APJAI
Asian Pacific Journal of
Allergy and Immunology

REVIEW ARTICLE

Chikungunya vaccines: An update in 2023

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Abstract

A recent chikungunya outbreak affected 1.5 million cases in more than 60 countries. The virus causes low mortality but moderate to severe morbidities such as high fever, myalgia, and polyarthritis. The chikungunya virus is transmitted by *Aedes spp*. Mosquitoes, of which the population has increased due to urbanization and global warming. Currently, no commercial vaccine is available, but several candidates are being tested in clinical trials. This review aimed to summarize the recent updates of candidates on each platform, ranging from traditional inactivation, live attenuation with reverse genetics, virus-like particles, viral vectors, and mRNA, mainly focusing on the candidates in clinical trials or recently developed.

Key words: Chikungunya virus, viral vector vaccines, mRNA vaccines, preclinical chikungunya vaccines, clinical trials

Citation:
Roongaraya, P., Boonyasuppayakorn, S. (2023) Chikungunya vaccines: An update in 2023. *Asian Pac J Allergy Immunol*, 41(1), 000-000. https://doi.org/10.12932/ap-271222-1520

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Background

The chikungunya virus is one of the significant re-emerging arboviruses in recent decades. The virus was first identified in Tanzania in 1952, and sporadic infections have been recorded in Africa and Asia over the past 50 years.[1] In 2004-2007, an outbreak started in Kenya and spread across the Indian Ocean to more than 60 countries, affecting 1.5 million people in Maldives, India, Sri Lanka, Indonesia, Myanmar, and Thailand.[1,2] La Reunion Island in the Indian Ocean reported 255,000 cases, representing more than one-third of the population.[3] The first local transmissions in Europe and America were reported in 2007[4] and 2013,[5] respectively. In the past ten years, there have been several outbreaks of chikungunya around the world, mainly caused by the East/Central/South African (ECSA)-diverged or Indian Ocean Lineage (IOL) strain.[6,7] Currently, the geographical distribution of the chikungunya virus covers all tropical regions,[8] resembling that of the ecological niche of *Aedes albopictus*.[9]

Chikungunya virus belongs to the *Togaviridae* family, the genus *Alphavirus*, consisting of four genotypes; West African, East-Central-South Africa (ECSA), ECSA-diverged or Indian Ocean Lineage (IOL), and Asian.[10] Recent vaccine candidates such as VLA1553, MV-CHIK, and ChAdOxI -Chik were developed under La Reunion (LR2006 OPY1) of the ECSA strain. Until now, none of the vaccine candidates has solidly proven a cross-protection to the other CHIKV genotypes. The genome is a single-stranded, positive sense, 11.8 kb RNA with a 5′-cap and a poly-A tail surrounded by an icosahedral capsid and a lipid envelope with trimer-shaped spikes E1-E2 (**Figure 1A**).[11] The virus exists in both sylvatic and urban life cycles, in which its mosquito vectors mainly are *Aedes spp*. The urban cycle is maintained by *Aedes aegypti* and *Aedes albopictus*. The virus is transmitted through mosquito saliva, mainly targeting the epithelial fibroblast and dermal macrophage. Maternal-fetal transmissions are also reported, but no differences in pregnancy outcomes were observed.[12] The initial attachment of CHIKV to the host cell is mediated by the binding of the viral envelope protein E1 to various cell surface receptors, including the DC-SIGN, CLEC5A, GRP78, and Mxra8 receptor,[13] which subsequently initiates receptor-mediated endocytosis. Low pH at the endosome triggers E1-E2 rearrangement for endosomal membrane fusion.[14] The initial translation yields four nonstructural proteins (nsp 1-4) responsible for the subsequent replication and translation of five structural proteins (C-E3-E2-6K-E1) (**Figure 1B**, **Table 1**).

