4) DP filled in PFS on long-term stability testing at 2 - 8°C each year. The (b) (4) DP stability specifications are presented in Tables 14 and 15 in Section 3.2.P.5.1 of this memo. (b) (4) samples will be tested at intervals of (b) (4) DP samples will be tested at 0, 12, 24, 36, (b) (4) -month intervals.

Overall Reviewer's Assessment of Section 3.2.P.8:

- On October 17, 2024, the following IR was sent to the applicant:

Please define the "date of manufacture" for the Drug Product of Chikungunya Vaccine, Recombinant.

The applicant responded on October 28, 2024 under STN 125820/0.33. The “date of manufacture” of the CHIKV VLP DP is defined as the date that the (b) (4) (b) (4) The response is acceptable.

- ❑ For post-approval stability lot selection, we noted during inspection that the (b) (4) lot produced in each product stage for the calendar year is chosen, provided it is representative. If a critical deviation occurred during the production of the scheduled annual stability lot, the next acceptable lot is chosen for the annual stability study.
- ❑ The stability data support the proposed shelf life of 36 months at 2 - 8°C for DP. The post-approval stability protocol is acceptable.

3.2.A APPENDICES

3.2.A.1 Facilities and Equipment

Facilities and Equipment is reviewed by DMPQ.

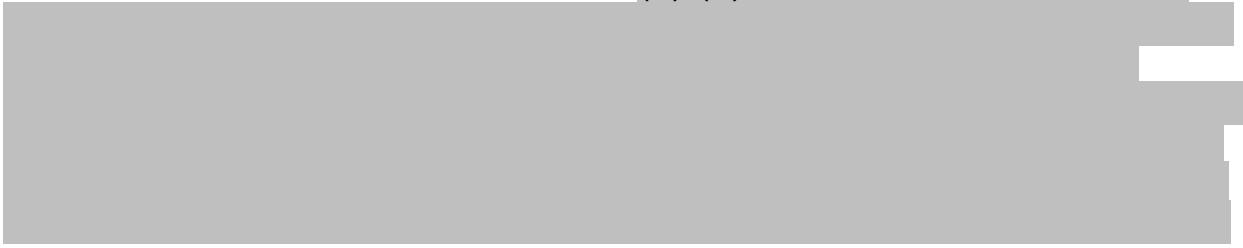
Overall Reviewer’s Assessment of Section 3.2.A.1:

- ❑ Please refer to DMPQ reviewer’s memo for review information pertaining to this section.

3.2.A.2 Adventitious Agents Safety Evaluation

See Section 3.2.S.2.3 in this memo for assessment of materials of biological origin in (b) (4) and for the adventitious agent’s safety evaluation of the (b) (4) cells and (b) (4) plasmid lots used to produce (b) (4). No raw material of human or animal origin has been used at any manufacturing stage.

The safety of the vaccine with regard to viral and non-viral contamination has been assessed through the following approaches: (b) (4)



Viral Clearance Studies

The CHIKV VLP vaccine does not contain live or inactivated viruses. Therefore, viral clearance studies were performed to demonstrate the removal and/or inactivation of potential adventitious viral agents. The applicant stated that the (b) (4)

(b) (4) is the (b) (4) step for viral clearance. To assess the (b) (4) ability to remove viruses, (b) (4)

The non-specific model viruses tested in the clearance studies were selected to represent a broad range of viruses and are listed as follows:

(b) (4)

(b) (4)

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

The executed batch records were reviewed during the pre-license inspection. The provided batch records were deemed acceptable, and no objectionable findings were noted.

☐ Method Validation Package

Method 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

VIMKUNYA is supplied in a single dose 1-mL pre-filled syringe. Per FDA Guidance for Industry and FDA Staff “Current Good Manufacturing Practice Requirements for Combination Products”, the pre-filled syringe is considered as a combination product. Please refer to the memo from the device reviewer Andrea Gray.

❑ Comparability Protocols

There are no comparability protocols for this BLA.

Overall Reviewer’s Assessment of Section 3.2.R:

- ❑ The information provided is acceptable.

Other eCTD Modules

Module 1

A. Environmental Assessment or Claim of Categorical Exclusion

Bavarian Nordic 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

The applicant has requested reference product designation in section 1.3.5.3 of the BLA. No biological product that has the same molecular target or that shares some of the same principal molecular structural features as CHIKV VLP vaccine has previously been the subject of a BLA. The FDA has not previously approved under Section 351(a) of the Public Health Service (PHS) Act any biological product that is structurally related to the biological product that is the subject of the 351(a) application being considered. Specifically, no biological product containing the active ingredient of CHIKV VLP vaccine, the CHIKV Senegal strain 37997, has been approved in any other application under Section 351(a) of the PHS Act. Therefore, reference product designation was agreed upon.

