

Toxicology Review of BLA/STN 125820 of VIMKUNYA Chikungunya Vaccine, Recombinant

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File: BLA 125820/0, also reviewed amendment 31, 39 and 61

Product: VIMKUNYA, Chikungunya Vaccine, Recombinant

Subject: Review of toxicology data

Reviewer: Claudia Wrzesinski

Reference: BLA sections reviewed:
4.2.3.2 Repeat-dose Toxicity
4.2.3.5 Reproductive and Developmental Toxicology

Sponsor: Bavarian Nordic, Inc.

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EXECUTIVE SUMMARY:

The sponsor is developing a Chikungunya Virus Virus-Like Particle (CHIKV VLP) vaccine for the prevention of disease caused by chikungunya virus infection in individuals 12 years of age and older. The CHIKV VLP vaccine is a sterile aluminum hydroxide adjuvanted enveloped VLP. The safety and toxicity of unadjuvanted CHIKV VLP vaccine was evaluated in a repeat-dose toxicity study in rabbits (study MRI 110752.01.001) and two Developmental and Reproductive Toxicology (DART) studies, one in rabbits (CHIK-DART-001) and another one in rats (CHIK-DART-002). The toxicology program evaluated the intended dose of 40 µg applied to humans.

The repeat-dose toxicity study did not demonstrate any safety concerns and confirmed that the rabbit is a pharmacologically relevant species. A dose of 1 mL CHKVLP059 (40 µg VLP) was given four times at 3-week intervals. No CHIKV VLP-attributable adverse effects were observed for body weight changes, clinical signs, body temperature, injection site reactions, ophthalmic assessments, hematology, clinical chemistry, necropsy, and histopathology. Any changes observed were either within the normal variation observed for the species or procedure-related (IM injection trauma). The test article in this repeat-dose toxicity study was unadjuvanted CHIKV VLP vaccine (CHKVLP059). However, aluminum hydroxide has been safely used over six decades in other licensed vaccines (FDA Common Ingredients in U.S. Licensed Vaccines (FDA, 2019). Therefore, a second repeat-dose toxicity study using adjuvanted CHIKV VLP vaccine, was not considered necessary.

The first DART study (CHIKDART-001) was performed in rabbits and included a C-section cohort as well as a natural delivery cohort. Subsequently the sponsor performed a second DART study (CHIKDART-002) which was performed in rats and only included a natural delivery cohort. Both DART studies were similar in design and tested the full human dose. Female rabbits received a full human dose (0.8 mL) of VIMKUNYA by intramuscular injection on five occasions: 28 and 14 days prior to start of cohabitation, on Gestation Days 7 and 21 and on Lactation Day 7. The study did not demonstrate any adverse effects on the fertility and embryo-fetal development. An increased kit mortality was observed on Lactation Day 4 over the concurrent control group and the overall mean postnatal survival at PND28 (last evaluated time point) of kits was 69.0% for the control group and 42.3% for the vaccinated group with a (p-value of 0.0008262), the historical control data showed a range for postnatal survival from 47.6% to 91.4% with a mean of 71%, from 18 studies. Additionally, a decrease in activity was seen in kits of the vaccinated group. Since it cannot be excluded that the effect on the kits is treatment related, it should be reported in the package insert. Additionally, the sponsor is planning to perform a pregnancy registry. Subsequently, the sponsor performed a second reproductive toxicity study (CHIK-DART-002) in a second species ^{(b) (4)} rats). In the CHIK-DART-002 study female rats received a full human dose (0.8 mL) of VIMKUNYA by intramuscular injection on five occasions: 28 and 14 days prior to start of cohabitation, on Gestation Days 7 and 21 and on Lactation Day 7. No serious test article related effects were observed on fertility or postnatal development, and importantly, no increased pup mortality was observed.

Overall, the sponsor submitted sufficient nonclinical toxicology data for the approval of BLA 125820.

PRODUCT: VIMKUNYA, Chikungunya Vaccine, Recombinant

PROPOSED USE: Prevention of disease caused by chikungunya virus infection in individuals 12 years of age and older.

INTRODUCTION:

Chikungunya disease is caused by infection with chikungunya virus (CHIKV), an arthritogenic alphavirus in the *Togaviridae* family and is transmitted to humans through the bite of infected mosquitoes. The virion contains a positive-sense single-strand ribonucleic acid (RNA) genome with a long open reading frame coding for C, envelope (E1, E2, E3) and 6K structural proteins, together with four nonstructural proteins (nsP1–4). Chikungunya virus is transmitted to humans through the bite of mosquitoes infected with CHIKV, primarily *Aedes aegypti* and *Aedes albopictus*. Once infected, a mosquito is infectious for the rest of its life and can transmit virus to multiple hosts. Although mosquitoes are the primary mode of transmission of CHIKV, blood-borne transmission via needle stick is possible and maternal-fetal transmission has been documented during pregnancy. Globally, over two million cases have been reported since 2005. Chikungunya disease has now been identified in over 110 countries in Asia, Africa, Europe, and the Americas (WHO, 2024a). The onward transmission of CHIKV in mainland US and Europe is mostly linked to importation of the virus by viremic travelers into receptive areas with established and active competent vectors (*Aedes* mosquitoes), which are established in Europe and the US (Leta, 2018; Grabenstein, 2023). While most US and European cases of chikungunya remain imported, locally acquired cases were reported sporadically in the European Union (EU) (Italy and France in 2007, 2010, 2014, and 2017 [ECDC, 2023]) and in US or US Territories (CDC, 2024b) and are susceptible to increase. The recent surge in cases in Latin America, partially attributed to warmer than average temperatures in the region (Giovanetti, 2023), underscores the multiple factors contributing to the global persistence of CHIKV as a public threat.

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

age and more severe acute symptoms are associated with worse arthritic sequelae (van Aalst, 2017).

Chikungunya virus (CHIKV) virus-like particle (VLP) vaccine, abbreviated as CHIKV VLP vaccine (previously designated PXVX0317), is a sterile aluminum hydroxide adjuvanted vaccine. The CHIKV virus-like particle (VLP) is comprised of three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997: Capsid (b) (4) Envelope 1 (b) (4) and Envelope 2 (b) (4) which self-assemble to form a spherical, highly ordered VLP. The CHIKV VLP drug product is a sterile aqueous buffered suspension comprised of 40 µg CHIKV VLP adsorbed on aluminum hydroxide adjuvant (300 µg aluminum) and stabilized with formulation buffer. It is supplied as single dose 1-mL pre-filled glass syringe with 0.8 mL deliverable dose volume of CHIKV VLP vaccine for intramuscular administration.

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

STUDIES SUBMITTED:

Repeat dose toxicity study:

1. Study no. 110752.01.001 “AV7909: Repeat-dose Toxicity Testing of VRC-CHKVLP059-00-VP”

Reproductive and developmental toxicity study:

2. Study no. 20313401 “A Fertility and Postnatal Developmental Toxicity Study of PXVX0317 Vaccine by Intramuscular Injection in Female Rabbits”
3. Study no. 2256-019 “An Intramuscular Vaccine Reproductive Study of PXVX0317 in Rats”

IRs sent to the sponsor

- 1) IR23 was sent regarding DART study no 20313401 and was acceptable answered by the sponsor with amendment 31.
- 2) IR 32 was sent as a follow up IR to amendment 31 and was acceptable answered by the sponsor in amendment 39.
- 3) Amendment 60 was submitted by the sponsor as response to advice given by CBER regarding their label, the sponsors response in the amendment was acceptable.

Proposed labeling text:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in individuals exposed to VIMKUNYA during pregnancy. Individuals who receive VIMKUNYA during pregnancy are encouraged to contact, or have their healthcare provider contact, 1-800-XXX-XXXX to enroll in or obtain information about the registry.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are no clinical studies of VIMKUNYA in pregnant individuals. Data on VIMKUNYA administered to pregnant individuals are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study was performed in female rabbits, administered the equivalent of a single human dose of VIMKUNYA on 5 occasions, twice prior to mating, twice during gestation and once during lactation. In this study, postnatal survival of the kits was reduced; there were no adverse effects on other postnatal development parameters. There were no adverse effects on female fertility; and there was no evidence of harm to the fetus due to the vaccine. A developmental toxicity study was performed in female rats administered the equivalent of a single human dose of VIMKUNYA on 5 occasions, twice prior to mating, twice during gestation and once during lactation. In this study there were no adverse effects on postnatal survival and on other postnatal development parameters. There were no adverse effects on female fertility. [see Data].