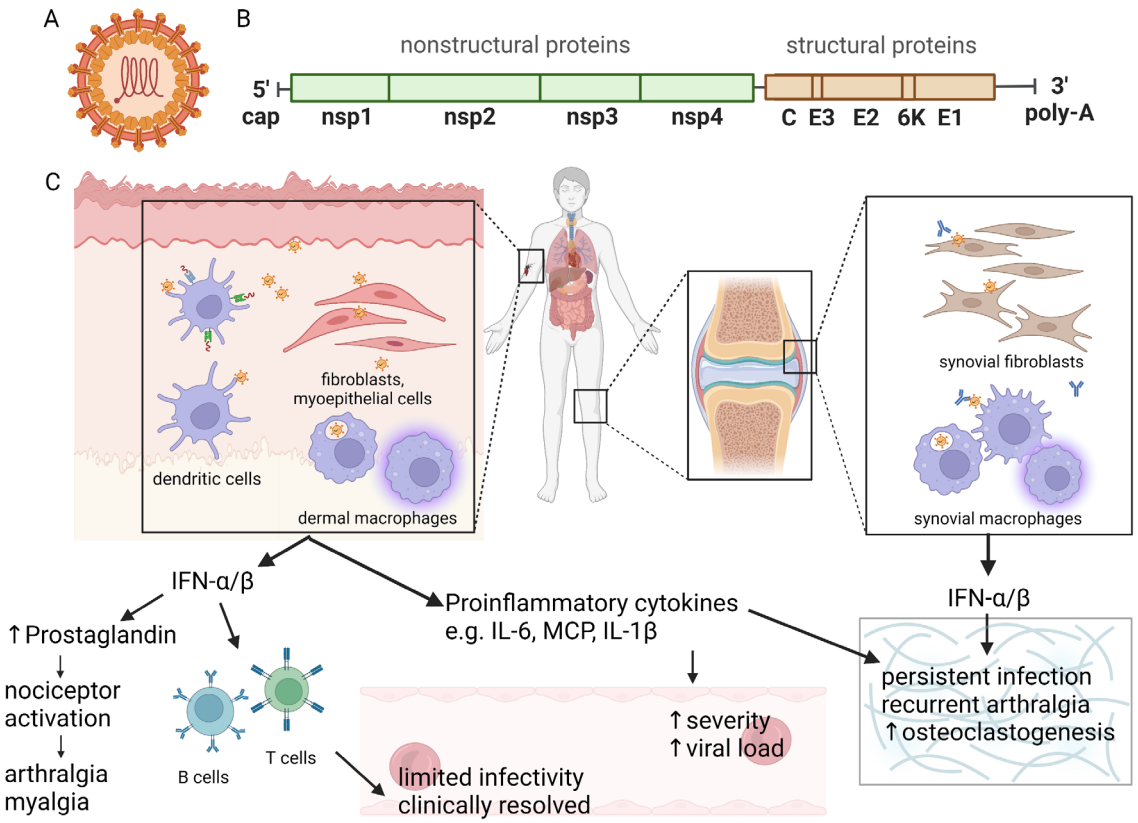

Figure 1. Diagrams of Chikungunya virus A) structure B) genome C) pathogenesis in acute and chronic phases, created by Biorender.

Table 1. Chikungunya proteins and their functions.

Viral protein	Function	Reference
nsp1	Methyltransferase, guanylyltransferase (mRNA capping)	16
nsp2	papain-like cysteine protease, NTPase, RNA triphosphatase, helicase, nuclear localization signal, inhibit host protein synthesis	15, 17, 18
nsp3	Scaffolding, associated with host-derived replication factors	19-21
nsp4	RNA-dependent RNA polymerase	22
C	Capsid (240 copies/virion), two nuclear localization signals, capsid protease	23, 24, 25
E3	Facilitate E1-E2 heterodimer (inhibit E1 homotrimer formation), E.R. signal sequence	26
E2	Component of a spike (three copies of E1-E2 heterodimers, 80 spikes/ virion)	14, 21
6K	Mediates E1 translocation to the E.R., membrane permeabilization (ion channel), virus budding, and virus assembly	21, 27
E1	Component of a spike (three copies of E1-E2 heterodimers), membrane fusion at low pH (E1 homotrimer)	14, 21

The assembly occurs in the plasma membrane, where the capsid interacts with the C-terminal region of E2 to initiate virion development. Moreover, the nsp2 is a key factor for a host translation shut-off.[15] The E3 contains a signal sequence for ER localization, thus directing pE2 (premature E2)-6K-E1 to the ER lumen. Furthermore, E3 hinders the E1 fusion loop and inhibits E1 trimerization, thus preventing premature fusion while traveling through low pH trans-golgi network (TGN). The host-derived furin protease is responsible for the cleavage of pE2, followed by the formation of a mature spike containing three copies of the E1-E2 heterodimers.

The primary targets of the chikungunya virus are dermal fibroblasts, skin macrophages, and local epithelial and endothelial cells.[28-30] Local infection triggers pathogen-recognition receptors (for example, TLR and RIG-1)[31] to up-regulate the expression of type 1 interferon (IFN-α/β) expression (**Figure 1C**), which is critical for viral clearance.[32] Inadequate IFN-α/β responses could lead to progression to hemorrhagic fever and shock, as demonstrated by adult mice lacking interferon response factors 3 and 7-deficient (IRF3/7$^{-/-}$) adult mice.[33] The IRF3/7$^{-/-}$ mice challenged with the chikungunya virus clinically manifested similar to hemorrhagic shock, including increased virus replication, edema, vasculitis, hemorrhage, oliguria, thrombocytopenia, and hemoconcentration despite the ~50- and ~10-fold increases in levels of IFN-γ and tumor necrosis factor (TNF), respectively.[33] Furthermore, dermal macrophages secrete various proinflammatory cytokines to initiate inflammation, including TNF-α, IL-1, IL-6, and monocyte chemoattractant protein-1 (MCP-1/CCL2). The level of IL-1β, IL-6, and MCP-1 correlate directly with disease severity.[34,35] In addition, mosquito-derived salivary components modified immune responses towards TH_2 to accommodate the blood-feeding purpose.[36] Therefore, the chikungunya virus replication could benefit from this saliva-induced TH_2 as the proinflammatory cytokines and antiviral responses are inhibited.

The incubation period is usually 3-7 days and the symptomatic period is 2-12 days.[37] The most common clinical features are high-grade fever (>39°C), arthralgia, maculopapular rash, conjunctivitis, myalgia, and edema of the upper and lower extremities. Duration and intensity of acute infection correlate with viral load, and IgM and IgG levels correlate with the disease severity. Serious complications such as encephalopathy and encephalitis, cardiovascular and respiratory disorders, renal failure, hepatitis, and myocarditis are rare but often involve extreme ages with underlying diseases. About 5% of acute infections could develop prolonged polyarthritis similar to chronic inflammatory rheumatism such as rheumatoid arthritis and spondyloarthritis.[38] A recent finding showed that human synovial tissues are CHIKV sanctuaries, as the viral RNA persists in joint fluid.[39] The persistent chikungunya-associated arthritis could be related to CHIKV-infected fibroblast-like synoviocytes and induced the migration of primary human monocytes.[40] Monocytes/macrophages were transformed into osteoclast-like cells and secreted high levels of TNF-α and IL-6 proinflammatory cytokines (**Figure 1C**). Furthermore, intraarticular IL-6 activated the receptor activator of the nuclear factor κ-B ligand (RANKL) and inhibited osteoprotegerin (OPG), thus promoting osteoclastogenesis and osteoclast activation. Other cytokines, including GM-CSF, IFN-α, IL-17, IL-1β, and IL-18, were reported to be associated with chronic arthritis. In addition, the NLR family pyrin domain containing 3 (NLRP3), the NOD-like receptor (NLR) subfamily of PRRs, was activated, and caspase-1 was recruited to the inflammasome complex to activate proinflammatory cytokines IL-1β and IL-18. Furthermore, proinflammatory molecules were reported in patients with acute polyarthritis caused by Ross river virus (RRV), an alphavirus closely related to CHIKV. The increased level of C3a anaphylatoxin in synovial fluid suggests that a complement pathway is activated during chronic inflammation. Furthermore, silica-administration macrophage depletion reduced mononuclear infiltrations in skeletal muscle, thus alleviating the severity of the disease in RRV-infected mice. In summary, sufficient direct and indirect evidence confirms the critical role of macrophages in CHIKV-induced chronic polyarthritis.