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 6: Adverse Reactions
- Section 11: Description
- Section 12: Clinical Pharmacology
- Section 13: Nonclinical Toxicology
- Section 16: How Supplied/Storage and Handling

Carton and Container Label:

The CMC information provided on carton and container labels 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

Preclinical evaluations of the CHIKV VLP vaccine have been studied in academic and industry laboratories using a variety of animal models. Table 16 summarizes the nonclinical studies submitted to Section 4.2.1.1 of the BLA.

Table 16 Nonclinical Studies

Report Number	Animal Used	Study Objective
RPT062501	Mice, NHPs	To evaluate the immunogenicity and protection of the unadjuvanted CHIKV VLP vaccine in mice and NHPs
PTR-TRD-CHIK-004	Mice	To evaluate the immunogenicity CHIKV VLP formulated with and without alum vaccine in mice
PAS-Mouse-CHIK-001	(b) (4) mice	To determine the titer of human serum neutralizing antibodies required to confer protection against CHIKV lethal challenge
PAS-NHP-CHIK-001P	NHPs	To determine the challenge dose of CHIKV strain (b) (4) in cynomolgus macaques
PAS-NHP-CHIK-001	NHPs	To evaluate the immunogenicity and efficacy of both nonadjuvanted and alum-adjuvanted (b) (4) PXVX0317 in NHPs
PAS-NHP-CHIK-002PK	NHPs	Pharmacokinetics study

Report Number	Animal Used	Study Objective
PAS-NHP-CHIK-002	NHPs	To test the efficacy of CHIKV hyperimmune IgG, purified from human volunteers of the clinical trials of PXVX0317, in cynomolgus macaques
PAS-NHP-CHIK-003	NHPs	To establish a threshold titer for protection after vaccination with the CHIKV VLP vaccine.

RPT062501: Chikungunya Virus Virus-Like Particle: Early-Stage Immunogenicity and Efficacy Studies Conducted by the Vaccine Research Center

The CHIKV VLP vaccine was developed by the VRC, NIH as a non-adjuvanted formulation. The VRC evaluated the immunogenicity and protection of the vaccine in several animal models including normal and immunodeficient mice, and nonhuman primates (NHPs).

Report RPT062501 contains the following 5 studies:

1. Immunogenicity of NIH VRC CHIKV VLP in (b) (4) Mice

Groups of female (b) (4) mice (n=5) were intramuscularly injected with 19 µg of CHIKV VLP twice at a 28-day interval. Sera were collected 10 days after the second dose and tested for neutralizing antibodies using pseudotyped lentiviral particles. The measured IC₅₀ titer was 9,600 for CHIKV strain 37997 and 12,031 for CHIKV strain LR2006 OPY-1, demonstrating that the antibodies elicited by the CHIKV VLP vaccine neutralize both homologous (strain 37993) and heterologous (strain (b) (4) (b) (4) CHIKV strains.

2. Immunogenicity and Protectiveness of NIH VRC CHIKV VLP in (b) (4) (b) (4)

Six (b) (4) were immunized with 20 µg of CHIKV VLP three times on Day 0, 1 month, and 6 months. Sera were collected 10 days after each immunization for neutralization testing. A single immunization with CHIKV VLP stimulated the production of neutralizing antibodies, and the response was enhanced after a second injection. A third injection did not appear to further boost the immune response. Sera from immunized animals were able to neutralize both homologous (strain 37997) and heterologous (strain LR2006 OPY-1) strains with similar neutralization titers.

Animals were intravenously challenged with 1 x 10¹⁰ PFU of CHIKV strain (b) (4) (b) (4) 15 weeks after final immunization. Viremia was measured 24 hours after challenge with a plaque assay. Animals immunized with CHIKV VLPs had no detectable viremia after challenge whereas control unimmunized animals (n=3) had a viral load of 10⁴-10⁵ PFU/mL.

3. Protection Against Heterologous CHIKV Challenge in Immunodeficient Mice by Passive Transfer of IgG from (b) (4)

Purified IgG from either control or CHIKV VLP-immunized NHPs was IV transferred to Ifnar -/- mice (2 mg IgG per mouse). After 24 hours, animals were challenged with a lethal dose of 30 PFU CHIKV strain (b) (4) via intradermal administration. Mice that received IgG from CHIKV VLP-immunized NHPs had no detectable viremia after challenge, and all mice survived the challenge, whereas mice that received control IgG developed high level of viremia and died within 3 days of the challenge.