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

Vertical transmission of wild-type CHIKV to neonates from pregnant individuals with viremia at delivery is common and can cause severe, potentially fatal CHIKV disease in neonates, with neurologic (e.g., encephalopathy, intracranial hemorrhage) and myocardial manifestations) [citation].

Data

Animal Data

In a pre- and postnatal developmental study with an embryo-fetal development toxicity phase performed in female rabbits, a full human dose (0.8 mL) of VIMKUNYA was administered by intramuscular injection on five occasions: 28 and 14 days prior to start of cohabitation, on Gestation Days 7 and 21 and on Lactation Day 7. Among kits born in the control group, 69% of the kits [95% confidence interval (CI) (57.8%, 80.1%)] survived compared to the 42% of kits [95% CI (31.5%, 52.8%)] born to vaccinated mothers (the historical control data showed a range for postnatal survival from 47.6% to 91.4% with a mean of 71%, from 18 studies); all other postnatal development parameters were not affected. There were no adverse effects on female fertility; and there was no evidence of harm to the fetus due to the vaccine.

In a pre- and postnatal developmental study performed in female rats, a full human dose (0.8 mL) of VIMKUNYA was administered by intramuscular injection on five occasions: 28 and 14 days prior to start of cohabitation, on Gestation Days 7 and 21 and on Lactation Day 7. No vaccine related adverse effects on female fertility or postnatal development were observed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

VIMKUNYA has not been evaluated for the potential to cause carcinogenicity, mutagenic potential, or for impairment of male infertility in animals.

Repeat dose toxicity study:

AV7909: Repeat-dose Toxicity Testing of VRC-CHKVLP059-00-VP

Study number: (b) (4) No. 110752.01.001

Performing laboratory: (b) (4)

Final Report date: January 4, 2012

Test article batch/lot: VCR-CHKVLP059-00-VP Chikungunya Virus Vaccine: lot number: (b) (4) (b) (4) in formulation buffer

(b) (4)

Animal species and strain: (b) (4) rabbits

Breeder/supplier: (b) (4)

Number of animals per group and sex: 5

Age: 12 to 16 weeks

Body weight range: 2.5 to 4kg

Route and site of administration: Intramuscularly

Volume of injection: 2x 0.5 mL

Frequency of administration and study duration: Day 1, 22, 43, and 64

Dose: 40 µg

Stability: Means of administration: Needle and syringe

Report status: Final

Experimental design:

Group	Treatment	Vaccine dosage	Total rabbits per group (M/F)
1	(b) (4)	0	(10/10)
2	VR-CHIKVLP-059-00-VP	40 µg in 2x0.5 mL	(10/10)

Table 1: Study design

Randomization procedure: The animals arrived at the test facility 11 days prior to study initiation. They were individually housed and underwent an 11-day quarantine/acclimation period during which the rabbits were allowed to recover from the stress of transport, new housing, potential dietary changes, and new handlers. All of the animals were weighed within 72 hours of arrival. The (b) (4) Veterinarian examined the animals and released them for the study. All rabbits were in good health and free from malformations and signs of clinical disease. The animals were received from a source that monitored for specific pathogens and the health status records from the source animals were reviewed prior to study initiation to ensure their suitability.

Statistical analysis plan:

Statistical analyses were conducted for the following in-life parameters: clinical observations, physical/orthopedic exams, body weight (change from baseline), body

temperature (change from baseline), dose site evaluation, food consumption, clinical chemistry and hematology and organ weights. The following sections describe the statistical analyses in detail for each set of data.

Statistical analyses were not performed for immunoassay results or histopathology findings results. Summary statistics were generated by sex and treatment group. Continuous-scale parameter summaries included the number of observations, mean, standard deviation, median, minimum and maximum values. The number of observations and corresponding frequencies of observation types were provided for qualitative/discrete-valued parameters. Plots were provided for group means (average plots) and individual animal values (profile plots) for all continuous outcomes measured at multiple time points. Reference ranges were included when available. Statistical analyses of study outcomes were performed using analysis of variance (ANOVA) with a treatment factor (treated versus control) and a sex factor and a sex by treatment interaction factor for continuous-scale parameters. Analysis of parameters that were measured at multiple time points included a time component and an appropriate covariance structure to account for repeated measures for the same animal and potential time-dependence. Frequency data were analyzed based on odds ratios between the treatment and control group and accounted for potential sex differences, if needed. All analyses were performed in (b) (4) of the (b) (4) for (b) (4)

Parameters	Frequency of Testing
Cage side observation	Once daily upon receipt through the quarantine/acclimation phase; two times daily through Study Day 66/78.
Clinical observations	Physical examinations with orthopedic assessments were performed on each animal on Study Days 1, 8, 22, 29, 43, 51, 64, and prior to euthanasia.
Body weight	Within 72 hours of arrival, within a day of randomization, prior to each vaccination, weekly between vaccinations and prior to euthanasia.
Food consumption	Food consumption will be measured daily during the 7 days following each vaccination.
Body temperature	Prior to each vaccination, 4 hours, and 24 hours post-dose. If an elevated body temperature ($\geq 40^{\circ}\text{C}$ or $\geq 1^{\circ}\text{C}$ increase above baseline) is noted at any of these three timepoints, measurements will be obtained every 24 hours until the fever resolves.
Ophthalmologic exam	Study Day -2 and a second time within 5 days prior to necropsy.
Clinical chemistry*	Prior to dosing (baseline) on Study Days 3, 8, 24, 45, and prior to euthanasia.
Hematology*	Prior to dosing (baseline) on Study Days 3, 8, 24, 45, and

	prior to euthanasia.
Coagulation*	Prior to dosing (baseline) on Study Days 3, 8, 24, 45, and prior to euthanasia.
Immunological response	Study Day 1, 66 and 78
Evaluation of site of inoculation (e.g., the Dermal Draize scoring method)	Prior to each injection, 4-6 hours post-injection, once daily for the next 7 days, and then weekly until the next dose.
Necropsy	Study Day 66 or 78.
Tissues for histopathology	Study Day 66 or 78 (Day 78 will only be evaluated if adverse findings are noted in the necropsy of Day 66).

Table 2: Parameters measured in the repeat dose toxicity study *(central ear artery or lateral saphenous vein)

Postmortem procedures:

Pituitary gland*	Mammary gland (male and female)
Thyroid glands*	Liver* (with drained gallbladder)
Parathyroid glands*	Gallbladder
Adrenal glands*	Kidneys*
Pancreas	Urinary bladder
Ovaries*	Heart*
Uterus*	Sternum
Testes*	Femur (including distal articular surface)
Prostate	Bone marrow smear (from sternum)
Epididymis	Spleen*
Thymus*	Stomach
Lymph nodes (mandibular, mesenteric)	Brain* (cerebrum, cerebellum, mid brain, brain stem)
Spinal cord (cervical, mid thoracic, lumbar)	Eyes
Jejunum	Duodenum
Ileum	Colon
Cecum	Lung with mainstem bronchi
Muscle, skeletal (biceps femoris)	Skin
Mandibular salivary glands	Injection site(s) including underlying muscle
Optic nerves	Aorta (thoracic)
Cervix	Esophagus
Harderian gland	Lacrimal glands
Rectum	Seminal vesicles
Sciatic nerve	Trachea
Tongue	Vagina
Gross lesions	

Table 3: Organs collected for histopathology – (All tissues in the control group and treatment group were examined at day 66, only tissues with test article related findings were evaluated on day 78), *organ weight

Results:

Morbidity and mortality: No treatment related mortality or morbidity was observed.

