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Authors

Weber, Whitney C

Streblow, Daniel N

Coffey, Lark L

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LEADING ARTICLE

Chikungunya Virus Vaccines: A Review of IXCHIQ and PXVX0317 from Pre-Clinical Evaluation to Licensure

Whitney C. Weber[1,2] · **Daniel N. Streblow**[1,2] · **Lark L. Coffey**[3]

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Abstract

Chikungunya virus is an emerging mosquito-borne alphavirus that causes febrile illness and arthritic disease. Chikungunya virus is endemic in 110 countries and the World Health Organization estimates that it has caused more than 2 million cases of crippling acute and chronic arthritis globally since it re-emerged in 2005. Chikungunya virus outbreaks have occurred in Africa, Asia, Indian Ocean islands, South Pacific islands, Europe, and the Americas. Until recently, no specific countermeasures to prevent or treat chikungunya disease were available. To address this need, multiple vaccines are in human trials. These vaccines use messenger RNA-lipid nanoparticles, inactivated virus, and viral vector approaches, with a live-attenuated vaccine VLA1553 and a virus-like particle PXVX0317 in phase III testing. In November 2023, the US Food and Drug Administration (FDA) approved the VLA1553 live-attenuated vaccine, which is marketed as IXCHIQ. In June 2024, Health Canada approved IXCHIQ, and in July 2024, IXCHIQ was approved by the European Commission. On August 13, 2024, the US FDA granted priority review for PXVX0317. The European Medicine Agency is considering accelerated assessment review of PXVX0317, with potential for approval by both agencies in 2025. In this review, we summarize published data from pre-clinical and clinical trials for the IXCHIQ and PXVX0317 vaccines. We also discuss unanswered questions including potential impacts of pre-existing chikungunya virus immunity on vaccine safety and immunogenicity, whether long-term immunity can be achieved, safety in children, pregnant, and immunocompromised individuals, and vaccine efficacy in people with previous exposure to other emerging alphaviruses in addition to chikungunya virus.

1 Chikungunya Virus

Chikungunya virus (CHIKV) was first described in 1952 after an outbreak in people in Tanzania [1–3]. CHIKV is an alphavirus (*Togaviridae, Alphavirus chikungunya*) that comprises four major genetic lineages (West African, East Central South African [ECSA], Asian, and Indian Ocean Lineage). Despite this genetic diversity, CHIKV comprises a single serologic group. CHIKV is an enveloped, single-stranded, positive sense RNA virus. The viral

Key Points

Two chikungunya virus vaccines have recently or are approaching regulatory approvals in 2024. One vaccine is a live attenuated vaccine, VLA1553, marketed as IXCHIQ, and the other is a virus-like particle, PXVX0317.

Pre-clinical and clinical data reviewed here support the safety, immunogenicity, and protective efficacy of the two chikungunya virus vaccines.

Outstanding questions for both vaccines include impacts of pre-existing chikungunya virus immunity on vaccine safety and immunogenicity, long-term immunity, safety in children, pregnant and immunocompromised people, and efficacy in individuals previously exposed to other alphaviruses in addition to chikungunya virus.

✉ Lark L. Coffey
lcoffey@ucdavis.edu

1 Vaccine and Gene Therapy Institute, Oregon Health and Science University, Beaverton, OR, USA

2 Department of Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, OR, USA

3 Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis One Shields Avenue, Davis One Shields Avenue, 5327 VM3A, Davis, CA 95616, USA

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RNA is translated from the full-length genomic RNA or a subgenomic RNA as two polyproteins; one encodes the four non-structural proteins (nsP1-4) to form a replication complex that synthesizes the genome, and the other encodes the structural proteins (capsid, 6K peptide, and E1, E2, and E3 envelope proteins). The envelope proteins are the dominant antibody targets of the host immune response with E1 conferring membrane fusion and E2 responsible for cell receptor (MXRA8) binding to target cells [4]. CHIKV is a member of the Semliki Forest virus antigenic complex that affords cross-reactive adaptive immunity to other emerging pathogenic alphaviruses including O'nyong nyong (ONNV), Mayaro (MAYV), Una (UNAV), and Ross River viruses (RRV) [5].

2 Chikungunya Disease and Management

Chikungunya virus causes chikungunya (CHIK) disease. Chikungunya virus is transmitted to humans during blood feeding by infected *Aedes aegypti* or *Aedes albopictus* mosquitoes that are common in urban tropical and subtropical regions and spreading globally owing to various factors including climate change [6, 7]. After deposition by a mosquito, CHIKV spreads cell- free or in infected fibroblasts in the skin, leading to dissemination through the blood and infection of and replication within liver, muscle, joint, lymphoid tissues including lymph nodes and spleen, and brain [8]. Although rarely fatal, CHIKV infection causes symptomatic disease in most infected people, presenting primarily as fever, myalgia, arthralgia, headache, stiffness, rash, and fatigue, with less frequent neurologic and ocular symptoms [9]. Many patients report chronic arthritic joint pain that persists 1 month or longer after acute disease [10]. Congenital infection also occurs, usually via intrapartum fetal infection [9], and infection of neonates can lead to severe and sometimes fatal disease. Acute CHIK disease is treated with rest, oral hydration, and pain mitigation using analgesics and non-steroidal anti-inflammatory drugs [11–13]. Chronic CHIK disease is treated with non-steroidal anti-inflammatory drugs or disease-modifying antirheumatic drugs [14–16]. A variety of antiviral and monoclonal antibody therapies have been tested in pre-clinical models for mitigation of disease, but none has been licensed for use in humans.

3 Unmet Need for a CHIKV Vaccine

Chikungunya disease presents a global health problem. The absence of CHIKV-specific therapies restricts treatment to supportive care. From 2010 to 2019, CHIK caused average annual loss of >100,000 disability-adjusted life-years in endemic areas, mostly due to chronic rheumatic manifestations [17]. Areas without prior CHIKV circulation are often especially affected, as evidenced by explosive and often unpredictable outbreaks that debilitated public health infrastructures in Reunion Island in 2005 [18], India in 2008–9 [19], and Paraguay in 2022–23 [20]. People more than 35 years of age and obese persons are also more prone to severe or atypical CHIK [21–23]. Chikungunya virus outbreaks present a clear need for licensed CHIKV vaccines. In the absence of vaccines, strategies to reduce CHIKV transmission focus on limiting exposure to mosquito vectors, which include removing standing water where immature mosquitoes develop, installing screens on buildings, wearing long clothing, using mosquito repellents, and aerosol insecticide applications [24].

4 CHIKV Vaccines in Development

There are many CHIKV vaccines in development using various platforms. The nature of the platform and evaluation data has been extensively reviewed elsewhere [2, 25–29]. This review focuses only on the two CHIKV vaccine candidates, the IXCHIQ live attenuated vaccine (LAV) and the PXVX0317 virus like particle (VLP), that have advanced farthest in human trials as of 2024.

5 CHIKV-Specific Neutralizing Antibody as a Target for CHIKV Vaccine Development

Chikungunya virus-specific antibodies play an important protective role against CHIKV infection and disease [8, 30–32]. After a natural CHIKV infection, anti-CHIKV immunoglobulin M (IgM) antibody develops within 1 week and mediates early control of infection. An immunoglobulin G antibody (IgG), which develops after IgM, persists for months to years. A CHIKV-specific antibody recognizes viral components, especially the CHIKV structural envelope proteins E1 and E2. Passive transfer studies using serum or plasma containing CHIKV neutralizing antibody (NAb) from naturally infected or vaccinated people protects against CHIKV infection and disease in mice [33, 34], supporting a role for NAb in protection. These data are also used to support circulating NAb as a correlate of protection from CHIKV infection and disease that can be used in vaccine efficacy predictions.