4. Comparative Immunogenicity of NIH VRC-manufactured Research vs Development CHIKV VLP Lots in Mice

In this study, the sponsor compared the immunogenicity of a research CHIKV VLP lot with two development lots (b) (4) of CHIKV VLP produced using the NIH VRC GMP manufacturing process. Groups of (b) (4) mice (n=5) received two IM immunizations of CHIKV VLP 28 days apart at one of three dose levels (0.2, 2.0 and 20 µg). Analysis of serum samples collected 10 days after the second immunization revealed that the research and development lots were similarly immunogenic and were able to induce dose-dependent neutralizing antibody responses in mice.

5. Comparative Immunogenicity of NIH VRC-manufactured Research vs Development and Clinical CHIKV VLP Lots in Mice

In this study, the sponsor compared the immunogenicity of a development lot (b) (4) with (b) (4) GMP lots (Lots (b) (4) of CHIKV VLP in mice. Groups of (b) (4) mice (n=5) received two IM immunizations of VLPs at one of four doses levels (0.02, 0.2, 2.0 and 20 µg) 28 days apart. Serum samples were obtained 10 days after the second immunization and tested for anti-VLP binding antibodies by (b) (4) and for neutralizing antibodies using (b) (4) (b) (4) (b) (4). Analysis of sera from CHIKV VLP immunized mice indicated that the development and the (b) (4) GMP lots were similarly immunogenic and were able to induce dose-dependent neutralizing antibody responses in mice.

PTR-TRD-CHIK-004: Immunogenicity Assessment of Chikungunya VLP Vaccine in Mice

This study was conducted to evaluate immunogenicity of CHIKV VLP Research Lot formulated with and without alum (b) (4) in (b) (4) (b) (4) mice.

Groups of (b) (4) hybrid mice (n=6 per group) were first immunized on day 0 and then boosted on day 35 with 0.2, 2, or 20 µg of PaxVax CHIKV VLP (research lot: (b) (4) with or without (b) (4) of alum. The 2 and 20 µg of VLP doses with (b) (4) of alum were prepared immediately before, 4 hours prior to, or one

day in advance of administration for comparison. All animals were immunized intramuscularly with a 0.1 mL of volume containing the vaccine dose. For comparison, mice were given CHIKV VLP generated by the VRC (Lot (b) (4)) with the same dose titration with and without alum, or alum only.

Two weeks after the first immunization, serum CHIKV-specific binding antibody titers were measured by (b) (4) . The obtained EC50 titers in pooled serum were 209, 1417, and 3899 for the PaxVax CHIKV VLP vaccine doses of 0.2, 2, and 20 µg without alum, respectively. The same doses formulated with alum resulted in higher EC50 titers of 1669, 4145, and 10423. The VRC CHIKV VLP vaccine induced similar responses compared to PaxVax CHIKV VLP. Responses to the 2 and 20 µg vaccine doses prepared at 4 and 24 hours prior to immunization were comparable to the same doses formulated just prior to administration. Neutralizing antibody titers against CHIKV were determined by a CHIKV-Luc neutralization assay. At Day 14, NT80 titers were 80, 385, and 1754 for the PaxVax CHIKV VLP vaccine doses of 0.2, 2, and 20 µg without alum and 1168, 2975, and 7075 with alum. The VRC CHIKV VLP vaccine material resulted in titers comparable to the PaxVax CHIKV VLP vaccine. Analysis of serum samples from days 28 and 56 showed that NT80 titers increased approximately 8-fold following the second immunization, with antibody responses rising in a vaccine dose dependent manner. Moreover, alum increased antibody response levels by approximately 6-fold after the first immunization and 3-fold following the second immunization, compared to the vaccine formulated without alum and supported the inclusion of alum in the vaccine.

PAS-Mouse-CHIK-001: Testing Normalized Volumes of Passively Transferred Human Sera Against Chikungunya Virus Challenge in (b) (4) Mice

In this study, human sera collected from human subjects vaccinated with Emergent's PXVX0317 chikungunya vaccine were passively transferred to (b) (4) mice (interferon alpha/beta receptor null), to determine the titer of human serum neutralizing antibodies (SNA) required to confer protection against CHIKV lethal challenge. The study was conducted at (b) (4) .