CLINICAL CHEMISTRY		
MEASUREMENT RELATED TO	END POINTS DIFFERENT THAN THE CONCURRENT CONTROL	NOT OF NOTE
ELECTROLYTE BALANCE		Calcium, chloride, phosphorus potassium, sodium
CARBOHYDRATE METABOLISM		Glucose
LIVER FUNCTION: A) HEPATOCELLULAR		Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) Total bile acids
B) HEPATOBILIARY		Alkaline phosphatase (ALP) Total bilirubin
ACUTE PHASE REACTANTS	C-reactive protein: (b) (4) 3): ♀ G2: ↑6x (b) (4) 24): ♀ G2: ↑3x (b) (4) 45): ♀ G2: ↑2.4x (b) (4) 3): ♂ G2: ↑9x (b) (4) 8): ♂ G2: ↑5.6x (b) (4) 24): ♂ G2: ↓0.6x (b) (4) 45): ♂ G2: ↑1.8x	Fibrinogen (also under coagulation),
KIDNEY FUNCTION		Creatinine Blood urea nitrogen
OTHERS (ACID/BASE BALANCE, CHOLINESTERASES, HORMONES, LIPIDS, METHEMOGLOBIN, AND PROTEINS)		Albumin (A) Globulin (G, calculated) or A/G Ratio Total cholesterol Total protein Creatine kinase

Table 4: Table of clinical chemistry, (b) (4) Study Day, G: Group, fold change is listed if greater than 1.5 or lower than 0.7.

There were several Group 2 animals with an elevated CRP level on Study Day 3 and 24; two Group 1 males had an elevated CRP on Study Day 24. The average CRP for Group 2 was noticeably higher on Study Day 3 for Group 2 males and females. On Study Day 45 and 66, the average CRP was noticeably higher for Group 2 females. Overall, Group 2 animals had a significantly higher CRP than Group 1 animals ($p = 0.0331$).

No test article related changes in hematology or clinical chemistry have been observed.

Systemic toxicity:

No treatment-related, mortality, nor any toxicologically relevant changes in clinical signs, body weight (gain), relative food consumption, ophthalmoscopic parameters, hematology, clinical chemistry, gross anatomy, histopathology or organ weight were observed.

Organ Weight:

<i>SEX</i>	<i>MALE</i>		<i>FEMALE</i>	
<i>GROUPS</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>
<i>NUMBER OF ANIMALS</i>	<i>9</i>	<i>10</i>	<i>10</i>	<i>10</i>
BODY WEIGHT (TERMINAL)	3941	4095	3921	4054
BRAIN	10.18	9.88	10.10	9.85
ADRENALS	0.47	0.45	0.44	0.46
HEART	9.10	9.10	9.45	9.24
KIDNEYS	21.45	19.96	21.51	20.78
LIVER	91.77	85.36	97.44	92.86
PITUITARY	0.2066	0.0440	0.0360	0.0889
SPLEEN	1.44	3.46	1.37	1.55
THYROID AND PARATHYROID	0.6787	0.7635	0.6776	0.6787
THYMUS	8.01	5.42**	6.09	5.92
TESTES	6.15	5.98		
OVARIES			0.429	0.357
UTERUS			11.40	10.24

Table 5: Mean organ weights after treatment phase: absolute weights are expressed as mean (grams); *different from controls at $P \leq 0.05$; **different from controls at $P \leq 0.01$.

There was a statistically significant decrease in absolute thymic weight, thymus-relative-to body weight, and thymus-relative-to-brain weight for only male rabbits vaccinated with CHKVLP059-00-VP (Group 2) as compared to control male rabbits administered (b) (4) (Group 1).

Gross Pathology:

No test article-related gross findings were noted. The gross findings observed were considered incidental, of the nature commonly observed in this strain and age of rabbits and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of VRC-CHKVLP059-00-VP.

Microscopic findings:

<i>Treatment Group Finding – Test article related</i>	<i>1M</i>	<i>2M</i>	<i>1F</i>	<i>2F</i>
Mixed cell infiltration in the epididymis (minimal)	1	-		
Mixed cell infiltration in the Harderian gland (minimal)	1	-	-	-
Injection site A: mixed cell infiltration (minimal)	-	1	-	-

Injection site A: mixed cell infiltration, dermis (mild)	-	-	1	-
Injection site B: mixed cell infiltration, dermis (minimal)	-	1	-	1
Injection site C: mixed cell infiltration (minimal)	-	-	-	2
Injection site C: mixed cell infiltration, dermis (minimal)	-	1	-	2
Injection site D: mixed cell infiltration (minimal)	-	1	-	1
Injection site D: mixed cell infiltration, dermis (minimal)	-	1	-	-
Injection site E: mixed cell infiltration, dermis (minimal)	-	-	-	1
Injection site F: mixed cell infiltration, dermis (minimal)	-	-	-	3
Injection site G: histiocytic cell infiltration (minimal)	-	1	-	-
Injection site G: mixed cell infiltration, dermis (minimal)	-	-	1	1
Injection site H: histiocytic cell infiltration (minimal)	-	1	-	-
Injection site H: mixed cell infiltration, dermis (minimal)	-	-	1	-
Mixed cell infiltration in the duodenum (minimal)	1	-	1	2
Injection site I: mixed cell infiltration (minimal)	-	1	-	-
Injection site J: mixed cell infiltration (minimal)	-	1	-	-

Table 6: Microscopic findings in treatment group

Mixed cell infiltrates, composed of heterophils and lymphocytes or histiocytic infiltrates were seen at the injection site of vaccinated animals and control animals with a higher incidence in the vaccinated animals.

Since no adverse test article-related findings were not present at Day 66, tissues from the Day 78 scheduled euthanasia were not examined microscopically.

Body temperature:

Group	Males	Females
1	2	0
2	0	0

Table 7: Table of occurrences for body temperature $\geq 40^{\circ}C$

There were no statistically significant changes after dose administration as compared to the pre-dose phase in body temperature for the male or female rabbits administered VRC-CHKVLP059-00-VP, and as compared to control placebo rabbit body temperatures.

Local toxicity:

The administration of VRC-CHKVLP059-00-VP vaccine to rabbits via IM injection did not result in a significant treatment-effect on edema or erythema at the dose site following four administrations of each test article to two sites on each administration day. Microscopically a mixed cell infiltrates, composed of heterophils and lymphocytes or histiocytic infiltrates were seen at the injection site of vaccinated animals and control animals with a higher incidence in the vaccinated animals.

Serology:

Immunoassay via (b) (4) was performed on Study Day 1 and Necropsy Day (Study Day 66 or 78) samples from Groups 1 (control) and Group 2 (vaccinated) rabbit sera to

determine the relative concentrations of antibodies to Chikungunya VLP antigen (CKVLP_37997).

All control animal but one showed sera titers below the limit of detection for CKVLP_37997. It was unclear why this animal showed a positive serum titer, the animal was excluded from the analyses.

Test article related effects
<ul style="list-style-type: none">• Slightly higher incidence of mixed cell infiltrates at the injection site of vaccinated animals• Slight transient increase in CRP levels

Table 8: *Test article related effects.*

Reproductive and developmental toxicology study:

Title: A Fertility and Postnatal Developmental Toxicity Study of PXVX0317 Vaccine by Intramuscular Injection in Female Rabbits

Study no.: Testing Facility Study No. 20313401; Sponsor Reference No. CHIK-DART-001

Conducting laboratory and location: (b) (4)

Final report: 13 Dec 2022

GLP compliance: Yes

QA reports: Yes

Drug, lot #: PXVX0317 vaccine mixed with (b) (4) adjuvant Batch/Lot No.: (b) (4)

(b) (4) Control Article: Placebo for CHIKV VLP: Batch/Lot No.: (b) (4)

Animal species: (b) (4) rabbits

Doses: PXVX0317 vaccine mixed with (b) (4) adjuvant: 40 µg

Chikungunya Virus Like Particles given in 800 µl

Number/sex/group: 22

Route, formulation, volume, and infusion rate: 5 intramuscular injections of test article, PXVX0317 vaccine mixed with (b) (4) adjuvant, or the placebo for CHIKV VLP

Time of dosing: Doses were administered 28 and 14 days prior to the initiation of the mating phase, on Gestation Days (GD) 7 and 21 for all rabbits and again on Lactation Day (LD) 7 for rabbits assigned to the natural delivery phase.