6 IXCHIQ

IXCHIQ (Valneva, Vienna, Austria), formerly called Δ5nsP3 and VLA1553, was created from an infectious clone of CHIKV strain LR2006-OPY1 that was isolated from a patient in 2006 in Reunion Island. This strain belongs to the ECSA CHIKV genotype. The clone was genetically modified by deleting 62 amino acids in the C terminal region of nsP3 that is part of the viral replication complex (Fig. 1) [35]. The rationale for this approach is that a LAV is immunostimulatory much like wild-type CHIKV, but mutations or deletions at specific locations in the alphavirus replication complex attenuate virulence by reducing viral replication efficiency and fitness. The linker sequence AYRAAAG was inserted to replace the deleted nsP3 sequence. The deletion leads to decreased murine [35], macaque [36], and human virulence [37, 38], which will be discussed below, and forms the basis for attenuation of the LAV.

6.1 Pre-Clinical Evaluations

6.1.1 Genetic Stability

In the first study after its generation, IXCHIQ, then identified as Δ5nsP3, was serially passaged at a low multiplicity of infection in Vero cells and then the genome region flanking the deletion was sequenced to assess genetic stability of the deletion over time [35]. No increases in titers were detected and the introduced deletions were genetically stable after five [35] or ten [36] passages.

6.1.2 Immunogenicity and Efficacy in Animal Models

Both humoral and cell-mediated immune responses protect against alphavirus-mediated disease. To evaluate immunogenicity and protective efficacy of CHIKV vaccines, mice and non-human primates (NHP) that each recapitulate features of human CHIK have been used.

Mice In the first studies evaluating IXCHIQ [35], then identified as Δ5nsP3, female inbred C57BL/6 mice aged 5–6 weeks were administered a single immunization of 10^4

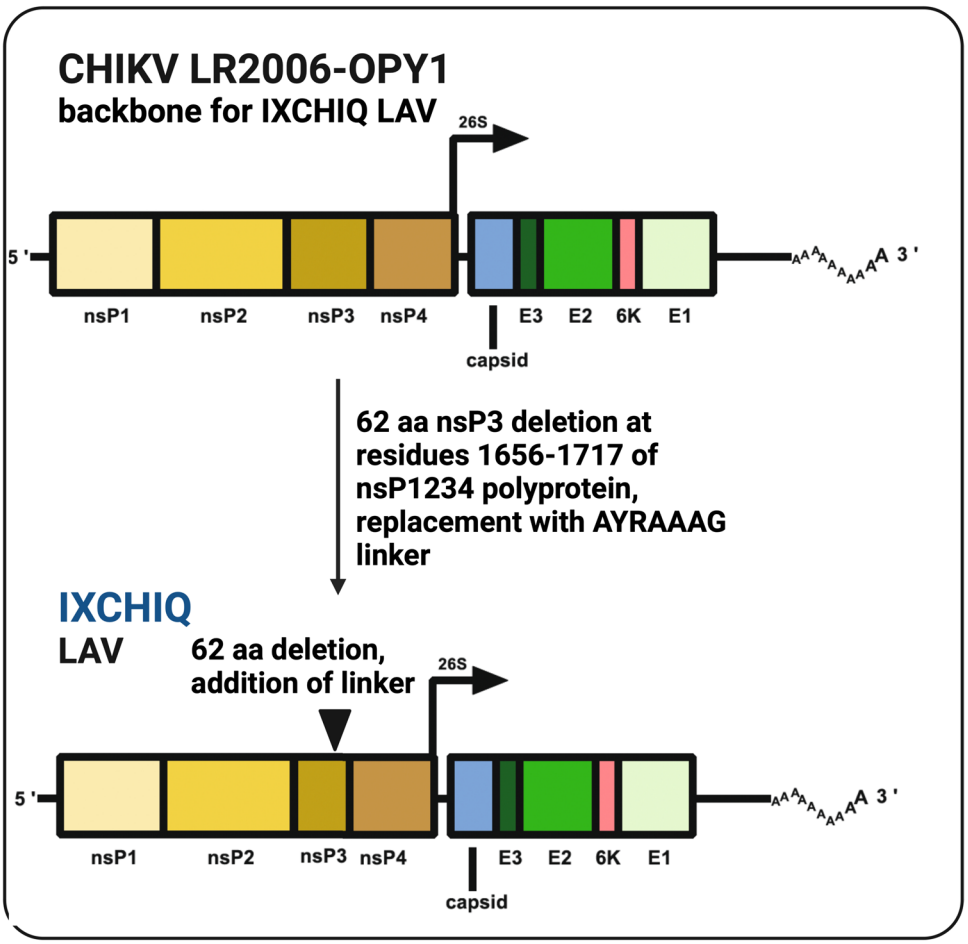

Fig. 1 Design of the IXCHIQ live attenuated vaccine (LAV). A 62-amino acid (aa) deletion was made in the chikungunya virus (CHIKV) non-structural protein (nsP) 3. The deleted region was replaced by a linker. A publishing license was granted for the image created in Biorender

or 10^5 Vero cell plaque forming units (PFU) of IXCHIQ subcutaneously in both upper thigh flanks. Mice vaccinated with 10^4 PFU did not develop detectable viremias 1 or 3 days after vaccination, but two of five mice vaccinated with 10^5 PFU developed detectable viremias. Footpad swelling, a proxy for arthritic disease in this model, was noted but it was suggested to not to occur at a significant level. Vaccinated mice developed IgG antibody responses measured by enzyme-linked immunosorbent assay, NAb titers assessed by 50% neutralization test (NT50) assays and CD8 T-cell responses measured by interferon-γ ELISPOT assays. Fifty percent neutralization dilution titers displayed a wide range, from less than 10^1 to $< 10^4$. Seven weeks after vaccination, mice were challenged in the feet with 10^6 PFU of the wild-type CHIKV LR2006-OPY1 strain that serves as the backbone for the vaccine. The challenged mice did not develop detectable viremias or footpad swelling. An inverse correlation was measured between IgG and NT50 titers and both viremia and footpad swelling, implicating both antibody measures as correlates of protection. Mice immunized with 10^5 PFU IXCHIQ challenged 8 or 20 weeks after immunization showed no detectable viremia and no differences in footpad swelling. Immunoglobulin G titers were not different at either post-immunization timepoint.

Non-Human Primates Following successful demonstration of immunogenicity and protection of the vaccine in mice, safety and efficacy studies in cynomolgus macaques were next performed [36], where the vaccine was then identified as Δ5nsP3. Adult cynomolgus macaques aged 3–4 years were vaccinated subcutaneously in the right upper backside with one injection of 1×10^5 PFU IXCHIQ. Vaccinated animals developed viremias between 1 and 9 days post-vaccination that peaked between days 2 and 4 at titers of 10^3 to $>10^6$ genomes/mL. These viremias were delayed in peak by 1–2 days, where macaques inoculated with wild-type CHIKV LR2006-OPY1 peaked 1 day after inoculation. The area under the viremia curve in vaccinated animals was also lower than in macaques that received wild-type CHIKV LR2006-OPY1. The vaccine induced high-titer CHIKV binding and NAb that did not decline between vaccination and challenge, 81 days later. The NAb responses against a heterologous strain of CHIKV representing a different genetic lineage that was isolated from a patient in the Caribbean were similar to those for the homologous CHIKV LR2006-OPY1, suggesting vaccine-mediated cross-neutralization across different CHIKV genotypes. Each animal was challenged intravenously in the saphenous vein with 100 animal infectious dose 50%, corresponding to 7000–10,000 PFU of wild-type CHIKV. After challenge, none of the vaccinated animals developed detectable viremias, fever responses, lymphopenia, or monocytosis; these are disease signs that were observed in challenged animals that were not vaccinated. Further analyses showed that cytokines including interferons and tumor necrosis factor-α that correlate with disease in people and are upregulated in macaques infected with wild-type CHIKV were not increased after IXCHIQ vaccination.