Before passive transfer, human serum was diluted in sterile PBS to the appropriate concentration or used neat. Groups of A129 mice (n=4) were passively transferred with varying concentrations of human sera in a 2.0 mL volume via intraperitoneal injections on Day -1. Control mice (n=4) were given 2.0 mL non-immune human sera. All mice were challenged with a lethal dose of 10 PFU of CHIKV strain (b) (4) through a footpad injection the next day. Just prior to challenge, mice blood was collected for SNA analysis. Following challenge, animals were observed and scored daily for 14 days.

Two sets of experiments were performed. In experiment 1, it was observed that administering large volume of human serum at concentrations higher than a 1:1 dilution in PBS was lethal to mice. However, mice that survived the passive immunization were

protected from lethal challenge, regardless of the pre-challenge SNA titers. Due to the serum toxicity, experiment 2 was performed with more diluted sera administered in a 2.0 mL volume. Partial protection was achieved by both high and low titer sera and no correlation between circulating SNA titer and survival of the CHIKV challenge was observed.

These findings indicated that this model is not suitable for use to determine the titer of human serum neutralizing antibodies required to confer protection against lethal challenge.

PAS-NHP-CHIK-001P: Pilot Study in Cynomolgus Macaques to Evaluate the Effect of Different Challenge Doses of Chikungunya Virus Strain (b) (4)

This study was conducted to determine the challenge dose of CHIKV strain (b) (4) (b) (4) in cynomolgus macaques that induces high viremia, clinical symptoms and pathology that recapitulate the infection and disease in humans.

Groups 1 - 3 of adult cynomolgus macaques (n=3) were inoculated intravenously with 10^6 , 10^7 or 10^8 PFU of CHIKV strain (b) (4) respectively. Animals were monitored closely for 10 days, at which time animals were euthanized for tissue analysis.

The sponsor evaluated the magnitude and kinetics of viremia (viral RNA and infectious virus), clinical symptoms (fever, rash, physical examination including ankle and wrist for joint effusion, other clinical signs indicative of pain or discomfort), pathology, kinetics of hematology, serum biochemistry and plasma inflammatory cytokines and chemokines.

Considering the virological, clinical, and pathological data, and balanced with the need to minimize the risk of fulminant infection with excessive inflammation and immune activation, an inoculation dose of 10^7 PFU was chosen for the subsequent active immunization studies.

PAS-NHP-CHIK-001: Immunogenicity and Protective Efficacy of PXVX0317 (Chikungunya Virus Virus-Like Particle Vaccine [CHIKV-VLP], Unadjuvanted or Alum-adjuvanted) against Chikungunya Virus Challenge in Cynomolgus Macaques

This study was performed to evaluate the immunogenicity and efficacy of both nonadjuvanted and alum-adjuvanted (b) (4) PXVX0317 in cynomolgus macaques.

Cynomolgus macaques were immunized intramuscularly on days 0 and 28 with one of three doses of CHIKV VLP (1.25, 6 or 20 µg, n=5 per group) with alum (300 µg), CHIKV VLP alone (20 µg, n=5), or alum alone (300 µg, n=4). Animals were challenged intravenously with 10^7 PFU of CHIKV (b) (4) on day 56 and euthanized on day 66.

Immunogenicity of PXVX0317: All animals immunized with CHIKV VLP (with or without Alum) developed high NT80 titers, which peaked at 2 weeks after the 2nd immunization. On day 56 before CHIKV challenge, NT80 titers in the immunized animals ranged from 3.2 to 4.5 log₁₀, with the groups' GMTs in the following order: 20 µg VLP only < 1.25 µg VLP/alum < 6 µg VLP/alum < 20 µg VLP/alum. In contrast, the alum only control group had no detectable NT80 titers prior to CHIKV challenge.

Efficacy of PXVX0317: Efficacy of the vaccine was evaluated by challenging both vaccinated and control macaques with CHIKV strain (b) (4) and assessing viremia, clinical symptoms, and pathology. After challenge, all control animals showed high levels of viral RNA and infectious virus in plasma, peaking within the first 2 days post-challenge and decreasing to undetectable levels by Day 10. In contrast, vaccinated animals exhibited no detectable infectious viremia and had reduced viral RNA levels. Accordingly, most control animals exhibited clinical signs, elevated levels of several liver and muscle enzymes, increased levels of C-reactive protein, and numerous inflammatory cytokines/chemokines. In contrast, vaccinated animals showed fewer symptoms, and had smaller changes in liver and muscle enzymes, C-reactive protein, and inflammatory cytokines/chemokines. At the time of euthanasia, all control animals exhibited joint pathology and high levels of viral RNA in joint-associated and muscle tissues, while immunized animals showed minimal or no joint and muscle pathology, with no detectable viral RNA in the tissues.