CHIKV infection triggers a sufficiently adaptive immune response and protects against later infections. Functional T and B cell-depleted or RAG-1$^{-/-}$ mice displayed higher viral load and persistent viremia than wild-type suitable for vaccine safety trials.[41] The pathological strain exhibited a highly conserved glycine (G82) in the CHIKV-E2 protein, contributing to inadequate viral clearance and persistent infection. The G82R mutation in a CHIK 181/25 vaccine strain attenuated persistent infection in wild-type mice. However, the G82R strain generated persistent infection in mature B cells-depleted (μMT) mice, suggesting that B cells played an important role against persistent infection.[42] Moreover, the CHIK 181/25 infection with interferon (IFN)-α/β -deficient mice resulted in temporary morbidity, while the infection with IFN-α/β and IFN-γ -deficient mice was lethal.[43] Moreover, the (IFN)-α/β -deficient mice expressed significantly increasing levels of IFN-γ and IL-12 while the inflammatory cytokines, TNFα and IL-6, remained low.

The role of B and T cells has been investigated in Rag1$^{-/-}$ mice, which lack T and B cells. After infection in the footpad, Rag1$^{-/-}$ mice in various tissues than wt mice, suggesting that adaptive immunity controls tissue specificity and helps eliminate CHIKV infection.[44] The role of B cells was also explored in B cell-deficient (μMT) knockout mice infected with CHIKV on the footpad. In these animals, viremia persisted for over a year, indicating a direct role for B cells and antibodies mediating CHIKV clearance.[42] Furthermore, these infected mice exhibited a more severe disease than wild-type mice during the acute phase.[42] The roles of T cells were explored in adult mice deficient in T cells and infected in the footpad.[45] Interestingly, CHIKV-specific CD4+ but not CD8+ T cells were found to be essential for the development of joint swelling development without any effect on virus replication and dissemination, suggesting that T cells are involved in inflammation.[45] This corroborates observations made from human muscle biopsies

in which T cells were detected but not B cells.[46] The importance of B and T cells in protection against CHIKV infections has also been demonstrated by vaccine studies. In fact, vaccination can induce robust cellular and humoral responses that protect mouse and non-human primate models against lethal challenges.[47,48] The role of host T and B cell responses has been investigated in rhesus macaques. Interestingly, aged animals have delayed and reduced T and B cell immunity compared to adult animals.[49] Furthermore, while adult animals can control viral infection, aged animals show persistent viruses in the spleens.[49] These data support clinical findings of CHIKV susceptibility in elderly humans and provide evidence that effective T and B cell responses against the virus are required to prevent persistent CHIKV infection.

Examples of animal models of CHIKV infection are mice and non-human primates.[50] In mice, acute phase models were 1) lethal neonatal challenge that develops lethal encephalitis representing severe neonatal encephalitis; 2) mice deficient in type I IFN that is also susceptible to a lethal challenge for pathogenesis study and vaccine safety and efficacy tests; 3) CHIKV-induced arthritis in adult immunocompetent mice to test CHIKV vaccines and therapies. The chronic mouse model was not fully established, but ineffective T and B cell responses were related to persistent CHIKV infection, as discussed earlier. Furthermore, cynomolgus and rhesus macaques demonstrated clinical manifestations to acute infection in human; thus commonly used in vaccine and immunotherapeutics studies.

Laboratory investigations are required for a definitive diagnosis of acute and chronic infections.[51] Reverse transcription PCR (RT-PCR) for viral RNA is highly sensitive and specific 5-7 days after the onset of the disease. Furthermore, specific IgM and IgG antibodies can be detected after the acute phase and last for months. Therefore, detecting chikungunya RNA in blood and paired sera for specific IgM and IgG antibodies is a definitive diagnosis of acute infection. Rapid antigen and antibody detection tests are available, but cross-reactivity with other mosquito-borne viruses should be a concern.[52] A recent report showed that two PCR-confirmed CHIKV infections were positive for dengue NS1 immunochromatography and fluorescent immunoassay.[53] In addition to acute phase detection, the viral genome detected in synovial fluid strongly suggested persistent chikungunya-associated arthritis.[54] In addition, ELISA CHIKV-IgG, rheumatoid factor, anti-CCP (anti-citrullinated peptide antibody), and HLA B27 (human leukocyte antigen B27) are suggested for differential diagnosis with rheumatoid arthritis and spondyloarthritis. Supportive treatments such as adequate rest, hydration, and acetaminophen are mainly recommended. Furthermore, early administration of disease-modifying antirheumatic drugs (DMARD) was recommended in cases with chikungunya-associated polyarthritis that mimics rheumatoid arthritis.[54] No specific treatment is currently available, but some antivirals, such as antifungal itraconazole and andrographolide, are under development.

In vaccine research, surrogate markers are biomarkers to predict the effect of a vaccine on the clinical endpoint.[55] The level of CHIKV-specific neutralizing antibodies against viral structural proteins, especially the E2, was an important surrogate marker to evaluate the vaccine efficacy.[56] Examples of immunoassays are the plaque reduction neutralization test (PRNT), and microneutralization (μNT), in which their endpoints are evaluated by inhibition of 50-90% plaque titration ($PRNT_{50}$, or $PRNT_{90}$) and 50-100% cytopathic effect (CPE), respectively.[56] Seroconversion is defined by the presence of viral-specific antibodies, which were previously negative prior to infection. Therefore, seroconversion could correlate with protection if the viral-specific antibodies were proven to have neutralization activities.