6.1.3 Animal Toxicology

Toxicology of IXCHIQ is based on data from the Summary Basis for Regulatory Action [39]. Rabbits administered a human dose of IXCHIQ twice on days 1 and 15 showed no clinical signs including changes in body weight, temperature, dermal appearance, ophthalmic appearance, blood chemistry, coagulation, gross pathology, organ weight, and histopathology. Rabbits that received the vaccine showed mild hematologic changes including increased monocyte, eosinophil, and neutrophil counts compared with control rabbits that were not vaccinated. A three-fold to 5-fold increase in C-reactive protein was measured in vaccinated rabbits. Chikungunya virus antibody responses were detected in vaccinated rabbits. In rats, a human dose of IXCHIQ did not produce adverse effects on fetal development, fecundity, or pre-or post-natal infant development.

6.2 Clinical Trials

Based on the success of the vaccine in animals, safety and immunogenicity of IXCHIQ were next evaluated in two clinical trials that were conducted at vaccine trial sites across the USA with almost 3200 healthy participants aged 18 years and older. These data are reported in two publications [37, 38], which are detailed below.

6.2.1 Design and Protection from Disease

In the first study (ClinicalTrials.gov, NCT03382964), a phase I trial was conducted in 2018 in Illinois and Alabama, USA [37]. A total of 120 healthy volunteers between 18 and 45 years received one of three doses in a single shot intramuscular immunization of IXCHIQ, then identified as VLA1553, followed by re-vaccination with the highest dose 6 or 12 months later. The low dose was 3.2×10^3, the medium dose was 3.2×10^4, and the high dose was 3.2×10^5 50% tissue culture infectious dose ($TCID_{50}$) per mL. Safety data and laboratory parameters were collected using the US FDA grading guidance [40]. Participants reported daily temperature, solicited injection, and systemic reactions for up to 14 days after vaccination. Participants were also monitored for signs of CHIKV-like disease that were recorded separately as adverse events of special interest (AESI) and included rapid-onset fever, myalgia, headache, back pain, rash, edema in the face and extremities, acute adenopathy, acute arthritis, tenosynovitis, neurological symptoms, or cardiac symptoms lasting for more than 3 days. The majority of

adverse events across all dose groups were mild or moderate and occurred after the single vaccination. In the first 14 days after vaccination, 7% (4/59) in the high-dose group reported any local adverse event, which was most often injection-site tenderness. Systemic adverse events including short-term fever, fatigue, headache, and muscle pain were also reported. Severe fever exceeding 102.1 °F (38.9 °C) was reported in seven participants, all of whom were in the high-dose group, beginning 2–4 days after vaccination and lasted for 1–3 days. Systemic adverse events were less common in the low-dose and medium-dose groups compared with the high-dose group. Leukopenia, neutropenia, and lymphopenia were reported in one third of vaccinees after the single vaccination but not after revaccination. No CHIKV-like disease or vaccine-related serious events occurred in any vaccinee. Together, these metrics showed that the vaccine is safe and well tolerated for up to 12 months after the single vaccination in the low-dose and medium-dose groups, and safe in the high-dose group. Provided that a single vaccination induced antibody titers that plateaued in all dose groups, no phase II clinical trial was deemed necessary [41].

Building on the promising data from the phase I trial, another study (ClinicalTrials.gov, NCT04546724) was conducted in 2020–21 [38]. This study was a double-blind phase III trial performed at 43 sites in the USA in healthy volunteers aged 18 years and older. Based on the medium-dose safety and immunogenicity data from the phase I clinical trial in 2018, a final dose of 1×10^4 $TCID_{50}$ was selected. A total of 3082 participants received IXCHIQ, identified as VLA1553 in the study, and 1033 participants received the placebo. Participants were excluded if they were pregnant, had evidence of prior CHIKV infection, immunodeficiencies, or had received any inactivated vaccine within 2 weeks prior or any live vaccine within 4 weeks prior to receiving IXCHIQ. Adverse events to 180 days after vaccination were reported more frequently in vaccinated (63%) than placebo (45%) participants. Five of ten participants with AESI who received IXCHIQ experienced CHIK-like disease in the form of a fever of 102.2 °F (39 °C) or higher for 2–4 days. Other adverse events in order of decreasing frequency reported in both vaccinated and placebo participants were headache (32% vaccinees, 16% placebo), fatigue (29%, 13%), myalgia (24%, 8%) and arthralgia (18%, 6%). Significant adverse events occurred in 1.5% of vaccinated and 0.8% of placebo participants. Two participants who received the vaccine were hospitalized but then recovered fully; one had myalgia and the other had high fever and atrial fibrillation and hyponatremia that was associated with a syndrome of inappropriate antidiuretic hormone secretion; both events were assessed as probably related to vaccination. Changes in hematologic parameters were not significant. The safety profile was similar in participants stratified by age in older (≥65 years) compared to the younger (18–64 years) groups. An independent Data Safety Monitoring Board did not raise major concerns about vaccine safety in the period after vaccination to 180 days.

6.2.2 Viremia and Urinary Shedding

In the phase I multi-dose trial, IXCHIQ produced viremias in study participants who received any of the vaccine doses. The mean viremia peak was 3 days after vaccination and viremia was detectable until 7 days. A caveat of this assessment is that blood was only collected 3, 7, and 14 days after vaccination, barring evaluation of whether the true peak was on day 3; NHP models with daily assessments show an earlier peak on day 2 [42]. The 3-day mean viremia titers were 2.3×10^5 in the high-dose group, 7.4×10^4 in the medium-dose group, and 8.9×10^4 genome copy equivalents/mL in the low-dose group. After re-vaccination, viremia above the 1087 genome copy equivalents/mL limit of detection was only detected 7 days after vaccination in the high-dose group. Urinary shedding was only detected in a single participant who received the low dose 7 days post-vaccination. Viremia and urinary shedding were not assessed in the phase III study.

6.2.3 Immunogenicity, Breadth, and Durability of Protection

In the phase I study, IXCHIQ was immunogenic and induced CHIKV-specific NAb titers in vaccinated study participants [37]. The micro plaque reduction neutralization test (μPRNT) was performed with CHIKV strain 181/25, which represents a heterologous strain of CHIKV that belongs to the Asian genotype. One hundred percent (103/103) of participants seroconverted by 14 days and all 91 participants that were followed remained seropositive for up to 1 year. The mean peak micro neutralization antibody titer at day 28 ranged from 592 to 686 in the low-dose and high-dose groups, respectively. Individuals that were revaccinated did not show anamnestic responses after revaccination and did not develop detectable viremias. In the phase III study [38], 98.9% of (263/266) participants vaccinated once with IXCHIQ developed $\mu PRNT_{50}$ titers of ≥ 150 by day 28. The geometric mean titer (GMT) on day 29 was 3362. There was no difference in rates of seropositivity or magnitude of titers between participants aged 18–64 years versus participants aged older than 65 years. At 180 days, the GMT decreased to 752. There were no differences in GMT $\mu PRNT_{50}$ based on sex, body mass index, ethnicity, or race [43]. One year after vaccination, 98.9% of participants had a μPRNT50 titer of ≥150 (the metric used to define seroprotection), and at two years, 96.8% of participants remained seroprotected [44]. In serum from 39 people in the Philippines who were naturally infected with the Asian genotype CHIKV, the GMT was

1341 (range: 170–5297) [31], which is similar to levels after IXCHIQ.

Neither of the clinical trials evaluated the breadth of NAb responses across genetically divergent CHIKV lineages or related alphaviruses. However, in studies we performed in the period since the IXCHIQ licensure, we detected cross-NAb using 50% plaque reduction neutralization test ($PRNT_{50}$) against divergent CHIKV lineages as well as related Semliki Forest virus antigenic complex alphaviruses ONNV, MAYV, and RRV viruses in participants from phase III trials [45]. There was little difference in NAb potency in vaccinees ($n = 30$) based on similar $PRNT_{50}$ titers for CHIKV LR2006-OPY1, 181/25, or a 2021 Brazilian isolate (ECSA genotype) up to 1 year post-vaccination. Neutralizing antibody responses against MAYV and ONNV were detected in 100% of vaccinees at 1-year post-vaccination. Neutralizing activity in participant sera was much lower against RRV but was present in ~80% of the participants. This cross-neutralizing activity in vaccinees was directly compared with CHIKV infection-elicited antibodies in serum collected from individuals 8–9 years post-infection living in endemic Puerto Rico, revealing consistency in potency and breadth of cross-NAb between these groups. These findings are consistent with our previous study that demonstrated the breadth and durability of CHIKV cross-NAb specific to the E2B domain in humans infected in Puerto Rico [46].