Study design:

Group No.	Test material	Dose level (µg/mL)	Dose Concentration (µg/mL)	Dose volume (mL)	No of rabbits/cohort	
					Cohort 1 (Caesarean-Sectioning)	Cohort 2 (Natural Delivery)
1	Control Article	0	0	0.8	22	22
2	PXVX0317	40	50	0.8	22	22

Table 9: Study design of (b) (4) rabbit DART study. Five intramuscular (IM, bolus) injections were administered into the thigh muscle, PXVX0317 vaccine mixed with (b) (4) adjuvant, or the placebo, Placebo for CHIKV VLP, on 2 occasions before mating, on 2 occasions during the gestation phase, and on 1 occasion during the lactation phase (Cohort 2 only). Doses were administered 28 and 14 days prior to the initiation of the mating phase, on Gestation Days (GD) 7 and 21 for all rabbits and again on Lactation Day (LD) 7 for rabbits assigned to the natural delivery phase. The F0 rabbits were euthanized on GD 29 (Cohort 1) or Lactation Day (LD) 29 (Cohort 2). The F1 kits (Cohort 2) were euthanized on Day 29 postpartum.

Parameters and endpoints evaluated: Caesarean-sectioning [Cohort 1], Natural Delivery [Cohort 2]).

The following parameters and end points were evaluated in all F0 rabbits: viability, clinical signs (including dermal scoring), body weights, body weight changes, food

consumption, reproductive performance (mating and fertility), and macroscopic observations. F0 rabbits assigned to Cohort 1 were examined for ovarian and uterine contents on GD 29, gravid uterine weights were recorded, and the fetuses were sexed, weighed and examined for changes in external, visceral, and skeletal morphology. F0 rabbits assigned to Cohort 2 were allowed to naturally deliver their litters, maternal/litter observations were recorded, and the F1 kits were weighed, assessed for clinical signs, physical development (hair growth, incisor eruption, eye opening, auditory startle, or pupil constriction), and macroscopic observations.

Blood samples were collected from the F0 females 31 days before mating (DS-3, “Predose”), and on DS 22, GD 14, and GD 29. Fetal blood samples were collected from the F1 litters of Cohort 1 females on GD 29 (1 per sex pooled). On LD 29, 2 kits per litter from Cohort 2 females were bled for serum SNA analysis.

Results:

F0 Generation:

Clinical Observations: There were no PXVX0317 vaccine-related clinical signs or skin reactions (erythema or edema) in the F0 females during the premating, gestation, or lactation phase.

Abortions: There were two abortions at the end of the gestation phase, one female each assigned to Cohort 1 (GD 25, control group) and Cohort 2 (GD 26, control group). These abortions were considered unrelated to the PXVX0317 vaccine because they were limited to the control group and the incidence of abortions in this species and strain was within the historical range of the Testing Facility.

Body Weight: There were no PXVX0317 vaccine-related effects on mean body weights or body weight gains during the premating, gestation, or lactation phase.

Food Consumption: There were no PXVX0317 vaccine-related effects on mean food consumption during the premating, gestation, or lactation phase.

F0 does with no surviving kits: During the natural delivery phase, two Cohort 2 F0 females (one female in control group and one female in PXVX0317 group) did not deliver a litter and were euthanized on GD 34 and three other Cohort 2 F0 females (one female in control group and two females in PXVX0317 group) that did deliver a litter met the protocol requirements for euthanasia based on no surviving kits on LD 4 or LD 5. These events were considered unrelated to PXVX0317 because they occurred in the control group and/or the number of females affected was similar to the control group.

Female 109 (Cohort 2) in the control group was euthanized on LD 4 due to no surviving kits. Prior to euthanasia, this rabbit was administered the control article on 4 occasions (2 during the premating phase and 2 during the gestation phase). There were no clinical

signs or dermal observations noted in this female during the lactation phase. Body weights for this female were unremarkable, but this female consumed 1 g to 89 g of food per day (180 to 185 g offered per day) from GD 16 to GD 29. There were no macroscopic observations detected during necropsy examination. At necropsy, this female had 10 former implantation scars in the uterus.

Female 203 (Cohort 2) in the PXVX0317 dose group was euthanized on LD 5 due to no surviving kits. Prior to euthanasia, this rabbit was administered the control article on 4 occasions (2 during the premating phase and 2 during the gestation phase). Clinical signs observed during the lactation phase were limited to ungroomed coat and thin fur cover. Body weights for this female were unremarkable, but this female consumed 3 g to 113 g of food per day (180 to 185 g offered per day) from GD 14 to GD 29. There were no macroscopic observations detected during necropsy examination. The litter for the female consisted of 9 conceptuses.

Female 221 (Cohort 2) in the PXVX0317 dose group was euthanized on LD 4 due to no surviving kits. Prior to euthanasia, this rabbit was administered the control article on 4 occasions (2 during the premating phase and 2 during the gestation phase). There were no clinical signs or dermal observations noted in this female during the lactation phase. Body weights for this female were unremarkable, but this female consumed 0 g to 126 g of food per day (180 to 185 g offered per day) from GD 10 to GD 29. There were no macroscopic observations detected during necropsy examination. The litter for the female consisted of 8 conceptuses and there were 11 former implantation scars in the uterus at necropsy examination.

Mating and fertility: (Cohorts 1 and 2)

Reproductive Performance:

Parameter		F0 generation	
		Group 1	Group 2
Female animals paired	N	44	44
Mated females	N	42	41
Pregnant females	N	41	41
Female Mating Index	N/N (%)	42/44 (96.6)	41/44 (93.2)
Female Fertility Index	N/N (%)	41/44 (97.6)	41/41 (100.0)
Female Pregnancy Index	N/N (%)	41/44 (93.2)	41/44 (93.2)

Table 10: Summary of mating and fertility: F0 generation female rabbits

Mating performance (mating, fertility, and pregnancy indices) were unaffected by PXVX0317 vaccine.

Ovarian and Uterine Examinations and Litter Observations (Cohort 1):

Parameter		F0 generation	
		Group 1	Group 2
Rats tested	N	21	22
Pregnant	N (%)	19 (90.5)	20 (90.9)
Female with resorptions	N (%)	10 (52.6)	5 (25.0)
Female with all nonviable	N (%)	1 (5.3)	0 (0.0)
Number of Corpora Lutea	Mean	11.1	9.2**
Number of Implantations	Mean	9.9	8.5*
Pre-implantation Loss	(%)	9.87	9.30
Total Number of Fetuses	Mean	9.3	8.2
Number of Live Fetuses	Mean	9.3	8.2
Number of Live Male Fetuses	Mean	4.1	4.3
Number of Live Female Fetuses	Mean	5.2	3.9*
Mean Fetal Weight males (g)	Mean	44.16	42.30
Mean Fetal Weight females (g)	Mean	41.69	41.59

Table 11: Summary of C-section group observations.

There were no PXVX0317 vaccine-related effects on any ovarian, uterine, and litter parameter. Pregnancy was confirmed in 19 and 20 F0 females in the control and PXVX0317-treated group, respectively. Due to the single abortion in the control group, ovarian and uterine examinations on GD 29 were based on 18 and 20 females in the control and PXVX0317-treated group, respectively. In the PXVX0317 dose group, the mean number of corpora lutea and implantation sites was statistically significantly lower ($p \leq 0.05$ or $p \leq 0.01$) when compared with the control group means. Upon review of the individual data, F0 females in the PXVX0317 dose group had 5 to 12 corpora lutea and 3 to 12 implantation sites while F0 females in the control group had 6 to 15 corpora lutea and 1 to 13 implantation sites. Overall, the mean number of corpora lutea and implantation sites, were within the historical range of the Testing Facility, and there was no impact on the mean percent pre-implantation loss. As a result of the mean number of corpora lutea and implantation sites, there were fewer live fetuses available for evaluation in the PXVX0317-treated group. The embryo-fetal viability was not impacted.