NHP Passive Transfer Studies

Since chikungunya outbreaks are sporadic and unpredictable, the feasibility of conducting randomized, controlled clinical disease endpoint efficacy trials for demonstrating the effectiveness of chikungunya vaccines is uncertain. A VRBPAC meeting was held on November 08, 2019, recommending that a NHP model using passive transfer of human sera or purified IgG prior to challenge with wild-type CHIKV could be used to identify a CHIKV-neutralizing antibody titer that is reasonably likely to predict clinical benefit in vaccinated humans (i.e., to support accelerated approval of this CHIKV vaccine), provided that the NHP model is sufficiently stringent. To establish a threshold neutralizing antibody titer for protection after vaccination with the CHIKV VLP vaccine, Bavarian Nordic conducted three NHP passive transfer studies: PAS-NHP-CHIK-002PK, PAS-NHP-CHIK-002, and PAS-NHP-CHIK-003.

1. PAS-NHP-CHIK-002PK: Report for Chikungunya Virus Neutralizing Antibody Titers in the NHP Pharmacokinetics (PK) Study

The objective of the NHP PK study was to determine the serum NT80 titer levels in NHPs after IV administration of CHIKV neutralizing IgG. The resulting NT80 titers at 24 hours post-IgG administration from the PK study would be used to set the three IgG dose levels in the subsequent NHP passive transfer study PAS-NHP-CHIK-002.

The human IgG was purified from subjects who had received two doses of 20 µg CHIKV-VLP vaccine PXVX0317 in Phase 2 clinical trial PXVX-CV-317-001. CHIKV seronegative cynomolgus monkeys (n = 3 per dose) were administered with a single IV injection of purified human IgG (CHIKV NT80 titer of 32,532.8 at 100 mg/mL) to reach final dose levels of 10, 30, or 100 mg human IgG/kg (Groups 1, 2, and 3, respectively). Serum samples were collected from each animal before injection (Predose) and then at 1, 12, 24, 48, 72, 96, 168, and 240 hours post-injection. The resultant NT80 titers at each time point were determined using a CHIKV-Luc neutralization assay. The CHIKV-Luc neutralization assay was performed and validated by Emergent Travel Health. The validation report was provided in Section 4.2.2.1 of this BLA and is reviewed below.

All Predose NT80 titers were found to be below the assay LOD of 19. At 24-hour post-administration, when NHPs were challenged during the NHP passive transfer studies, the GMTs for the 10, 30, and 100 mg/kg groups were 51.6, 191.6, and 536.8, respectively, indicating a linear relationship with the administered IgG dose ($R^2 = 0.989$). Based on the data, to achieve NT80 titers of 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) at the time of challenge, doses of 5, 15, and 100 mg/kg from this IgG lot should be used in the following passive transfer study.

2. PAS-NHP-CHIK-002: Protective Efficacy of Passively Transferred Human IgG Against Chikungunya Virus Challenge in Cynomolgus Macaques

The objective of this study was to test if circulating CHIKV serum neutralizing antibodies alone protect cynomolgus macaques against viremia following CHIKV challenge. The study was performed based on study protocol PAS-NHP-CHIK-002 submitted in IND 17998 Amendment 16. This protocol was reviewed and found to be acceptable.

Study Approach

The human IgG was purified from volunteers who had been vaccinated with two doses of 20 µg CHIKV VLP vaccine in the Phase 2 clinical trial PXVX-CV-317-001.

On day 0, four groups of cynomolgus macaques (n=6, 3 males, 3 females) were passively transferred the purified human IgG at three dosages (5, 15 or 100 mg/kg body weight) or negative control IgG in the right saphenous vein.

NHPs were challenged IV with 10^7 PFU of CHIKV LR2006-OPY1 strain 24 hours later.

Just before challenge, a blood sample was drawn from each animal for the determination of neutralizing anti-CHIKV antibody titers analyzed by the CHIKV-Luc

neutralization assay. This measured titer was used to determine the neutralizing antibody titer required to protect NHPs from CHIKV viremia.

Following infection, animals were monitored for 10 days for clinical signs of infection, and blood was drawn at various timepoints to measure viremia, cytokine levels and SNA titers. Plasma viremia (viral RNA) was quantified via a validated RT-qPCR assay by Battelle Biomedical Research Center. The validation report was provided in Section 4.2.2.1 of this BLA and is reviewed below.

On day 11, NHPs were euthanized to collect tissues for virological and histological analyses.