6.2.4 Serologic Correlates of Protective Efficacy

Late-stage vaccine evaluations typically require randomized controlled human efficacy trials in regions of virus endemicity with ongoing virus activity. However, CHIKV outbreaks are by nature unpredictable, sporadic, and explosive, with low case numbers during interepidemic periods. This presents challenges to performing efficacy trials [47]. One approach to circumventing this hurdle is studies that establish a serologic surrogate of protection. For IXCHIQ, studies were performed where the protective efficacy of serum from IXCHIQ vaccinated humans passively transferred to NHP was evaluated, where the vaccine was identified as VLA1553 in that study [42]. Pooled human sera from IXCHIQ vaccinees in the phase I trial was passively transferred into cynomolgus macaques. One day later, animals were challenged with 100 animal infectious dose 50 wild-type CHIKV LR2006-OPY1 and protection from viremia, fever, and hematologic changes were assessed for 28 days and compared with control macaques that received human non-CHIKV immune sera. Serum from IXCHIQ vaccinated people reduced the magnitude of viremia by 3 to 5 log_{10} genome copies/mL and delayed the peak and duration. None of the animals that received serum from IXCHIQ vaccinees developed fevers, lymphopenia, or neutropenia, and a $\mu PRNT_{50}$ titer of ≥ 150 was defined as a surrogate of protection from viremia and development of fever.

6.3 Regulatory Agency Approvals for Human Use

On 9 November, 2023, the FDA approved IXCHIQ for use in people aged 18 years and older who are at risk of exposure to CHIKV [48]. On 28–29 February, 2024, the US Centers for Disease Control Advisory Committee on Immunization Practices recommended IXCHIQ for use in people aged $\geq$ 18 years traveling to a country or territory with a CHIKV outbreak or evidence of CHIKV transmission within the last 5 years. In addition, IXCHIQ may be considered in people aged >65 years with at least 2 weeks of mosquito exposure, and in travelers to CHIKV endemic areas who will be staying for cumulative periods of more than 6 months [49]. The Advisory Committee on Immunization Practices also recommended IXCHIQ for laboratory workers with potential exposure to CHIKV. On 24 June, 2024, Health Canada announced approval of IXCHIQ in Canada [50]. On 1 July, 2024, the European Commission granted marketing authorization of IXCHIQ in Europe [51].

6.4 Components and Storage

IXCHIQ is propagated in African green monkey kidney (Vero) cells in a growth medium that contains fetal bovine serum, amino acids, vitamins, and minerals. The LAV harvested from infected Vero cells is pooled, clarified, concentrated, purified by chromatography, and ultracentrifuged. The resulting vaccine is then mixed with formulation buffer and lyophilized. Healthcare providers receive a vial of lyophilized IXCHIQ that should be stored in a refrigerator at 2–8 °C, prior to reconstitution for use in sterile water [52]. Each reconstituted 0.5-mL IXCHIQ dose contains at least 10^3 $TCID_{50}$ of CHIKV LAV.

7 PXVX0317

PXVX0317 is a VLP vaccine that was initially developed by the US National Institutes of Health Vaccine Research Center. The vaccine (Fig. 2) contains recombinant CHIKV structural proteins capsid, E3, E2, 6K, and E1, which were derived from Senegalese CHIKV strain 37997, a member of the West African genotype. Following in vitro expression of the CHIKV structural gene cassette, the structural proteins self-assemble into a particle that is highly similar to wild-type CHIKV but that cannot replicate because of the absence of a viral genome [34]. The rationale for the VLP approach is that structural proteins enable attachment, entry, and fusion into host cells to resemble a CHIKV virion and induce NAb responses that are similar to wild-type CHIKV.

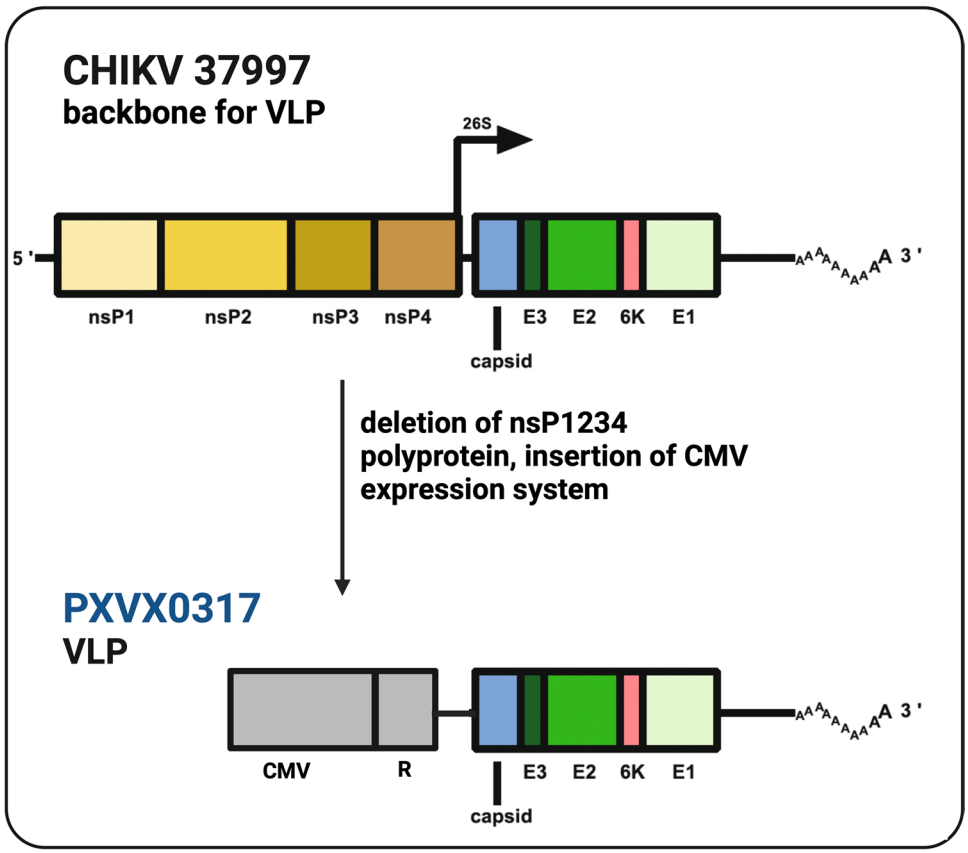

Fig. 2 Design of the PXVX0317 virus-like particle (VLP) vaccine. The chikungunya virus (CHIKV) non-structural genes were removed and the structural proteins: capsid, E2 and E1, along with accessory proteins E3 and 6K, were expressed from a human cytomegalovirus (CMV) R vector that comprises the human CMV early enhancer/promotor, a human T-cell leukemia virus-1 R region containing a splicing donor, a CMV immediate early-splicing acceptor, and a bovine growth hormone poly A signal. *nsP* is non-structural protein. A publishing license was granted for the image created in Biorender

In initial studies, NHP immunized with VLP-generated NAb and were protected from viremia after a wild-type CHIKV challenge [34]. These data served as the precedent for further development of this vaccine, discussed in detail below.

7.1 Pre-Clinical Evaluations

7.1.1 Immunogenicity and Efficacy in Animal Models

Mice Initial immunogenicity studies for PXVX0317 were performed in female BALB/C mice aged 6–8 weeks that were injected intramuscularly in the right and left quadricep muscles two times with a 1-month interval in between injections [34]. For some animals, the immune stimulatory adjuvant Ribi was also included together with PXVX0317. Compared with mice that received saline, mice administered adjuvanted PXVX0317 developed high titer NAb responses against the homologous 37997 strain and a heterologous CHIKV LR2006-OPY1 strain. Mice that received non-adjuvanted PXVX0317 also mounted NAb responses, although titers were lower than in mice that received adjuvanted vaccine.