Fetal Examinations (Cohort 1):

Fetal abnormalities were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development). Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

Parameter: developmental landmarks F1		F0 generation	
		Control	Adjuvant
Number of Fetuses Examined:		167	163
Number of Litters Examined:		18	20
Eye bulge, Both, Depression - Malformation	Fetuses N(%)	0(0.00)	1(0.71)
	Litters N(%)	0(0.0)	1(5.0)
Fat Pad, Large - Malformation	Fetuses N(%)	1(0.46)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Tongue, Protruding - Malformation	Fetuses N(%)	1(0.46)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Brain: Third ventricle, Dilatation, Moderate - Variation	Fetuses N(%)	1(0.93)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Diaphragm, Thick - Malformation	Fetuses N(%)	1(0.46)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Eye, Small - Malformation	Fetuses N(%)	0(0.00)	1(0.71)
	Litters N(%)	0(0.0)	1(5.0)
Great vessels: Truncus arteriosus, Persistent - Malformation	Fetuses N(%)	0(0.00)	1(0.45)
	Litters N(%)	0(0.0)	1(5.0)
Heart, Ventricular Septal Defect - Malformation	Fetuses N(%)	0(0.00)	1(0.45)
	Litters N(%)	0(0.0)	1(5.0)
Kidney, Right, Malpositioned - Malformation	Fetuses N(%)	0(0.00)	1(0.71)
	Litters N(%)	0(0.0)	1(5.0)
Lung, Misshapen - Malformation	Fetuses N(%)	1(0.46)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Forepaw phalanges, 1 or more, Small - Variation	Fetuses N(%)	1(0.62)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Rib, 1 or more, Detached - Variation	Fetuses N(%)	1(0.93)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Rib, 1 or more, Fused - Malformation	Fetuses N(%)	1(0.51)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Rib, 1 or more, Misshapen - Variation	Fetuses N(%)	1(0.46)	1(0.45)
	Litters N(%)	1(5.6)	1(5.0)
Scapula ala, Both, Misshapen - Variation	Fetuses N(%)	1(0.62)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Skull: Hyoid ala, Both, Bent - Variation	Fetuses N(%)	1(0.93)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Skull: Hyoid ala, Both, Short - Variation	Fetuses N(%)	0(0.00)	1(0.45)
	Litters N(%)	0(0.0)	1(5.0)
Skull: Hyoid ala, Right, Bent - Variation	Fetuses N(%)	0(0.00)	1(0.71)
	Litters N(%)	0(0.0)	1(5.0)
Skull: Nasal, Both, Fused - Malformation	Fetuses N(%)	1(0.62)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Skull: Parietal, Both, Hole - Malformation	Fetuses N(%)	0(0.00)	1(0.63)
	Litters N(%)	0(0.0)	1(5.0)
Skull: Parietal, Both, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.63)
	Litters N(%)	0(0.0)	1(5.0)
Skull: Zygomatic arch, Both, Misshapen - Variation	Fetuses N(%)	0(0.00)	1(0.71)
	Litters N(%)	0(0.0)	1(5.0)
Sternebra, 1 or more, Fused - Variation	Fetuses N(%)	1(0.46)	3(1.62)
	Litters N(%)	1(5.6)	3(15.0)
Sternebra, 1 or more, Misshapen - Variation	Fetuses N(%)	2(1.08)	3(1.36)
	Litters N(%)	2(11.1)	1(5.0)
Sternebra, 1 or more, Incomplete	Fetuses N(%)	0(0.00)	2(1.34)

ossification - Variation	Litters N(%)	0(0.0)	2(10.0)
Sternebra, 1 or more, Isolated	Fetuses N(%)	1(0.46)	1(0.45)
ossification site - Variation	Litters N(%)	1(5.6)	1(5.0)
Supernumerary rib: Cervical, 1 or more, Short - Variation	Fetuses N(%)	1(0.46)	1(0.71)
	Litters N(%)	1(5.6)	1(5.0)
Vertebra: Caudal vertebra, 1 or more, Misaligned – Variation	Fetuses N(%)	0(0.00)	1(0.71)
	Litters N(%)	0(0.0)	1(5.0)
Vertebra: Cervical arch, 1 or more, Misshapen - Variation	Fetuses N(%)	1(0.62)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Vertebra: Cervical arch, 1 or more, Small - Variation	Fetuses N(%)	1(0.46)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Vertebra: Cervical centrum, 1 or more, Unossified - Variation	Fetuses N(%)	2(1.23)	0(0.00)
	Litters N(%)	2(11.1)	0(0.0)
Vertebra: Cervical centrum, 1 or more, Incomplete ossification - Variation	Fetuses N(%)	2(1.31)	0(0.00)
	Litters N(%)	2(11.1)	0(0.0)
Vertebra: Lumbar arch, 1 or more, Misshapen - Variation	Fetuses N(%)	1(0.46)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Vertebra: Thoracic arch, 1 or more, Misshapen - Variation	Fetuses N(%)	1(0.46)	0(0.00)
	Litters N(%)	1(5.6)	0(0.0)
Vertebra: Thoracic arch, 1 or more, Small - Variation	Fetuses N(%)	0(0.00)	1(0.45)
	Litters N(%)	0(0.0)	1(5.0)
Vertebra: Thoracic centrum, 1 or more, Incomplete ossification - Variation	Fetuses N(%)	0(0.00)	1(0.45)
	Litters N(%)	0(0.0)	1(5.0)
Vertebra: Thoracic vertebra, 1 or more, Hemivertebra - Malformation	Fetuses N(%)	3(1.89)	1(0.45)
	Litters N(%)	3(16.7)	1(5.0)

Table 12: Summary of fetal examinations – Cesarean section; * $p < 0.05$; ** $p < 0.01$

There were no PXVX0317 vaccine-related external, visceral, or skeletal malformations or variations. In addition, all ossification site averages were similar between the control and PXVX0317 dose groups.

There were no PXVX0317 vaccine-related malformations or variations observed during external examination. These external malformations were considered unrelated to the vaccine because the findings were limited to individual fetuses, and/or were limited to the control group, and/or the number of fetuses/litters affected was within the historical range of the Testing Facility.

There were no PXVX0317 vaccine-related malformations or variations observed during visceral examination. These visceral malformations and variations were considered unrelated to the vaccine because the findings were limited to individual fetuses, and/or were limited to the control group, and/or the number of fetuses/litters affected was within the historical range of the Testing Facility.

There were no PXVX0317 vaccine-related malformations or variations observed during skeletal examination. All skeletal abnormalities were considered to be unrelated to PXVX0317 as: 1) the number of fetuses/litters affected was similar to controls; and/or 2) the finding was limited to the control group; and/or 3) the litter and/or fetal incidence was within the historical range of the Testing Facility (see Appendix XX, Historical Control Data).

Maternal Necropsy (Cohorts 1 and 2): There were no PXVX0317 vaccine-related macroscopic findings in the Cohort 1 F0 females euthanized on GD 29 or the Cohort 2 F0 females euthanized on LD 29.

Natural Delivery

Twenty-one F0 females were pregnant in the control and PXVX0317 dose groups. Of these pregnant females, 20 and 21 does delivered a live litter in the control and PXVX0317 dose groups, respectively.

In the PXVX0317 dose group, the gestation index (100%), mean number of total kits (9.6), mean implantation sites per delivered litter (10.9), and percent of post-implantation loss per litter (11.61%) were similar to or higher than when compared with the control group or were within the historical control range of the Testing Facility. All maternal grooming and nesting/nursing activities were normal. The mean gestation length in the PXVX0317 dose group reached statistical significance compared with the control group (31.6 days compared with 31.0 days in controls). However, longer duration of gestation (0.6 days) was considered unrelated to PXVX0317 because the mean value remained within the expected gestation period of this species and strain of laboratory animal historical control data.