Current vaccine development

General vaccine trials consist of two major steps: preclinical evaluation in two animal models and three phases of clinical trials. Safety and efficacy are mostly concerned in all steps. In this review, the level of efficacies is evaluated based on the following conditions; 1) protecting the animal from a lethal challenge, 2) activating robust immunogenicity in humoral responses, especially a high neutralizing antibody titer, and 3) eliciting robust cellular responses by interferon-γ level from specific CD4+ and CD8+ T cells. Safety concerns include potential adverse reactions, multiple doses or an invasive route of administration, the potential to revert to virulence, etc. Until now, vaccines in clinical trials use platforms including formalin inactivation, live-attenuated strains, virus-like particles, viral vectors, and mRNAs (**Table 2**, **Figure 2**).

Inactivated vaccine

Formalin and UV-inactivated vaccines were developed primarily during 1967-1973.[57,58] Both candidates were claimed to trigger neutralizing antibodies in the non-human primate.[58] The two doses, 28 days apart, formalin-inactivated CHIKV Asian (15561) strain were given to 16 military volunteers, and all subjects developed significant neutralizing antibodies on day 14 after the second dose. The vaccine did not show a local or systemic reaction.[57] Persistent antibodies were not determined. No further development was done with this candidate. After the re-emerging CHIKV outbreak in India and the Indian Ocean Islands, standard formalin inactivation was attempted with the ECSA strain (CHK/03/06) strain. Three doses of the hydrogel gel formulation were administered subcutaneously, and high neutralizing antibodies were evident in both the ELISA (1:51,200) and plaque reduction neutralizing antibodies (1:6,400) during 6 to 8 weeks after vaccination.[59] Furthermore, the TH_1 and TH_2 responses were suggested by high levels of pro- and anti-inflammatory cytokines. However, no further development was recorded afterward.

Table 2. Summary of safety and efficacy profiles of vaccines in their latest clinical trials.

List of vaccines	Safety	Efficacies	Clinical trial identifier
Δ5nsP3 (Valneva)	51.1% (1575/3082) mild to moderate side effects, 2 serious side effects	Neutralization antibody (day 29), single dose	III (NCT04546724)
CHIKV-VLP + adjuvant (PXVX0317PXV)	Mild to moderate side effects, no serious side effect	Neutralization antibody (day 14 and 28), 2 doses (14 or 28 days apart)	II (NCT03483961)
MV-CHIK	Mild to moderate side effects, no serious side effect	Neutralization and cell phagocytosis, 2 doses (1 month apart)	II (NCT02861586)
	Mild to moderate side effects, no serious side effect	High neutralization (2 doses, 28 days apart); vaccine/placebo GMFR = 1.6 (day 28), 2.1 (day 56)	II (NCT03101111) II (NCT03807843) Previously endemic area (Puerto Rico)
ChAdOxI -Chik/ (CHIK001)	Mild to moderate side effects, no serious side effect	Neutralization (day 14), single dose Cross-protection to ECSA IOL, West Africa, and Asian strains	I (NCT03590392) Ib (NCT04440774)
mRNA 1388	Well tolerated	no seroconversion (day 28, 1st dose) 100% seroconversion (days 56 and 196, 2nd dose of the 100 μg only)	I (NCT03325075)
mRNA 1944	Mild to moderate side effects	mean terminal half-life (t1/2) = 69 days, CHKV-24 IgG serum levels 1.8 fold linear accumulation (2 doses, 1 week apart)	I (NCT03829384)