Non-Human Primates Immunogenicity studies for PXVX0317 were performed in rhesus macaques [34]. Adult animals aged 3–4 years were administered PXVX0317 intramuscularly three times at weeks 0, 4, and 24. All developed NAb against homologous and heterologous CHIKV after the first immunization and titers increased with second and third immunizations. To assess protective efficacy, 15 weeks after the third immunization, animals were challenged intravenously with heterologous CHIKV LR2006-OPY1. Vaccinated animals did not develop detectable viremia 2 days post-inoculation, the time of peak viremia non-vaccinated rhesus macaques. Monocyte levels were also unchanged in vaccinated animals, contrasting with non-vaccinated controls, which showed increased levels. To evaluate the protective role of antibody after VLP vaccination, purified total IgG from immunized or control animals was passively transferred via an intravenous infusion into immunodeficient mice with defective type 1 interferon signaling (interferon-α/β receptor-1 knockout, Ifnar1$^{-/-}$), followed by a challenge with a lethal dose of CHIKV LR2006 OPY-1 1 day later. Mice that received IgG from vaccinated macaques did not develop detectable viremias and were protected from lethal disease, demonstrating the protective role of vaccine-stimulated IgG against CHIK.

7.1.2 Animal Toxicology

No publicly available data evaluate the toxicology of PXVX0317.

7.2 Clinical Trials

7.2.1 Design and Protection from Disease

Safety and immunogenicity of PXVX0317 were evaluated in three clinical trials that were conducted at vaccine trial sites across the USA comprising a combined total of nearly 1000 healthy participants aged 18 years and older. These data are reported in three publications describing the trials and three additional publications reporting on antibody responses, all of which are detailed below.

In the first study [53], a phase I dose-escalation, open-label clinical trial (ClinicalTrials.gov NCT04189358) was performed to evaluate the safety and tolerability of PXVX0317, which was named VRC-CHKVLP059-00-VP at the time. A total of 25 healthy adults aged 18–50 years were enrolled at the NIH Clinical Center, Bethesda, MD, USA in 2011–12. Participants received three sequential doses of 10 µg, 20 µg, or 40 µg administered intramuscularly in the deltoid on weeks 0, 4 and 20, and a follow-up at 44 weeks. Safety monitoring was similar to parameters used for IXCHIQ clinical trials and was performed via clinical and laboratory assessments. Vaccine injections were well tolerated with no serious adverse events. Thirty-six percent (9/25) of participants reported local reactogenicity and 40% (10/25) reported systemic reactogenicity at least once, usually manifest as malaise, headache, chills, nausea, fever, or joint pain.

Following success of the phase I trial, a phase II clinical trial for PXVX0317 (ClinicalTrials.gov NCT02562482) was performed next [54], in the period from 2015 to 2018. The trial was a randomized, placebo-controlled, double-blind study in male and female individuals at clinics in Dominican Republic, Guadeloupe, Haiti, Martinique, and Puerto Rico with 400 healthy adults aged 18–60 years as participants. The goal of the study was to evaluate the safety and tolerability of the vaccine in people in CHIKV endemic regions. Study participants received two intramuscular injections of VLP 20 µg 28 days apart (N = 201) or placebo (N = 199) and were followed for up to 72 weeks (1.5 years). Safety monitoring was similar to metrics from the phase I study and included laboratory parameters, adverse events, and tolerability based on local and systemic reactogenicity. As the study was conducted in CHIKV endemic regions, CHIKV infection was also evaluated. Candidate subjects were excluded from the study if they showed CHIKV IgG/IgM antibodies prior to enrollment. Similar to the phase I study, PXVX0317 was well tolerated with no serious vaccine-related adverse events reported. Thirty two percent (64/201) of participants in the vaccine group reported local reactogenicity, including pain or tenderness and swelling, compared with 19% (37/199) in the placebo group. Solicited symptoms reported included malaise, headache, myalgia, chills, nausea, fever, and joint pain, with 44% (87/201) of vaccinees reporting at least one symptom, which was higher than in the placebo groups. Unsolicited adverse events included neutropenia, bradycardia, hypotension, viral infection, rash, chest pain, dry lips, light headedness, fever, myalgia, gastroenteritis, abdominal pain, anemia, increased alanine aminotransferase, and hematoma, and were more common in the vaccine group (75%, 12/16) compared with the placebo (25%, 4/16) group. Despite being in a CHIKV endemic area, CHIK was not reported in any study participants during the trial.

A second phase II trial [55] (ClinicalTrials.gov NCT03483961) was next performed for PXVX0317 from 2018 to 2020, with a goal of informing selection of dose, adjuvant formulation, and immunization schedule for phase III trials. Instead of using unadjuvanted PXVX0317 as in the prior two clinical trials, PXVX0317 was prepared in an aluminum hydroxide-adjuvanted formulation. Adjuvants are intended to produce higher, earlier, and longer lasting immune responses when added to vaccines compared with non-adjuvanted formulations; the specific adjuvant selected for this study was intended to increase the efficiency of antigen uptake and release at the injection site. The study consisted of a randomized, double-blinded, parallel-group trial and was conducted at three clinics in the USA in healthy male and female CHIKV-naïve adults aged between 18 and 45 years, with a 2-year timeline between the first vaccination and study end. Participants were assigned to one of eight vaccination groups: two doses of unadjuvanted PXVX0317 28 days apart (2 × 20 µg; standard); adjuvanted PXVX0317 at two doses 28 days apart (2 × 6 µg, 2 × 10 µg, or 2 × 20 µg); a booster dose 18 months after the first active injection (40 µg; standard plus booster); two doses 14 days apart (2 × 6 µg, 2 × 10 µg, or 2 × 20 µg; accelerated); or one dose (1 × 40 µg; single). Immunogenicity and safety were study endpoints. In most groups, the majority of vaccinated participants reported solicited adverse events including injection-site and systemic reactions, where events were more common after the first vaccination. The most common solicited adverse event was injection-site pain, reported in 15–49% of participants, depending on the vaccine group. Common solicited adverse reactions were fatigue, headache, and myalgia across all dose groups and more common after the first vaccination. No treatment-related severe adverse events were reported. Significant differences in adverse events across vaccine groups were not reported.

A multi-center, randomized, double-blind, placebo-controlled, parallel-group phase III trial was performed next (ClinicalTrials.gov NCT05072080); some unpublished results are available [56]. Study subjects aged 12–64 years received VLP (2790 participants) or placebo (464 participants) as a single intramuscular injection. Ninety eight

percent (2503/2559) of vaccinated participants achieved 80% neutralization test (NT_{80}) serum neutralizing antibody titers of ≥ 100 by 22 days after vaccination; by contrast, only 1% (5/424) of placebo-treated participants achieved this NT_{80} serum neutralizing antibody level. Antibody responses were detected in all age groups. The most common adverse events were myalgia, fatigue, and headache.

7.2.2 Immunogenicity, Breadth, and Durability of Protection

In the phase I trial [53], immunogenicity was evaluated by measuring CHIKV-specific NAb titers using heterologous genotype CHIKV antigens or viruses at multiple intervals in the study timeline. Neutralizing antibodies were detected in all dose groups after the second vaccination and levels were boosted after the third. One month after the third vaccination, the GMT of the half-maximum inhibitory concentration was 8745 for participants who received 10 µg, 4525 for the 20-µg group, and 5390 for the 40-µg group. A second study [57] evaluated antibody responses generated by vaccinated participants in the phase I trial against nine CHIKV strains representing West African (homologous to PXVX0317 VLP strain 37997), ECSA, and Asian genotypes. The goal was to evaluate whether vaccination elicits cross-reactive NAb against all three genotypes (where the fourth genotype identified in the introduction of this review, Indian Ocean Lineage, is derived from the ECSA genotype), which would suggest that the vaccine cross-protects against all CHIKV across the globe. Serum from 12 study participants 44 weeks after enrollment and 24 weeks after the third vaccination was analyzed in cross-neutralization assays with several CHIKV strains from each of the three genotypes. Genotype-specific differences in neutralization potency were not measured, showing that the West African strain used in PXVX0317 produces a cross-reactive NAb response against the two other genotypes. Serum samples after the first and second vaccination were also evaluated and cross-neutralized CHIKV strains from heterologous genotypes, indicating that three PXVX0317 immunizations were not necessary to achieve neutralization breadth.