Results

Neutralization Titer in Serum of NHPs Prior to Challenge

The NT80 titers measured for each individual animal on day 0 prior to IgG transfer and on day 1 before CHIKV challenge were presented on page 194 of the report. Twenty-two out of 24 pre-transfer NT80 titers (day 0) were found to be below the assay LOD of 19. The other two NT80 values, one from the 15 mg/kg group and one from the 100 mg/kg group, were 37.4 and 32.3, respectively. On day 1 just before the challenge, GMTs for the 100, 15 and 5 mg/kg dose groups were 644, 101, and 38, respectively. All 6 control IgG titers were below the assay LOD.

Plasma Viremia

Following CHIKV infection, viremia was monitored by RT-qPCR, giving a read out in copies/mL, from day 2 to day 11. The data generated were presented in Figure 2 and the raw data were included in Appendix 9 (page 35) of the report. CHIKV RNA was detected in all control group animals with peak titers of $2.8 - 7.2 \times 10^9$ RNA copies/mL observed on days 2 and 3, and then subsided to low or undetectable levels by 10 days after inoculation. For animals receiving anti-CHIKV IgG, 0 out of 6, 1 out of 6, and 5 out of 6 animals had no detectable viremia above the LOD ($2.46 \log_{10}$ copies/mL) in the 5, 15 and 100 mg/kg dose groups, respectively. Only 6 animals in the 5 mg/kg group and 1 animal in the 15 mg/kg group showed viral RNA levels above the assay LLOQ of $3.02 \log_{10}$ copies/mL, with peak RNA levels 3 - 6 logs lower than those in control animals.

The sponsor further measured infectious virus levels in plasma by plaque assay. All control animals had detectable infectious virus in plasma, with peak levels ranging from 6.44 to 7.27 \log_{10} PFU/mL on day 2. Infectious virus titers became undetectable by 6 days after virus inoculation. Conversely, all animals that received CHIKV IgG were completely protected from infectious virus.

These data suggest that circulating SNA alone can protect NHPs against viremia following CHIKV challenge.

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

The objective of study PAS-NHP-CHIK-003 was to establish a threshold titer for protection after vaccination with the CHIKV VLP vaccine.

This study was conducted at (b) (4) in (b) (4) under protocol STDY-21-0004 titled “Efficacy of serum from CHIKV VLP-vaccinated human volunteers against chikungunya virus in cynomolgus macaques.”

Study Approach

Pooled human sera were obtained from volunteers vaccinated with a single 40 µg dose of CHIKV VLP vaccine in the Phase 2 clinical studies PXVX-CV-317-001 Group 10 and EBSI-CV-317-002. The NT80 titer of the pooled human sera was determined to be 2470.

The purified CHIKV IgG lot (Lot: (b) (4)) used in this study was the same as that used in Study PAS-NHP-CHIK-002.

28 NHPs (Cynomolgus macaque) were randomized by gender and body weight into two challenge cohorts and six groups. NHPs were confirmed seronegative for CHIKV prior to serum or IgG transfer.

On day -1, one of 4 doses of CHIKV immune sera (0.3, 0.6, 1.2, or 2.4 mL/kg), purified CHIKV IgG (0.075 mL/kg) or non-immune control serum (2.4 mL/kg) were administered via IV injection. Groups receiving 0.3, 0.6, or 1.2mL/kg of CHIKV immune sera and the control group included 6 NHPs each, while groups receiving 2.4 mL/kg CHIKV immune sera or purified CHIKV IgG included 2 NHPs each.

On day 0, NHPs were challenged with 1.0×10^5 PFU of CHIKV strain (b) (4) in a volume of 0.5 mL via subcutaneous route.

After challenge, clinical scores, body weight, rectal body temperature, plasma viremia, neutralizing antibody titers, clinical chemistry, and hematology were assessed. In addition, animals were observed for 10 days for clinical signs of disease.

Study Endpoint

The primary endpoint was protection against viremia (RNAemia) as measured by RT-qPCR.

Protection against viremia was defined as RT-qPCR value of gc/mL below the LLOQ (9.31×10^2 gc/mL) of the validated RT-qPCR assay, encompassing all sampling timepoints post-challenge.

Results

Serum Neutralizing Antibody (SNA) Titer in NHPs Prior to Challenge

NT80 titers of SNA were measured for each individual animal on day -1 prior to administration of human serum or IgG and on day 0 before CHIKV challenge. The results were presented in Table 8 (page 21) of the report. On day -1, NT80 titers of all animals were \leq LOD of 12.3. On day 0 prior to challenge, Groups of NHPs which received CHIKV sera at doses of 2.4, 1.2, 0.6 and 0.3 mL/kg had GMTs of 61.3, 35.4, 21.3, and 11.4, respectively. Animals that were administered purified IgG had a GMT of 32.2, and control animals had no measurable SNA titer.