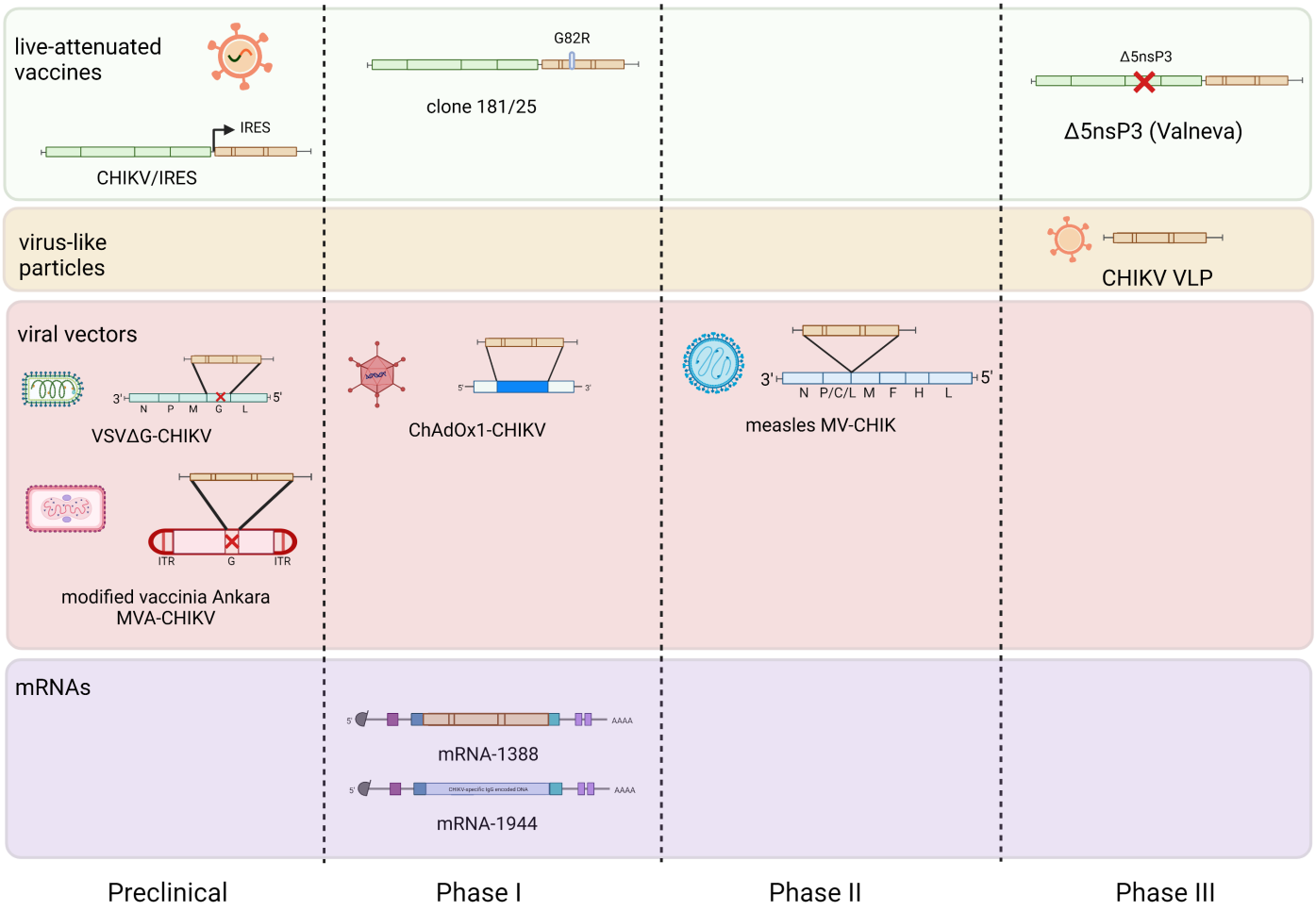

Figure 2. Diagrams of currently and recently active chikungunya vaccine candidates, their constructs, and developmental stages, created by Biorender.

Live attenuated vaccine

The 181/clone 25 (181/25 or TSI-GSD-218)

The 181/clone 25 (181/25 or TSI-GSD-218) was the first live attenuated CHIKV vaccine candidate developed in 1985 by the U.S. military.[60] The field isolate AF15561 (Thailand, 1962) was continuously subpassaged in primary grivet kidney cells and human embryonic lung cells (MRC-5) until it completely lost lethality in neonatal mice and rhesus monkey. Subsequent sequencing and genetic mutagenesis revealed that G82R at E2 was a significant attenuation.[61] Phase II randomized controlled trial (RCT) revealed that 98% (57/58) of vaccinees who received a single subcutaneous dose developed CHIKV neutralizing antibody on day 28 after vaccination.[62] However, another trial reported symptoms including headache (11–12%), fever (temperature > 38.1°C; 5–6%), myalgias or arthralgias (1%), odynophagia (5-6%), and local tenderness or erythema at the injection site (5–6%) in the CHIKV vaccine (19/38) and placebo (19/38) without significant differences.[63] Moreover, E2/R82G revertant mutant was detected in one of the subjects in Phase I / II studies.[60] No further clinical studies was registered after 2005, but preclinical studies with immunocompromised mice highlighted the importance of B cells and interferon signaling.[43]

The Δ5nsP3 or VLA1553

The VLA1553 is a live-attenuated vaccine candidate initially studied in a common European effort (ICRES FP7-HEALTH) project and further developed under the Valneva biotech company. The vaccine candidate is constructed from the La Reunion strain (LR2006 OPY1) with a 60 amino acid deletion in the nsP3 gene. The vaccine was granted the 'PRIME designation' by the European Medicines Agency (EMA) in 2020 to improve support for the development of the investigational vaccine. Recently, the US FDA granted a "Fast Track and Breakthrough designation" for rolling submission of Biologics License Application (BLA) in Aug 2022. The current status of this vaccine is still under investigation, and it is not approved for use in any jurisdiction.

The Phase 1 study (NCT03382964)[64] was carried out in 120 healthy 18–45-year-old adults administered intramuscularly with three doses at low (3×10^3 $TCID_{50}$, n = 31), medium (3×10^4 $TCID_{50}$, n = 30), and high (3×10^5 $TCID_{50}$, n = 59) levels. The vaccine was well tolerated in both the low-dose and medium-dose groups, but 7% (4/59) of the high-dose group reported local reactions. Additionally, 36% (11/31), 40% (12/30), and 68% (40/59) of the volunteers in the low-, medium- and high-dose groups, respectively, reported any solicited systemic reaction. No vaccine-related serious adverse events were reported. The vaccine showed a good immunogenicity profile with 100% seroconversion on day 14 (103/103), and the range of geometric mean peak antibody titers was 592.6 to 686.9 on day 28. Neutralizing antibodies (μNT50– target strain VLA1553) were still detected 1 year after vaccination in all dose groups. The high-dose group was re-vaccinated at 6 or 12 months, but vaccine-induced viremia was detected. The medium dose was selected for subsequent phase 3 clinical trials.

Furthermore, sera from phase I vaccinated volunteers were passively transferred to non-human primates (NHP) one day before the challenge with the wild-type CHIKV (LR2006-OPY1).[65] The sera-transferred NHPs showed lower to undetectable viremia than the control, suggesting successful neutralization by passive immunity. Additionally, NHPs were clinically observed throughout the experiment and sacrificed on day 28 after the challenge for the histopathology study. The researchers concluded that there was no CHIKV-associated clinical symptoms, histopathology, or any evidence of CHIKV persistence in tissue.