In the Phase II PXVX0317 trial [54], CHIKV NAb responses were measured in serum from blood collected at intervals after vaccination using Asian genotype CHIKV strain 181/25. All but 1 of the 192 participants (99.5%) who received both vaccinations developed NAb. The GMT in the vaccine group increased from baseline to week 8 and was higher than in the placebo group. At the 72-week study endpoint, 88% of the participants in the vaccine group who were seronegative at baseline had at least a 4-fold increase in NAb titer, and 96% were seropositive as assessed by a neutralization assay. Even though the study attempted to pre-screen and exclude participants who were CHIKV seropositive, there were regional differences in baseline NAb titers, where participants from 2 of the sites (Dominican Republic and Haiti) had higher baseline levels. Baseline timepoints were up to 56 days prior to enrollment, and many were IgG and IgM positive by IgG/IgM enzyme-linked immunosorbent assay, suggestive of either failure to exclude CHIKV participants who were seropositive at enrollment or CHIKV infection in the interval between blood collection at enrollment and vaccination. In participants who were CHIKV seropositive when vaccinated, NAb responses increased 2-fold, showing immunogenicity in spite of prior CHIKV exposure. In additional post hoc analyses from the trial [58], antibody responses post-vaccination were compared between 39 study participants with CHIKV neutralizing antibodies and 155 baseline seronegative participants. Baseline seropositive vaccinees showed stronger post-vaccination neutralizing antibody responses (peak GMT of 3594) compared to seronegative participants (1728), which persisted for 17 months. CHIKV seropositive vaccinees more frequently reported vaccine injection site swelling (10%) compared to seronegative recipients (0.6%). These data suggest that although it more frequently causes reactogenicity after administration VLP vaccine is immunogenic in people previously infected with CHIKV.

Similar to the clinical trials that preceded it, the immunology endpoint in the second Phase II trial [55] with adjuvanted PXVX0317 was assessed by measuring the GMT of CHIKV NAb, which was evaluated 28 days after the last vaccination. Neutralizing antibody titers in all vaccine groups rose within 7 days after PXVX0317 vaccination and persisted to the study end, 2 years, and a booster dose administered 18 months after the first dose augmented NAb levels. The adjuvant enhanced the magnitude of GMT 28 days after the first vaccination, with titers significantly higher in participants who received adjuvant formulations of either 2 × 10 µg or 2 × 20 µg at a 28-day interval, or 2× 20 µg at a 14-day interval, compared with the group that received 2 × 20 µg at a 28-day interval without an adjuvant. The adjuvanted formulations showed no advantage over the non-adjuvanted formulation after the second dose was administered. Geometric mean NAb titers were higher in groups that received 28 day compared with 14-day dosing intervals. Based on the results of this study, a single 40-µg injection of adjuvanted PXVX0317 is being further investigated in two phase III clinical trials. In trial NCT05072080 that was completed on 30 April, 2023, a safety, immunogenicity, and lot-consistency trial of PXVX0317 in healthy adults and adolescents was performed, for which the results are not yet available as of 29 August, 2024.

Using samples from the phase II adjuvant PXVX0317 trial, another study [59] characterized the B-cell response to evaluate the breadth of neutralization for three genotypes of CHIKV and related arthritogenic alphaviruses. This study

used serum collected 1, 29, and 57 days, corresponding to baseline before vaccination, 28 days after the first vaccination, and 28 days after the second vaccination, respectively, in 20 study participants who received 2 × 20 µg adjuvant VLP at the 28 day interval. Beginning 29 days after vaccination, antibody in serum was strongly and equally neutralizing against CHIKV strains from all three genotypes. Some of the participant sera also showed >50% or 80% neutralization tests against related alphaviruses including ONNV, MAYV, and RRV, where the magnitude of $PRNT_{50}$ or $PRNT_{80}$ titers and rates of positivity in the 20 participants paralleled genetic distance from CHIKV in the order ONNV>MAYV>UNAV or RRV. To evaluate induction and persistence of humoral responses, CHIKV-specific B cells were isolated from peripheral blood mononuclear cells at the times above and also at 182 days, which was 153 days after the second vaccination. Chikungunya virus-specific B cells were detected in day 29, 57, and 182 sera, where cells at the last time indicate activation markers consistent with a memory phenotype.

The study also identified broadly neutralizing monoclonal antibodies (mAbs) that bind multiple sites on the E2 glycoprotein, which could reduce potential for viral escape via mutation(s) at just a single antigenic site. When passively transferred 1 day prior to challenge with CHIKV LR2006-OPY1, some of the mAbs with neutralizing activity also protected against lethal disease in Ifnar1$^{-/-}$ mice; mAbs with reduced in vitro neutralizing capacity were less protective. A subset of the mAbs administered to C57BL/6 mice prophylactically reduced footpad swelling, virus levels in target tissues such as the ankle and calf, and histopathologic changes in myositis compared with isotype control mAbs. Using the same approach but for other alphaviruses, two of the mAbs that showed the greatest breadth of binding and cross-neutralization reduced virus levels and swelling in joints of mice after MAYV but not RRV infection, indicating that a greater cross-neutralizing potency is needed to achieve cross-protection against RRV, which is not unexpected given RRV is more distantly related to CHIKV than MAYV.

7.3 Components and Storage

The PXVX0317 VLPs are produced by transfection of human embryonic kidney VRC293 cells with a DNA plasmid encoding the CHIKV structural genes. VRC293 cells are a suspension cell line adapted to grow without serum that were derived from HEK-293 cells, which derive from human embryonic kidneys. VRC293 cells do not contain adventitious agents and lack tumorigenicity, which are criteria for use for vaccine production based on FDA guidance [60]. After the enveloped VLPs self-assemble, they are released into the culture medium as particles. The VLPs are concentrated and purified using centrifugation, filtration, and chromatography, then formulated at the appropriate dosage and stored in sterile vials before administration. The VLP manufacturer has not published storage requirements but in prior studies [59] purified VLPs were stored at −80 °C prior to use.

8 Comparing IXCHIQ and PXVX0317

Although LAV and VLP represent fundamentally different approaches to CHIKV vaccine design, the data reviewed here show common features of both vaccines revealed through animal studies and clinical trials (Fig. 3). Both vaccines confer rapid and durable immune responses for 2 years, the longest study timepoint to date. As a LAV, IXCHIQ produces infection and viremias in vaccinees, while, as a virus like particle, PXVX0317 does not. The advantage of LAV is that replicating vaccines in general produce more robust immune responses than non-replicating vaccines. Although safety risks posed by LAV are typically considered greater than other vaccine platforms, the nsP3 deletion renders virulent reversion unlikely, although no published studies have directly addressed this possibility or elucidated the mechanism by which the deletion attenuates virulence. Given the LAV nature of IXCHIQ, use of this vaccine may be more limited in scope than PXVX0317, given that it is contraindicated in immunocompromised individuals and people with a history of a severe allergic reaction such as anaphylaxis to components in IXCHIQ [52]. Contraindications for PXVX0317 have not yet been established.