Plasma Viremia

Following CHIKV infection, viremia was monitored for viable virus by plaque assay and for viral RNA by RT-qPCR.

Infectious virus was detected from all negative control animals. On day 1, 5/6 control NHPs developed viremia with a mean titer of 5.66×10^4 PFU/mL. On days 2 and 3 all controls were viremic with mean titers of 9.47×10^4 and 8.02×10^3 PFU/mL, respectively. Viremia resolved by day 4 in all animals. In contrast, no viable virus was detected in any of the samples from groups receiving CHIKV sera or IgG.

Analysis of viremia by RT-qPCR demonstrated that CHIKV sera protected animals from viremia in a dose-dependent manner.

In control animals, CHIKV RNA was detected in all 6 animals as early as day 1 post-challenge, the peak of viral RNA was observed on day 2 post exposure. At day 5, CHIKV RNA titers were above the LLOQ (9.31×10^2 gc/mL) in 4 out of 6 animals. At day 10, one animal was still viremic.

Viral RNA was not detected in the CHIKV IgG group, or the groups treated with 2.4 or 1.2 mL/kg of CHIKV sera.

Treatment with 0.6 mL/kg CHIKV sera resulted in no viral RNA detection in 4 out of 6 animals. Low levels of CHIKV RNA were detected in two animals on day 1, decreased to undetectable levels on day 2, increased again by day 3, and then continued to rise steadily on days 4 and 5. No viral RNA was detected on day 10.

CHIKV RNA was detected in all 6 NHPs treated with 0.3 mL/kg CHIKV sera. CHIKV RNA copy numbers were above LLOQ at days 1 through 5 for most animals. No animals had RNA levels $>$ LOD at day 10. In all animals, viral RNA loads were largely decreased compared with control animals.

Body Temperature

Following CHIKV challenge, the group that received control sera consistently exhibited higher body temperatures compared to the other groups. However, this group also displayed higher body temperatures from day -4 to day 0 prior to the

challenge. After CHIKV challenge, the body temperatures of the control animals remained within the normal range for *M. fascicularis*, which is 37.2 to 39.2 °C, indicating that no fever was observed in the study.

Reviewer comment: On December 11, 2019, the following Post-VRBPAC Comments were sent to the applicant: 4A, We consider complete protection against viremia and fever in NHPs challenged with wild type CHIKV as appropriate endpoints to assess vaccine effectiveness. The challenge dose should reliably produce high level viremia and fever in control animals and should be upwards of the estimated viral titer transmitted by mosquitos to humans during natural infection. 4C, We acknowledge that we previously agreed to your plans for IV administration of the challenge virus. Upon further internal discussion, we believe that the IV route is not sufficiently reflective of natural infection, as neutralization of the virus in the bloodstream by circulating passively transferred antibodies may interfere with initial steps of infection that may occur following inoculation from mosquito bites. Using the intradermal (ID) route to administer the challenge virus appears to induce similar viremia kinetics as intravenous challenge and more closely mimics the natural route of CHIKV infection. Thus, we consider the ID challenge route to be most appropriate for the NHP passive transfer model.

At the End of Phase 2/Pre-Phase 3 meeting, which took place on December 17, 2019, Emergent stated that due to its transient and variable nature, fever is not an appropriate endpoint to support the selection of the protective antibody threshold based on the NHP passive transfer study and questioned whether protection from viremia alone will be considered as an appropriate endpoint to support the selection of protective antibody threshold. After discussion, CBER agreed that protection from viremia alone can be considered as an appropriate endpoint to support the selection of protective antibody threshold. At the meeting, Emergent and CBER also discussed on the issue of challenge route. Emergent questioned that in the event they need to develop an alternate NHP model of CHIKV infection, whether a subcutaneous (SC) challenge route be acceptable, and CBER agreed that a SC challenge route is acceptable.

In addition to PAS-NHP-CHIK-003 study report, Module 4 of this BLA also includes three NHP studies, performed at the (b) (4)

(b) (4) None of these studies demonstrated consistent increase in body temperatures among cynomolgus macaques after receiving an intravenous inoculation of 10^7 PFU of (b) (4) (monitored for 10 days).

At the labeling meeting for STN 125820/0 on January 21, 2025, after discussion with OVR management, the review team decided not to include languages regarding fever in Section 13.2 – Animal Toxicology and/or Pharmacology of the prescribing information.