Phase III (NCT04546724)[66] was a multicenter, randomized, placebo-controlled, double-blind study conducted in the U.S. The trial enrolled 4,115 baseline negative adults and older adults (18-94 years of age) characterized to the vaccine arm (1×10^4 / 0.5 ml, n = 3,082) and in the placebo arm (placebo/0.5 ml, n = 1033) based on demographic data. The primary endpoint was 98.9% (263/266, 95%CI: 96.7-99.8) seroconversion in the vaccine arm determined by neutralizing the antibody titer (<150 by CHIKV $\mu PRNT_{50}$) at day 29 after a single dose vaccination, suggesting the vaccine efficacy at 50% was determined at the dilution of 1:150. The placebo was 0% (0/96, 95%CI 0.0-3.8) seroconversion. Furthermore, any related adverse events were observed at 51.1% (1575/3082) and 31.2% (322/1033) on the vaccine and placebo arms, respectively. The most common side effects were mild to moderate fatigue, headache, myalgia, arthralgia, and fever, which resolved in 3 days. Two vaccinees had serious side effects, including severe myalgia and syndrome of inappropriate antidiuretic hormone secretion (SIADH), requiring hospitalization. Overall, the vaccine candidate elicited 98.9% seroconversion in adults and older adults but retained 51.1% of adverse reactions. The protective efficacies in endemic areas are still under investigation.

CHIKV/IRES

This vaccine candidate genetically engineered a picornavirus-derived internal ribosome entry site (IRES) to replace a natural promoter of structural gene expression. The last updated status was in 2013-5 in preclinical studies.[47,67] The live CHIKV vaccine candidate showed a robust, protective immune response and no detectable diseases in A129 mice.[47,68] Furthermore, all vaccinated mice survived after a lethal challenge with wild-type CHIKV.[48] The vaccine candidate was incapable of infecting mosquito vectors.[68] Results in cynomolgus macaques showed no clinical signs but were highly immunogenic.[67] Moreover, the macaques showed no detectable viremia or significant changes in core body temperature or cardiovascular rhythm after challenging wild-type CHIKV, unlike sham-vaccinated animals.[67] These CHIKV/IRES vaccine candidates appear safe and efficacious, supporting their strong potential for further development in clinical trials. However, this CHIKV/IRES candidate has not been registered on clinicaltrial.gov. for a Phase I trail yet.

Virus-like particles

Virus-like particles (VRC-CHKVLP059-00-VP or CHIKV VLP)

The VRC-CHKVLP059-00-VP construct originated from structural genes of West African CHIKV strain 37997, thus lacking the self-replicating element. After two doses, the vaccine candidate elicited neutralizing antibodies against homologous 37997, and heterologous LR2006 OPY-1 (ECSA) strains in BALB/c mice and rhesus macaques intramuscularly.[69] Moreover, the vaccine candidate protected the non-human primates from viremia and inflammatory responses from a high titer LR2006 OPY-1 virus challenge. In addition, the purified total IgG from immunized or control monkeys was intravenously transferred to the mice. The results showed that the CHIKV-immunized monkeys'IgG-recipient mice survived a lethal challenge of CHIKV strain LR2006 OPY-1 with no detectable viremia. In contrast, the control monkeys'IgG-recipient mice showed severe infection and viremia, and all died.[69]

The Phase I (NCT01489358) dose-escalation trial was administered to 25 healthy volunteers at the National Institutes of Health Clinical Center (Bethesda, MD, USA) with 10, 20, or 30 µg per dose intramuscularly on weeks 0, 4, and 24.[70] The vaccine candidate was well-tolerated and provided high neutralizing antibodies at the levels comparable to natural infections. In the Phase II trial (NCT02562482), 71 recruited 400 participants in the endemic area to receive 2 intramuscular injections 28 days apart. The vaccine candidate (20 µg, n = 201) or placebo (n = 199) were randomized and the safety and tolerability were followed for 72 weeks. There were mild to moderate unsolicited adverse events potentially related to the vaccine, of which 75% (12/16) and 25% (4/16) were from the vaccine and placebo groups, respectively. All potentially related adverse events resolved without clinical sequelae. In addition, the level of neutralizing antibody at the eighth week after immunization was significantly different, in which the vaccine and the placebo groups showed the EC_{50} GMT of 2005 (95%CI, 1680-2392) and 43 (95%CI, 32-58; $P < 0.001$), respectively. The durability of the immune response was demonstrated through 72 weeks after vaccination.[71]

In addition, an aluminum hydroxide-adjuvant PXVX0317PXV was added to the formulation, and the safety and efficacy of the candidate was evaluated in the Phase II trial (NCT03483961). Aluminum hydroxide is a small insoluble particle mixed with the vaccine to form a gel-like substance. The substance enhances the uptakes by antigen-presenting cells, thus increasing adaptive immune responses and antibody production. The 415 healthy CHIKV-naïve adults were characterized into 8 groups varying the doses (6, 10, and 20 µg) and duration between the two doses (14 or 28 days apart). The level of neutralizing antibodies in volunteers receiving the adjuvant vaccine (10 or 20 µg dose, and 14 or 28 days apart) did not significantly differ from volunteers receiving the nonadjuvant vaccine (20 µg dose, 28 days apart).[72] The vaccine was well-tolerated, and no vaccine-related serious adverse events were reported. The most common solicited adverse event was pain at the injection site in 31% (111/356) and 23% (12/53) of subjects who received adjuvanted and nonadjuvanted vaccines, respectively.[72] Therefore, the adjuvanted vaccine had an advantage with the similar efficacy achieved by the lower dose (10 µg) and shortened the detection period of neutralizing antibody (14 days). The adjuvanted CHIKV VLP vaccine candidate (PXVX0317) is under investigation in phase 3 clinical trials (NCT05072080 and NCT05349617). The NCT05072080 started on 29 September 2021, recruiting 3258 participants between 12 and 64 years old in the United States, using up to 50 sites to validate safety, immunogenicity, and lot consistency in healthy adults. The NCT05349617 started on 12 May 2022, recruiting 413 participants 65 years and older in the U.S., using up to 10 sites to validate the safety and immunogenicity in elderlies. Both studies are expected to be completed in July 2023.