PXVX0317 NAb titers were evaluated using a luciferase neutralizing antibody assay [54], where values reported use the stringent NT80. With this NT80 cut-off, 72–98% of PXVX0317 recipients were seropositive within 7 days after the first dose and all participants were seropositive 28 days after one or two doses, which was sustained for 2 years in all participants. The IXCHIQ studies [37, 38] used a µPRNT test with a NT50 endpoint, which is less stringent than NT80. With NT50, IXCHIQ results in seropositivity in 30% of participants 7 days after vaccination, suggesting that the adjuvanted PXVX0317 leads to faster seroconversion. However, similar to the PXVX0317 data, NAb titers in participants who received IXCHIQ increased to 100% by day 14 and persisted for 2 years [37, 44]. Chikungunya virus 181/25, a different CHIKV LAV developed via serial passage of a wild-type strain, was evaluated in a prior clinical trial [61]. In that study, 8% (5/59) of participants developed transient arthralgia, which was noted with concern given that wild-type CHIKV infection frequently causes arthritic manifestations. In the phase I clinical trials reviewed here, IXCHIQ produced joint pain in 12% (14/120) participants within 14 days after vaccination, most frequently in the high-dose group. In part for these safety reasons, the medium

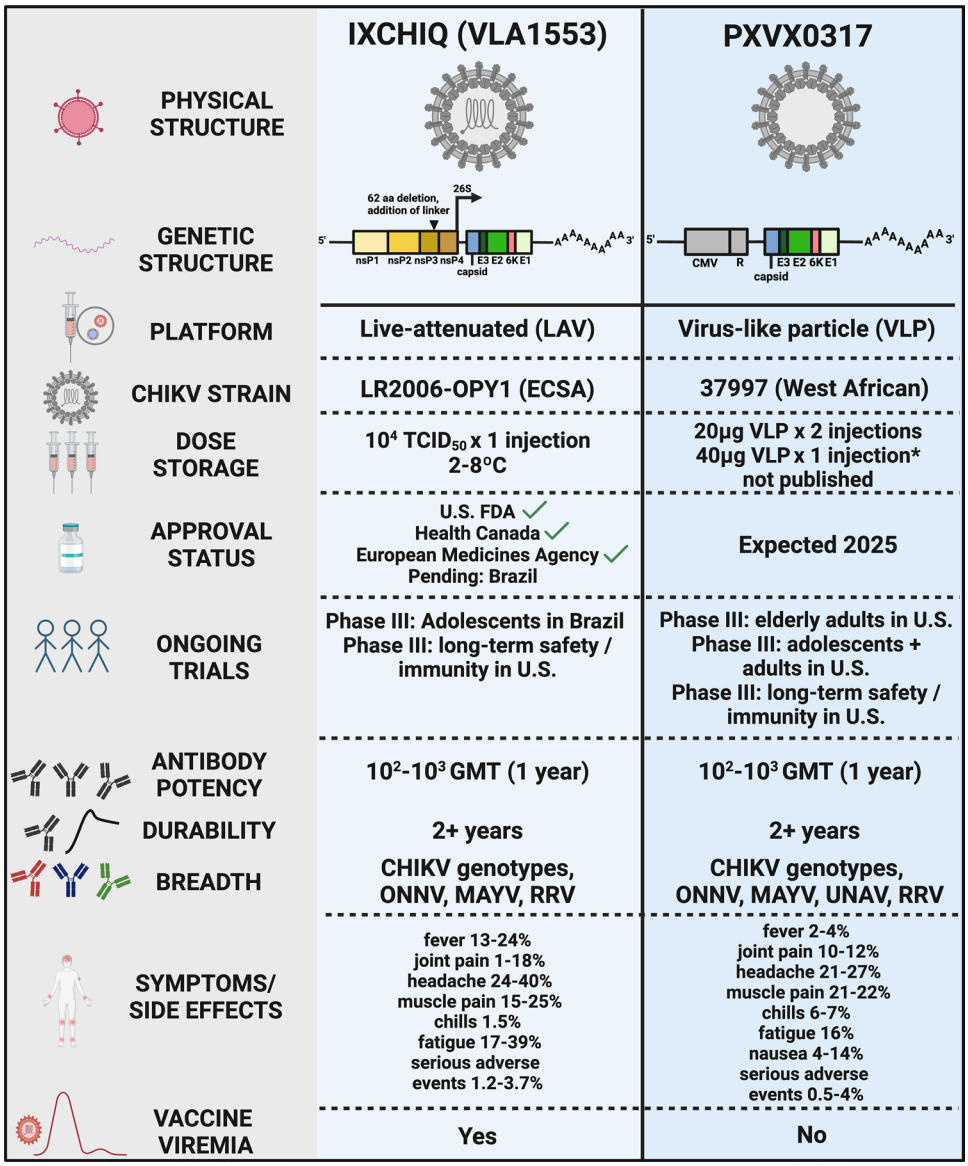

Fig. 3 Overview of IXCHIQ and PXVX0317 vaccines. Data are current as of August 30, 2024. Symptom ranges reported are compiled data for each level of symptom severity and vaccine dose across all reported clinical trials. *Indicates that the final dose has not yet been selected. *CHIKV* Chikungunya virus, *GMT* geometric mean titer, *ECSA* East Central South African, *MAYV* Mayaro virus, *ONNV* O'nyong nyong virus, *RRV* Ross River virus, *US FDA* US Food and Drug Administration. A publishing license was granted for the image created in Biorender

dose (1×10^4 $TCID_{50}$) was used in the phase III study. In the phase III study only, 0.06% (2/3082) of participants had a serious adverse event considered related to IXCHIQ. The PXVX0317 studies also noted joint pain. Six percent of participants reported joint pain after the first injection of PXVX0317 and 5% after injection of placebo, and three transient but severe events were reported in the PXVX0317 group.

9 Outstanding Unknowns

Licensure of IXCHIQ by US, Canadian, and European regulators and completion of phase III clinical trials for the VLP represent a significant step towards preventing CHIK. Furthermore, an agreement between Valneva and the Coalition for Epidemic Preparedness Innovations will aid in disseminating IXCHIQ to regions where outbreaks occur and will support WHO prequalification for widespread access in lower-income and middle-income countries [62]. Even with the licensure hurdle met or nearly met, unknown questions about both vaccines in context of endemic CHIKV circulation remain. Neither IXCHIQ clinical trial and only the phase II VLP study was conducted in a region with transmission of CHIKV or other alphaviruses (caveat: a subtype of Venezuelan equine encephalitis virus called Everglades virus is endemic to Florida where some of the clinical trials were performed; seroprevalence is very low [63, 64]). As such, the effects of pre-existing immunity to CHIKV or other alphaviruses on IXCHIQ and PXVX0317 safety and immunogenicity remain unknown. In addition to CHIKV, at least seven other alphaviruses, including MAYV, VEEV,

Eastern equine encephalitis and Western equine encephalitis, UNAV, ONNV, and RRV are considered significant emerging disease threats in regions that would be expected for CHIKV vaccine rollout.

Since 2005, the WHO estimates CHIKV has caused more than 2 million cases worldwide and is currently endemic in 110 countries [1]. The global spread of CHIKV extends into ranges of many of the other alphaviruses that threaten human health, especially in Latin America, where multiple alphavirus species share overlapping geographic distributions (Fig. 4) The shared range of CHIKV with other alphaviruses means that CHIKV vaccine delivery will be targeted to regions where people are exposed to other alphaviruses in addition to CHIKV. The impact of pre-existing alphavirus immunity on alphavirus cross-neutralization profiles has been examined in context of primary CHIKV infection [46, 65, 66], leaving open questions about how primary infection with other circulating alphaviruses shapes the neutralization breadth. Questions related to effects of pre-existing CHIKV or other alphavirus immunity and pan-alphavirus species protection can be initially addressed using experimental studies with existing animal models, where other human pathogenic alphaviruses that share geographic ranges with CHIKV also infect and cause disease in mice and NHP that model CHIKV.