Litter Observations:

Postnatal viability was numerically lower in the PXVX0317 group compared to the concurrent control group with an average of loss of 3.5 kits per litter between birth and Day 4 postpartum (day on which the litters were counted and weighed) compared to an average loss of 0.5 kits per litter in the concurrent controls during the same period. Thereafter, there were fewer kits surviving in the PXVX0317 dose group on Days 7, 14, 21, and 28 postpartum compared with the control group. Based on the lower survival on Day 4 postpartum, the mean values for kit survival in the PXVX0317 dose group remained lower than the concurrent control group mean and were statistical significance ($p \leq 0.05$) on Days 14, 21, and 28 postpartum. Overall, the pattern and timing of postnatal deaths was similar between two groups, except from birth to postnatal Day 4. The mean number of live newborn kits in the control group was 8.0 kits/litter at Day of birth and 7.5 kits/litter on postnatal Day 4 (5.6 kits/litter on postnatal Day 28), while the number of live newborn kits in the treatment group was 9.6 kits/litter at Day of birth and dropped to 6.1 kits/litter on postnatal Day 4 (4.3 kits/litter on postnatal Day 28). No maternal toxicity was identified, and all maternal grooming and nesting/nursing activities were normal. The overall survival of live born kits on postnatal Day 28 (latest day observed) was 69% for the control group and 42.3% for the treatment group with a p value of 0.0008262. The historical control data from 18 studies show a range for postnatal survival up to postnatal Day 28 or 29 from 47.6% to 91.4% with a mean on 71%. The cause of the difference in postnatal deaths in the PXVX0317 dose group is unknown. Additionally, decreased activity was observed in the kits of the treatment group compared to the control group. It cannot be excluded that these findings are treatment related and they should be included in the package insert.

Parameter	F0 generation	
	Group 1	Group 2
Mean No. Implantation Sites/Litter	9.3 ± 2.4	10.9 ± 2.5
Mean No. Live Newborn Kits	8.0 ± 2.9	9.6 ± 2.3
Post-Implantation Loss/Litter (%)	14.64 ± 23.36	11.61 ± 10.46
Mean No. Kits Surviving on Day 4 postpartum	7.5 ± 2.3	6.1 ± 2.9
Mean No. Kits Surviving on Day 7 postpartum	6.3 ± 2.5	5.3 ± 1.8
Mean No. Kits Surviving on Day 28 postpartum	5.6 ± 2.0	4.3 ± 1.4*
Mean Dead Kits Days 7 postpartum	2.2 ± 2.1	4.6 ± 2.5**
Mean Dead Kits Days 8 to 14 postpartum	0.5 ± 0.8	1.1 ± 1.1
% Survival (Days 4 to 28 postpartum)	77.28%	63.18%
% Survival (live birth to 28 postpartum)	69.0%	42.3%**

Table 13: Summary of litter parameters in natural delivery group. (*) = statistically significant at $p \leq 0.05$; (**) = statistically significant at $p \leq 0.01$.

F1 Generation Kits (Cohort 2):

Clinical Observations: PXVX0317-related clinical signs were observed in the F1 generation kits at a maternal dose of 40 µg/dose. The activity in kits was decreased across 9 litters (13 times recorded) in the PXVX0317 dose group compared with 3 litters (3 times recorded) in the control group, the impact of the vaccination is unclear. A higher occurrence of kits cold on touch was also observed in the treatment group (11 affected litters, 17 times recorded) compared to the control group (6 affected litters, 7 times reported). Splayed limbs were observed in 1 litter in the control group (observed 20 times) and in 5 litters in the treatment group (observed 181 times), splayed limbs were observed in the front legs, starting from PND 16. Splayed limbs are a common finding in (b) (4) rabbit kits and are considered to be caused by genetics and environmental factors (e.g. slippery cage floors).

Body Weights: There were no PXVX0317-related effects on mean kit body weights or mean body weight gain.

Reflex and Physical Development Parameters: There were no PXVX0317-related effects on hair growth, incisor eruption, eye opening, auditory startle, or pupil constriction in F1 generation preweaning male and female kits at the maternal dose of 40 µg/dose.

Serum Neutralizing Antibodies Evaluations: For the PXVX0317 group, pre-dose titers were all <10, consistent with CHIKV naïve animals. After the first dose, CHIKV 80% neutralizing antibody (NT80s) increased to between 3,913 and 35,763 titers consistent with successful vaccine take in all animals. NT80s further increased on GD 14 after the second dose but appeared to be maximal then. Further doses did not increase NT80s in most animals with a GMT of approximately 33,500 on GD 14 and then approximately 20,000 on GD 29 for maternal and fetal samples. Fetal pool NT80s were very similar to their corresponding maternal values and kit NT80s at Day 29 postpartum were approximately 10- to 20-fold lower than their corresponding maternal values at GD 29.

Assessment: Intramuscular administration of PXVX0317 vaccine (b) (4) with (b) (4) adjuvant before, during gestation, and during lactation to (b) (4) (b) (4) female rabbits was tolerated with no indication of maternal systemic toxicity. Overall, the NT80 titers in the placebo group were consistent with non-immune titers and in the PXVX0317 group with vaccine take in each animal and antibody transfer to F1 animals. In utero exposure of litters to PXVX0317 did not affect embryo-fetal viability or fetal body weights or cause any external, visceral, or skeletal malformations or variations in the females that were Cesarean-sectioned prior to delivery (Cohort 1). Cohort 1 females administered 4 doses of PXVX0317 had a reduced number of corpora lutea and implantation sites, but the overall significance of this finding was unable to be established since the opposite (i.e., an increase in implantation sites) was observed in the females allowed to naturally delivery (Cohort 2) and the numbers were within the historical control data. In the natural delivery group (Cohort 2), a reduction in viability (especially up to Lactation Day 4) and increased clinical signs (reduced activity, splayed limbs) in F1 kits (Cohort 2) from females administered PXVX0317 was observed compared to the concurrent control animals. The overall survival of live born kits on postnatal Day 28 (latest day observed) was 69% for the control group and 42.3% for the treatment group with a p value of 0.0008262. The historical control data from 18 studies show a range for postnatal survival up to postnatal Day 28 or 29 from 47.6% to 91.4% with a mean on 71%. Therefore, it cannot be excluded that this finding was treatment related.

Reproductive and developmental toxicology studies:

Title: An Intramuscular Vaccine Reproductive Study of PXVX0317 in Rats

Study no.: Testing Facility Study No. 2256-019, Sponsor Reference No. CHIK-DART-002

Conducting laboratory and location: (b) (4)

(b) (4)

Final report: 08 Nov 2023

GLP compliance: Yes

QA reports: Yes

Drug, lot #: PXVX0317 vaccine (b) (4) with (b) (4) adjuvant Batch/Lot No.: (b) (4)

(b) (4) Control Article: Placebo for CHIKV VLP: Batch/Lot No.: (b) (4)

Animal species: (b) (4) rats

Doses: 40 µg given in 800 µL

Number/sex/group: 22

Route, formulation, volume, and infusion rate: Groups 1 & 2: 28 days before pairing (Day 1), 14 days before pairing (Day 15) and on Gestation Days (GD) 0, 14 and Lactation Day (LD) 7. Females with no confirmed mating date will be dosed following the completion of the dosing period and will continue to be dosed once every 2 weeks following the completion of the pairing period until termination. Should an animal deliver a litter, the final dose will be on LD 7.

Group 3: Once 14 days prior to pairing (Day 1) and on GD 0 and 14.

Females with no confirmed mating date will be dosed following the completion of the dosing period and will receive one additional dose 2 weeks later.

Study design:

Group No.	Test material	Dose level (µg/mL)	Dose Concentration (µg/mL)	Dose volume ^a (mL)	No of rabbits: Natural Delivery
1 ^c	Control Article	0	0	0.8	22
2 ^c	PXVX0317	40	50 ^b	0.8	22
3 ^d	PXVX0317	40	50 ^b	0.8	22

Table 14: Study design of (b) (4) rat DART study. a) Total dose volume to be split over 4 approximately equal sites b) The total protein concentration was (b) (4) The target concentration was 50 µg/mL, with an intended dose of 40 µg from the 800 µl in each syringe. C) Dosed 28 days prior to pairing, 14 days prior to pairing, and on GD 0, 14, and LD 7. d) Dosed 14 days prior to pairing and on GD 0 and 14.