Viral vector vaccines

Measles-vectored vaccines (MV-CHIK)

This vaccine candidate has the backbone of the measles vaccine (Schwarz strain, MV/Schw) inserted with all structural genes of the CHIKV 06.49 La Reunion strain (ECSA). The measle vaccine has been widely used since the 1980s with proven efficacies inducing lifelong immunity. The vaccine is safe and feasible for large-scale production and distribution at low cost. Furthermore, the versatile MV backbone is used in cancer gene therapy[73] and other arboviral vaccines.[74] In a preclinical setting, a single dose of MV-CHIKV protected $CD46^{tg}$-$IFNAR^{-/-}$ mice from lethal CHIKV challenge, even in the presence of pre-existing immunity to MV. Moreover, the investigators showed that the vaccine candidate induced high titers of CHIKV-neutralizing antibodies and IFN-γ-producing T cells responses.[75] Later, the Phase I randomized, double-blind, placebo-controlled, active-comparator trial (NCT03028441) was conducted with 42 healthy volunteers in Vienna, Austria, during 2013-4.[76] Three doses of MV-CHIK (low, 1.5×10^4 $TCID_{50}$ / 0.05 ml, n = 12; medium, 7.5×10^4 $TCID_{50}$ / 0.25 ml, n = 12; high, 3×10^5 $TCID_{50}$ / 1 ml, n = 12), or the active comparator-Priorix (n = 6) were randomly assigned to participants. A booster injection was administered on day 28 or day 90 after the first vaccination. The results showed that the vaccine induced neutralizing antibodies with a seroconversion rate of 44% (4/12), 92% (11/12), and 90% (10/12), in low, medium, and high doses of MV-CHIK, respectively. Pre-existing anti-measles immunity did not interfere with CHIKV-induced immunogenicity of the candidate vaccine. The second vaccination resulted in 100% seroconversion in all vaccine groups. The candidate vaccine had an overall good safety profile and an acceptable tolerability profile with no serious adverse events recorded. The rate of adverse events increased with vaccine dose and volume.

The Phase II trial (NCT02861586) was also double-blind, randomized, placebo-controlled, and active-controlled and carried out at four study sites in Austria and Germany during 2016-7. The 263 healthy volunteers (18-55 years) were randomly assigned to receive intramuscular injections with

MV-CHIK (5×10^4 or 5×10^5 $TCID_{50}$) (n = 195), control vaccine (n = 34), or measles prime and MV-CHIK (n = 34), in two different administration regimens. All groups treated with MV-CHIK elicited neutralizing antibodies after one or two immunizations, but the prime-boost immunization led to significantly greater responses one month after immunization.[77] However, the remaining antibodies were similar in all groups 6 months after immunization. However, antibody avidity and antibody-dependent cellular phagocytosis remained significantly greater after booster immunization. Furthermore, adverse events, such as headache, nausea, pain, erythema at the injected site, and myalgia, were similarly common in the MV-CHIK and control vaccine groups at 73% (168/229) and 71% (24/34), respectively.[78] No serious adverse events related to the vaccine were reported. Interestingly, prime-boost-elicited antibodies decreased rapidly over time until 6 months. Both vaccine regimens displayed similar antibody profiles. The investigators suggested that a prime-boost administration of MV-CHIK could be more appropriate for CHIKV-endemic regions, while a prime-only regimen may be sufficient for travel or outbreak situations. Additional studies (NCT03101111 and NCT03807843) were carried out in a previously epidemic area (Puerto Rico) and previously exposed adults (V184-006), respectively. Adverse reactions were mild to moderate, similar to those results in NCT02861586. In a previously epidemic area, the vaccine-induced neutralizing antibodies on day 56 at 339.03 $PRNT_{50}$ (247.3 to 464.9) in a previously seronegative group, while the seropositive group maintained its high level of neutralizing antibodies at 1280 $PRNT_{50}$ (920.5 to 1779.9) (NCT03101111). Moreover, immunization in previously exposed adults showed significant differences on day 28 and day 56 as the ratios of V184 geometric mean fold rise (GMFR) / Placebo GMFR were 1.6 (CI 1.2-2.1, $p = 0.004$) and 2.1 (CI 1.5-2.8, $p < 0.001$), respectively. The results suggested that the vaccine should induce neutralizing antibodies (NCT03807843). In conclusion, the MV-CHIK vaccine completed Phase II clinical trials with tolerable safety and evidence of high immunogenicity. No further clinical trial has been registered until now.

Chimpanzee Adenovirus-vectored vaccines (ChAdOxI -Chik/ CHIK001)

This platform used a chimpanzee Adenoviral vector and two inserted constructs that included a full-length (C-E3-E2-6K-E1) and a cap-deficient (E3-E2-6K-E1, or Δcap) structural genes of the LR2006-OPY1 La Reunion strain (ECSA). Both candidates resulted in high titers of neutralizing antibodies[79] and protected the A129 mice from lethal challenges.[80] The construct with a full-length structural gene was used in Phase I (NCT03590392) trial. Twenty-four healthy participants received a single intramuscular injection of ChAdOx1 Chik at low, medium, or high doses and were followed up for 6 months. The vaccine candidate was safe with no serious adverse reactions in any tested dose. Neutralizing antibodies against the Indian Ocean (La Reunion), West African (37997), Asian (SV044-95), and Asian/American (YO-111213) strains were present on day 14 after injection. T-cell responses were also observed. Another Phase Ib (NCT04440774) trial completely enrolled 120 participants in Mexico to evaluate the safety and immunogenicity of the candidate Chikungunya Vaccine ChAdOx1 Chik & the Zika Vaccine ChAdOx1 Zika. The study was last updated on 23 March 2022, and the results were yet to be announced.