As a LAV, IXCHIQ produces viremias of 3–5 $\log_{10}$ genomes/mL in humans that peak at 3 days and usually last a week [37] but can extend up to 2 weeks [39]. These viremias exceed infection and transmission thresholds for CHIKV vector mosquito species *Aedes (Ae.) aegypti* and *Ae. albopictus* in laboratory studies we [67–69] and others [70] performed using the same or nearly identical CHIKV strains as the backbone used for IXCHIQ. However, no publicly available data have examined whether IXCHIQ is capable of transmission by mosquito vectors. It is not known whether the nsP3 deletion that attenuates vertebrate pathogenicity affects mosquito vector infection or transmission. Although regions of the C terminal portion of the CHIKV nsP3 that are required for mosquito infection and transmission have been defined [71], these are not in the region deleted in IXCHIQ. If vector infection and transmission are possible, even if only for a short period surrounding the viremia peak, IXCHIQ could be spread by mosquitoes from viremic vaccinees, producing mosquito-borne vaccine transmission in areas with vaccine rollout, which could pose safety risks for populations for which the vaccine is not approved, adding

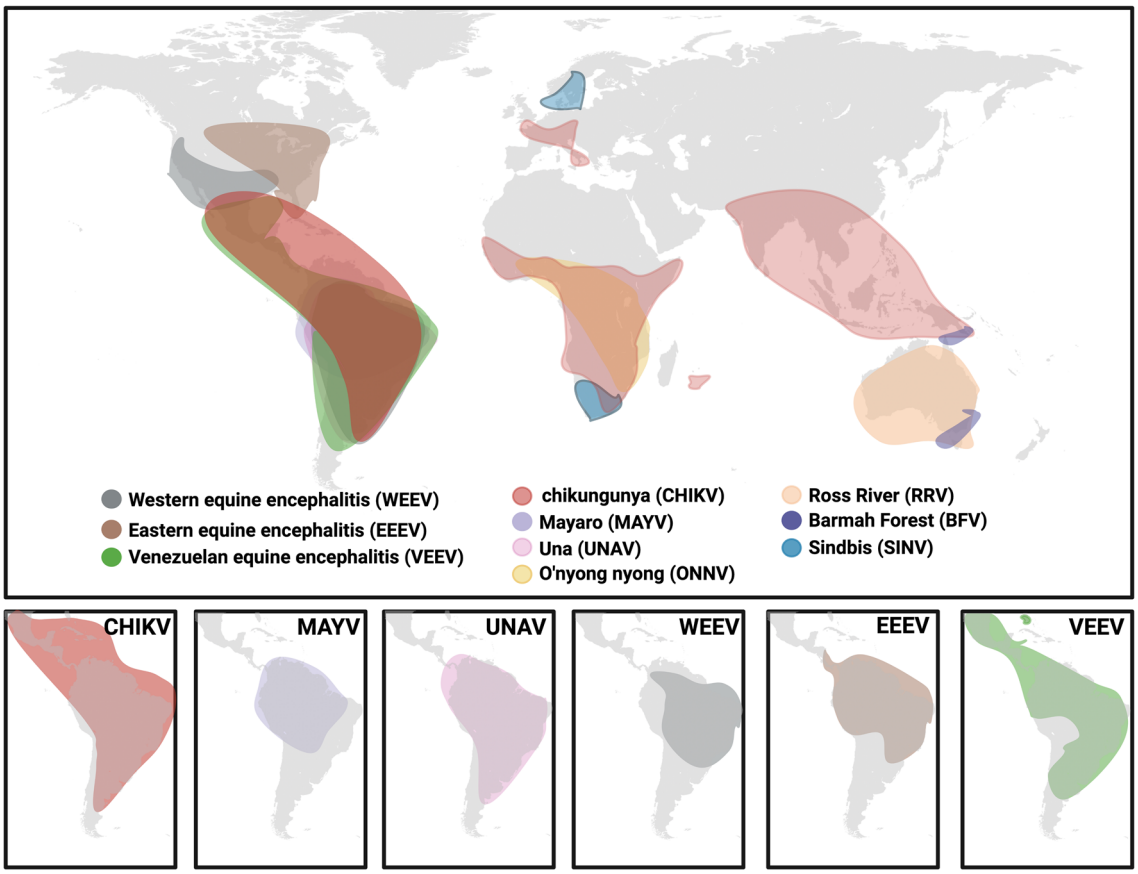

Fig. 4 Global distribution of medically important alphaviruses, 2024. A publishing license was granted for the image created in Biorender

another layer of complexity to dynamic population immunity. Infectiousness of IXCHIQ vaccinees to mosquito vectors could also lead to recommendations to protect recently vaccinated people from mosquito exposure. Experimental vector competence studies with IXCHIQ should be performed to address the possibility of IXCHIQ spread by CHIKV mosquito vectors.

Questions about the durability of IXCHIQ and PXVX0317 protection also remain. Although CHIKV antibody and memory B cells after natural infection in humans persist for several years [45, 46, 72], it is currently unknown whether long-term immunity beyond 2 years for both vaccines can be achieved. To address this question for IXCHIQ, a 5-year antibody persistence and long-term safety trial (ClinicalTrials.gov NCT04838444) that started in April 2021 is currently underway in the same participants in the phase III trial.

IXCHIQ and PXVX0317 safety and immunogenicity in children, elderly, immunocompromised individuals, and pregnant people are also not known. To study IXCHIQ in children, a prospective, double-blinded, multicenter, randomized phase III trial of 750 participants aged 12 to < 18 years was initiated in 2022 in Brazil (ClinicalTrials.gov NCT04650399). The goal of this study is to evaluate the safety and immunogenicity of the adult dose of IXCHIQ, 1×10^4 $TCID_{50}$, up to 180 days after a single immunization. On 13 May, 2024, Valneva reported positive immunogenicity and safety data where 99% (232/234) of juvenile study participants were seroresponsive 180 days after vaccination and GMT antibody titers exceeded the seroresponse threshold of a $\mu PRNT_{50}$ titer of ≥ 150 and study participants reported mild or moderate solicited adverse events [73]. To evaluate PXVX0317 in elderly persons, a phase III, randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov NCT05349617) to evaluate the safety and immunogenicity of in adults aged ≥ 65 years in the USA was completed on 8 August, 2023.

Absent efficacy trials in CHIKV endemic countries, vaccine licensing is based on NAb titers in human trials, animal studies, and serologic studies in areas with CHIKV. Some of these studies show apparently contradictory $\mu PRNT_{50}$ titers necessary to confer protection. Passive transfer of sera from IXCHIQ vaccinated humans into macaques established the $\mu PRNT_{50}$ titer of ≥ 150 as the threshold [42], but a human serosurveillance study indicated the protective titer against re-infection may be as low as 10 [31]. In an attempt to resolve this disparity, a WHO Expert Committee on Biological Standardization has established a WHO International Standard for CHIKV NAb that will allow for more direct comparisons of NAb titers between studies [74].

While NAbs play a key role in protection from disease, additional research is also warranted to understand the protective role of other contributing immune responses that mediate protection from infection and disease, especially in the context of pre-existing vaccine-elicited and/or natural infection-elicited alphavirus immunity.

10 Conclusions

Two CHIKV vaccines have recently reached or are approaching regulatory approvals in 2024. One vaccine is a LAV, VLA1553, marketed as IXCHIQ, and the other is a VLP, PXVX0317. Pre-clinical and clinical data reviewed here support the safety, immunogenicity, and protective efficacy of the two vaccines. Outstanding questions for both vaccines include impacts of pre-existing CHIKV immunity on vaccine safety and immunogenicity, long-term immunity, safety in young, pregnant, and immunocompromised people, and efficacy in individuals previously exposed to other alphaviruses in addition to chikungunya virus. Although many questions remain about how CHIKV vaccine coverage will shift the landscape of population-level alphavirus immunity and circulation, vaccine licensure represents a promising leap forward in global CHIKV disease prevention.

Declarations

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Consent to Participate Not applicable.

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Availability of Data and Material Not applicable.

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Authors' Contributions WCW, DNS, and LLC wrote the manuscript draft and generated figures. All authors read and approved the final manuscript.

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