Parameters and endpoints evaluated:

Parameter	Population(s)	Frequency
Mortality/Cage side Observations	All surviving animals	At least twice daily (morning and afternoon) beginning upon arrival through termination/release.
Detailed Clinical Observations	All surviving animals	At least once weekly during the acclimation. Twice daily on dosing days (predose and at 1hr [\pm 15minutes). Once daily on non-dosing days (including the day of euthanasia).
Evaluation of Skin Reaction (Draize scoring)	All surviving animals	4, 24, and 48 hours post each dose.
Individual Body Weights	All surviving animals	Once weekly prior to initiation of dosing, weekly during the premating dose period, and on GD 0, 6, 9, 12, 15, 18, and 21 & LD 1, 4, 7, 10, 14, 17, and 21. Body weight change will be calculated for GD 0-6, 6-9, 9-12, 12-15, 15-18, 18-21, and 0-21 and on LD 1-4, 4-7, 7-10, 10-14, 14-17, 17-21, and 1-21.
Food Consumption	All surviving animals	Recorded on the corresponding body weight days and calculated for the corresponding body weight intervals.
Breeding Procedures	All surviving animals	Pairing will begin on Day 15 (Grp 3) or Day 29 (Grps 1 and 2). One female will be housed with 1 male in the cage of the male. Positive evidence of copulation will be established by daily inspection for a copulatory plug in situ or vaginal lavage for sperm. The day in which positive evidence of copulation is observed will be considered gestation day (GD) 0. After mating, each female will be returned to an individual cage.
F0 Parturition	F0 females	Toward the end of the gestation period (GD 20), females will be examined twice daily for signs of parturition. Females that do not deliver a litter will be euthanized 25 days after the completion of the mating period.
Females that Fail to Deliver	F0 females	GD 25
F1 Litter Observations	F1 Generation	Beginning on PND 0
Static Righting Reflex	F1 Pups	PND 2
Pinna Detachment	F1 Pups	PND 3
Eye Opening	F1 Pups	PND 12
Auditory Response	F1 Pups	PND 21
Necropsy:	F0 main study animals and F1 pups.	Terminal (F0 Females): LD 21 Terminal (F1 Males/Females): PND 21

Table 15: Parameters evaluated in the ^{(b) (4)} rat DART study.

Results:**F0 Generation:**

Mortality: All F0 females survived to scheduled termination.

Clinical Observations: No test article-related clinical findings were observed in F0 females at any dose level evaluated.

Dermal Observations: No test article-related dermal findings (edema and erythema) were observed in F0 females at any dose level evaluated.

Body Weight: No test article-related effects were observed on mean F0 female body weights and body weight gain at any dose level evaluated.

Food Consumption: No test article-related effects were observed on mean F0 female food consumption at any dose level evaluated.

Mating and fertility:

Reproductive Performance:

Parameter		F0 generation		
		Group 1	Group 2	Group 3
Female animals paired	N	22	22	22
Mated females	N	21	15	17
Pregnant females	N	21	15	17
Pregnant No confirmed mating	N	1	2	4
Female Mating Index	N/N (%)	22/22 (100.0)	17/22 (77.3)*	21/22 (95.5)
Female Fertility Index	N/N (%)	21/22 (95.5)	15/17 (88.2)	18/21 (85.7)
Female Pregnancy Index	N/N (%)	21/22 (95.5)	15/22 (68.2)*	18/22 (81.8)
Gestation Index	N/N (%)	21/21 (100.0)		
Females Completing Delivery	N	21	15	17
Female with Liveborn	N	21	15	17
Female with no Liveborn	N	0	0	0
Live Newborn Pups	Mean	12.0	12.5	11.8
Live Male Pups/Litter (%) Birth	%	53.57	51.91	46.25
Implantation Sites - Total	Mean	12.9	13.7	12.8
Gestation Length (Days)	Mean	21.9	22.1	22.5

Table 16: Summary of mating and fertility: F0 generation female rabbits

F0 Reproductive Performance:

The mating index in the Group 2 F0 females was statistically lower (77.3% vs 100% in controls) in comparison to concurrent controls and outside recent historical control ranges (female mating index: 95.5%-100%). While the difference in mating index in the Group 2 F0 females was considered potentially test article related, it should be noted that the mating period utilized in this study was 7 days and no estrous cyclicity was conducted. The pregnancy index in the Group 2 F0 females was statistically lower (68.2% vs 95.5% in controls) in comparison to controls but this was attributed to the low number of mated F0 females, most importantly the fertility index was comparable to concurrent controls (88.2% vs 95.5% in controls). Additionally, this difference was not seen in group 3.

The mating, fertility, and pregnancy indices in the Group 3 F0 females ranged from 81.8% to 95.5% and were comparable to controls at 95.5% to 100%. The pre-coital interval (mean number of days to mating) in the treated groups ranged from 1.8 to 2.7 days and was comparable to controls at 2.7 days.

Litter observations:

Parameter		F1 generation		
		Group 1	Group 2	Group 3
Live Pups on Day 1	Mean	11.7	12.3	11.1
Live Pups on Day 4	Mean	12.2	12.3	10.8
Live Pups Postcull	Mean	7.4	7.9	7.5
Live Pups on Day 7	Mean	7.4	7.9	7.5
Live Pups on Day 14	Mean	7.4	7.9	7.5
Live Pups on Day 21	Mean	7.4	7.9	7.5
Viability Index (Birth-4)	(%)	92.87	98.63	93.17
Lactation Index 4Postcull-21	(%)	100.00	100.00	99.26

Table 17: Summary of litter observations

No test article-related effects on F1 pup survival were observed at any dose level evaluated. Mean viability (LD 0 to 4 pre-cull) and lactation (LD 4 post-cull to 21) indices in the treated groups ranged from 93.17% to 100% and were comparable to concurrent controls (92.87% to 100%).

No test article-related effects on F1 pup sex ratios (% male pups/litter) were observed at any dose level evaluated. Mean sex ratios at birth (LD 0) and weaning (LD 21) in the treated groups were comparable to mean concurrent control values.

No test article-related effects were observed on F1 pup clinical findings at any dose level evaluated. Mean F1 pup body weights on PND 1 throughout lactation (PND 4, 7, 10, 14, and 21) at all dose levels evaluated were comparable to concurrent controls and unaffected by treatment.

No test article-related effects were observed on surface righting reflex, pinna detachment, eye opening, and auditory startle in the F1 pups at any dose level evaluated.

No test article-related effects were observed from macroscopic examinations of F1 pups found dead during lactation, PND 4 culled pups (examined externally), and on PND 21 in the treated groups.

Assessment: In this vaccine reproductive study conducted in rats, IM administration of PXVX0317 at a dose level of 40 µg/dose on Day 1 (28 days prior to pairing), 15 (14 days prior to pairing), GD 0 and 14, and LD 7 (Group 2) or on Day 1 (14 days prior to pairing), and GD 0 and 14 (Group 3) resulted in no test article-related effects on F0 survival, clinical and dermal findings, body weights/body weight gain, food consumption, parturition parameters (gestation length, gestation index, live newborn pups/litter, live birth index, and uterine implantation sites), or macroscopic findings in the treated groups. No test article-related effects on reproductive performance (mating, fertility, and pregnancy indices) were observed in Group 3 or on pre-coital interval in Groups 2 and 3. The mating index in the Group 2 F0 females was statistically lower (77.3% vs 100% in controls) in comparison to concurrent controls and outside recent historical control ranges (female mating index: 95.5%-100%). While the difference in mating index in the Group 2

F0 females was considered potentially test article related, it should be noted that the mating period utilized in this study was 7 days and no estrous cyclicity was conducted. The pregnancy index in the Group 2 F0 females was statistically lower (68.2% vs 95.5% in controls) in comparison to controls but this was attributed to the low number of mated F0 females, most importantly the fertility index was comparable to concurrent controls (88.2% vs 95.5% in controls). The mating, fertility, and pregnancy indices in the Group 3 F0 females ranged from 81.8% to 95.5% and were comparable to controls at 95.5% to 100%. The pre-coital interval (mean number of days to mating) in the treated groups ranged from 1.8 to 2.7 days and was comparable to controls at 2.7 days. No test article-related effects were observed on F₁ pup survival, sex ratios, clinical findings, body weights, reflex and physical development (surface righting reflex, pinna detachment, eye opening, and auditory startle), or macroscopic findings in the treated groups.