Vesicular stomatitis virus-vectored vaccine (VSVΔG- CHIKV)

A VSVΔG- CHIKV is based on a vesicular stomatitis virus (VSV) replacing its glycoprotein (G) with the structural CHIKV envelope (E3-E2-6K-E1) of the S27 West African strain. The VSVΔG backbone was tested in Phase I/II clinical trials of the Ebola vaccine (rVSV-ZEBOV) involving 59 volunteers for safety and immunogenicity.[81,82] The preclinical study of VSVΔG- CHIKV suggested that a single intramuscular dose could generate robust neutralizing antibody and T cell-mediated responses in C57BL/6 mice.[83] Also, the VSVΔG vector platform generated more robust neutralizing antibody responses than the full-length VSV-CHIKV vector, possibly because of the greater expression of CHIKV glycoproteins in the absence of the VSV G protein.[83] The VSVΔG- CHIKV protected a CHIKV heterotype in a pathological immunocompetent model.[83]

Modified Vaccinia virus Ankara-vectored vaccine (MVA-CHIK)

The modified vaccinia virus Ankara (MVA) is a highly attenuated poxvirus generated by extensively subpassaging smallpox in chicken embryonic fibroblasts. The MVA itself is a third-generation live-attenuated smallpox vaccine used in Germany in the 1970s. It was approved by the U.S. Food and Drug Administration (FDA) on 24 September 2019 to prevent monkeypox. At least three research groups developed the MVA-CHIK vaccines using different CHIKV structural gene constructs such as E3-E2,[84] E3-E2-6K-E1,[85] and C-E3-E2-6K-E1.[86] The most promising candidate was the MVA-CHIKV using full-length or C-E3-E2-6K-E1 (LR2006-OPY1) that elicited neutralizing antibodies and CHIKV-specific CD8(+) T cell responses in C57BL/6 mice.[86] Moreover, the single-dose vaccine candidate protected the C57BL/6 mice after a high-dose virus challenge. In addition, the E3-E2-6K-E1[85] (S27 West African) provided 100% protection against lethal challenge in AG129 mice and showed neutralizing antibodies in all animals. Furthermore, the two doses of an E3-E2 candidate[84] also protected BALB/c mice from viremia after challenge after vaccination and protected A129 mice (deficient in IFNα/β) from viremia, footpad swelling, and mortality. However, the levels of neutralizing antibodies were low or undetectable. Furthermore, the passive transfer of MVA-CHIK immune serum to naïve mice did not protect against mortality. However, there is still no MVA-CHIKV clinical trial registered yet.

mRNA vaccines

mRNA-1388 (VAL-181388) vaccine

The mRNA-1388 (VAL-181388) is a Moderna Therapeutics in which the construct was a single mRNA encoding the complete native structural polyprotein (C-E3-E2-6k-E1). Phase I (NCT03325075) recruited 60 healthy volunteers from Bethesda, MD, USA, to receive two intramuscular doses of the vaccines (25, 50, 100 µg) or placebo, spaced four weeks apart (days 0 and 28). The primary and secondary outcomes were the safety and immunogenicity of this vaccine. The vaccine was well tolerated at all dose levels. $PRNT_{50}$ titers were undetected after the first vaccination in all dose groups (day 28). However, the titers increased to detectable levels after the second vaccination in the 50 and 100 µg dose groups (day 56 and day 196). Moreover, the 100 µg dose group showed 100% seroconversion on day 56 and day 196.[87]

mRNA-1944 vaccine

This mRNA-1944 vaccine is a lipid-encapsulated mRNA encoding a CHIKV-specific monoclonal antibody (CHKV24 IgG).[88] In fact, this approach is passive immunization. The Phase I study (NCT03829384) was conducted from January 2019 to June 2020 and was sponsored by Moderna. The study recruited 38 healthy participants to assess the safety and tolerability of an intravenous infusion of mRNA-1944 or placebo at 0.1, 0.3, and 0.6 mg/kg, or two doses, one week apart, at 0.3 mg/kg.[88] Results showed that dose-dependent levels of CHKV-24 IgG were observed at 12, 24, and 48 h after single infusions and persisted for 16 weeks in the 0.3 and 0.6 mg/ kg groups. Adverse effects were mild to moderate, and no exacerbating severity was observed from the two-dose regimen. Pharmacokinetics and pharmacodynamics showed that CHKV-24 IgG serum levels increased dose-relatedly and peaked at 36–48 h, then decreased with an overall mean terminal half-life (t1/2) of approximately 69 days. The two-dose regimen showed a 1.8-fold linear accumulation of CHKV-24 IgG concentrations of 1.8 times consistent with the extended terminal t1/2 of CHKV-24 IgG. More evaluation should be expected with this regimen.

Concluding Remarks

The most advanced vaccine candidate is a live-attenuated Δ5nsP3 (Valneva) that has already finished the Phase III trial and is currently in the licensing process. This vaccine has an advantage over a single-dose regimen and elicited 98.9% seroconversion in adults and older adults. Therefore, it could be beneficial for travelers before entering the endemic area. However, the protective efficacy in endemic areas is still under investigation. Furthermore, the vaccine showed apparent adverse reactions, and the live attenuated vaccine could revert to virulence. Therefore, it is likely that the vaccine might have limited use only in healthy adults. The second vaccine is an adjuvanted virus-like particle (CHIKV VLP) or PXVX0317PXV, which is expected to complete two Phase III clinical trials in July 2023. This vaccine has the advantage of not self-replicating and could elicit high immunogenicity. However, two doses are required, and the ongoing trial does not include people living in the endemic area. Therefore, this vaccine could be an option for immunosuppressed hosts. The mRNAs are still in early development, and additional data in the later Phase II-III trials should increase the validity of this vaccine's efficacy.

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