Protective SNA Threshold Titer

The relationship between pre-challenge circulating SNA titer and protection against viremia was evaluated using a logistic regression model, which was fit to the binary response data (where success is no viremia \geq LLOQ) with log₁₀ day 0 SNA antibody titer as a predictor.

From this model, an 80% probability of preventing viremia \geq LLOQ was associated with a day 0 SNA antibody titer of 23.6, while a 90% probability of preventing viremia \geq LLOQ was associated with a Day 0 SNA antibody titer of 25.9. Based on this analysis, the sponsor proposed that the protective antibody threshold level be set at 23.6.

Reviewer comment: After reviewing the information submitted to IND 17998 Am 73 in support of the proposed protective human SNA threshold titer (NT80) level of 23.6, CBER provided comments to the sponsor on February 3, 2022. CBER did not agree with the proposed SNA titer of 23.6 as a protective threshold because the proposed predicted probability of 80% is low and its corresponding 95% CI (34%, 97%) is too wide to provide sufficient assurance to support this titer as a surrogate endpoint. Based on the data from the NHP study, CBER calculated that an SNA titer of 50 has a 95% CI lower bound of $>80\%$. However, given the inherent variability of neutralization assays (which is 2-fold) and the small number of animals used to determine the threshold titer, CBER recommended a more conservative NT80 titer of 100 to be reasonably likely to predict protection in humans and therefore to be an acceptable surrogate endpoint to support licensure of the product by the accelerated approval pathway. In response to CBER comments, the sponsor provided their justifications to IND 17998 Am 80 on March 20, 2022, in support of retaining the originally proposed SNA threshold of 23.6. Nonetheless, CBER did not agree with the statistical and clinical arguments the sponsor offered in support of the justifications and provided follow up comments to the sponsor on April 14, 2022. Given the limitations of the supporting data and that this threshold titer will form the basis for licensure of the product via the accelerated approval pathway, CBER considered the more conservative value of 100, previously proposed by CBER, to be reasonable and appropriate. The sponsor responded on June 2, 2022 and asked if a threshold value of 48.5 or 62.0 is acceptable. In the comment sent on July 25, 2022, CBER maintained that a titer threshold value of 100 is still reasonable and appropriate. The sponsor agreed with the titer threshold value of 100 and a surrogate of protection with a NT80 titer of ≥ 100 was applied to clinical evaluation of CHIKV VLP in Phase 3.

4.2.2 Pharmacokinetics

4.2.2.1 Analytical Methods and Validation Reports

Study Number VA-5464: Validation of the Chikungunya Virus (CHIKV) Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) Assay in Non-Human Primate Plasma

This report describes the validation of the RT-qPCR that is used to quantify CHIKV-specific target RNA in NHP plasma samples. This assay was used in the NHP passive studies, which were conducted to determine a threshold titer for protection to be used as a surrogate in clinical trials.

Assay Description

The method for determination of CHIKV RNA includes (b) (4)
(b) (4) .

(b) (4)

Assay Validation

(b) (4)

6 pages have been determined to be not releasable: (b)(4)

(b) (4)

4.2.2.7 Other Pharmacokinetic Studies

On January 5, 2024, the following comment was sent to BN as part of the pre-BLA CMC written responses: “In your response to CBER comment 3b submitted under IND 17998 Amendment 47, you have committed to conducting additional animal immunogenicity studies to demonstrate that the (b) (4) assay (b) (4) measures the (b) (4) and is stability indicating. Please submit data and final study reports from relevant studies to the BLA.” In response to this comment, the applicant submitted the final study reports to Section 4.2.2.7.

The (b) (4) method is reviewed in Section 3.2.S.4.2 of this memo. This method uses a (b) (4) performed on the (b) (4)

(b) (4)

To establish a correlation between the (b) (4) assay and the (b) (4) (b) (4) model, (b) (4) studies were conducted as follows:

(b) (4)

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(b) (4)

Module 5 Clinical Study Reports

5.3.1.4 Reports of Bioanalytical and Analytical Methods for Human Studies

Validation of a Chikungunya Luciferase Neutralization Assay in Human Serum

The CHIKV luciferase neutralization method described in Module 4 is also used to analyze human sera from vaccinees participating in clinical trials including pivotal clinical trials.

Report RPT053048 titled “Validation of a Chikungunya Luciferase Neutralization Assay” summarizes the results of the validation of SOP043421 “Chikungunya Luciferase Neutralization Assay” following validation protocol PRO047111 “Validation of Chikungunya Luciferase Neutralization Assay.”

(b) (4)

4 pages have been determined to be not releasable: (b)(4)