OVERALL ASSESSMENT: The sponsor is developing a Chikungunya Virus Virus-Like Particle (CHIKV VLP) vaccine for the prevention of disease caused by chikungunya virus infection in individuals 12 years of age and older. The Chikungunya Virus Virus-Like Particle (CHIKV VLP) vaccine is a sterile aluminum hydroxide adjuvanted enveloped VLP comprised of three recombinant CHIKV structural proteins derived from CHIKV Senegal strain 37997, the capsid (C), envelope 1 (E1) and envelope 2 (E2) protein.

The safety and toxicity of unadjuvanted CHIKV VLP vaccine (CHKVLP059) was evaluated in a repeat-dose toxicity study in rabbits (study MRI 110752.01.001). Additionally, two DART studies were performed using adjuvanted CHIKV VLP vaccine: a fertility, embryo-fetal and postnatal development study in rabbits (CHIK-DART-001) and a fertility and postnatal development toxicity study in rats (CHIK-DART-002). The toxicology program evaluated the intended dose of 40 µg applied to humans.

The repeat-dose toxicity study did not demonstrate any safety concerns and confirmed the rabbit as a pharmacologically relevant species. A dose of 1 mL CHKVLP059 (40 µg VLP) was given four times at 3-week intervals. No CHIKV VLP-attributable adverse effects were observed for body weight changes, clinical signs, body temperature, injection site reactions, ophthalmic assessments, hematology, clinical chemistry, necropsy, and histopathology. Any changes observed were either within the normal variation observed for the species or procedure-related (IM injection trauma). The test article in this repeat-dose toxicity study was unadjuvanted CHIKV VLP vaccine (CHKVLP059). However, aluminum hydroxide has been safely used over six decades in other licensed vaccines (FDA Common Ingredients in U.S. Licensed Vaccines (FDA, 2019). Therefore, a second repeat-dose toxicity study using adjuvanted CHIKV VLP vaccine, was not considered necessary.

Two DART studies were submitted. The first DART study (CHIKDART-001) was performed in rabbits and included a C-section cohort as well as a natural delivery cohort. Subsequently, a second DART study, CHIKDART-002, was performed in rats and only included a natural delivery cohort. Both DART studies were similar in design and tested the full human dose. Rabbits are a standard species for reproductive toxicity studies in vaccines and the combined fertility, embryo-fetal and postnatal developmental toxicity study in rabbits followed the FDA guidance “Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications” covering the stages A-E of the ICH S5 guideline. A full human dose (0.8 mL) of VIMKUNYA was administered to (b) (4) rabbits by intramuscular injection on five occasions: 28 and 14 days prior to start of cohabitation, on Gestation Days 7 and 21 and on Lactation Day 7. The study did not demonstrate any adverse effects on the fertility and embryo-fetal development. Mating performance (mating, fertility, and pregnancy indices) were unaffected by the vaccine. There were no vaccine-related malformations or variations observed. In the treatment group of the natural delivery cohort the gestation index (100%), mean number of total kits (9.6), mean implantation sites per delivered litter (10.9), were similar to or higher than when compared with the control group or were

within the historical control range of the Testing Facility. All maternal grooming and nesting/nursing activities were normal. However, the postnatal viability of kits was numerically lower in the treatment group compared to the concurrent control group with an average of loss of 3.5 kits per litter between birth and Day 4 postpartum (day on which the litters were counted and weighed) compared to an average loss of 0.5 kits per litter in the concurrent controls during the same period. Thereafter, there were fewer kits surviving in the treatment group on Days 7, 14, 21, and 28 postpartum compared with the control group. Based on the lower survival on Day 4 postpartum, the mean values for kit survival in the treatment group remained lower than the concurrent control group mean and were statistically significant ($p \leq 0.05$) on Days 14, 21, and 28 postpartum. Overall, the pattern and timing of postnatal deaths was similar between two groups, except from birth to postnatal Day 4. The mean number of live newborn kits in the control group was 8.0 kits/litter at day of birth and 7.5 kits/litter on postnatal Day 4 (5.6 kits/litter on postnatal day 28), while the number of live newborn kits in the treatment group was 9.6 kits/litter at day of birth and dropped to 6.1 kits/litter on postnatal Day 4 (4.3 kits/litter on postnatal Day 28). The overall survival of live born kits on postnatal Day 28 (latest day observed) was 69% for the control group and 42.3% for the treatment group with a p value of 0.0008262. The historical control data from 18 studies show a range for postnatal survival up to postnatal Day 28 or 29 from 47.6% to 91.4% with a mean on 71%. The cause of the difference in postnatal deaths in the treatment group is unknown. Additionally, decreased activity was observed in the kits of the treatment group compared to the control group. It cannot be excluded that these findings are treatment related. There were no vaccine-related effects on hair growth, incisor eruption, eye opening, auditory startle, or pupil constriction in F1 generation preweaning male and female kits at the maternal dose of 40 µg/dose.

Following the results of the (b) (4) rabbit DART study, the sponsor performed a second DART study in a second species, (b) (4) rat. Rats are similarly a standard species for reproductive toxicity studies and immunogenicity analysis demonstrated robust antibody responses induced by CHIKV VLP vaccinations, verifying that the rat is a suitable species. In the CHIK-DART-002 DART study, female rats received a full human dose (0.8 mL) of VIMKUNYA by intramuscular injection on five occasions (treatment group 1): 28 and 14 days prior to start of cohabitation, on Gestation Days 7 and 21 and on Lactation Day 7. No test article related effects were observed on fertility or postnatal development, and importantly, no increased pup mortality was observed. The mating index in the Group 2 F0 females was statistically lower (77.3% vs 100% in controls) in comparison to concurrent controls and outside recent historical control ranges (female mating index: 95.5%-100%). While the difference in mating index in the Group 2 F0 females was considered potentially test article related, it should be noted that the mating period utilized in this study was 7 days and no estrous cyclicity was conducted. The pregnancy index in the Group 2 F0 females was statistically lower (68.2% vs 95.5% in controls) in comparison to controls but this was attributed to the low number of mated F0 females. Most importantly the fertility index was comparable to concurrent controls (88.2% vs 95.5% in controls). The mating, fertility, and pregnancy indices in the Group 3

F0 females ranged from 81.8% to 95.5% and were comparable to controls at 95.5% to 100%. The pre-coital interval (mean number of days to mating) in the treated groups ranged from 1.8 to 2.7 days and was comparable to controls at 2.7 days. No test article-related effects on F1 pup survival were observed at any dose level evaluated. Mean viability (LD 0 to 4 pre-cull) and lactation (LD 4 post-cull to 21) indices in the treated groups ranged from 93.17% to 100% and were comparable to concurrent controls. No test article-related effects were observed on F1 pup clinical findings at any dose level evaluated. Mean F1 pup body weights on PND 1 throughout lactation (PND 4, 7, 10, 14, and 21) at all dose levels evaluated were comparable to concurrent controls and unaffected by treatment. No test article-related effects were observed on surface righting reflex, pinna detachment, eye opening, and auditory startle in the F1 pups at any dose level evaluated.

Overall, the sponsor submitted sufficient nonclinical toxicology data for the approval of BLA 125820. The sponsor is planning to perform a pregnancy registry.

CONCLUSIONS: In the BLA (125820) adequate nonclinical toxicology data regarding VIMKUNYA, Chikungunya Vaccine, Recombinant have been presented for the safety. No issues regarding non-clinical toxicology have been identified that preclude approval of the BLA in healthy adults.