Chikungunya Virus Vaccines: Platforms, Progress, and Challenges

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Abstract Chikungunya is a clinically and economically important arbovirus that has spread globally in the twenty-first century. While uncommonly fatal, infection with the virus can lead to incapacitating arthralgia that can persist for months to years. The adverse impacts of viral spread are most severe in developing low- and middle-income countries in which medical infrastructure is insufficient and manual labor is an economic driver. Unfortunately, no prophylactic or therapeutic treatments are approved for human use to combat the virus. Historically, vaccination has proven to be the most efficient and successful strategy for protecting populations and eradicating infectious disease. A large and diverse range of promising vaccination approaches for use against Chikungunya has emerged in recent years and been shown to safely elicit protective immune responses in animal models and humans. Importantly, many of these are based on technologies that have been clinically approved for use against other pathogens. Furthermore, clinical trials are currently ongoing for a subset of these. The purpose of this review is to provide a description of the relevant immunobiology of Chikungunya infection, to present

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immune-stimulating technologies that have been successfully employed to protect against infection, and discuss priorities and challenges regarding the future development of a vaccine for clinical use.

1 Introduction: Chikungunya Ecology and Economic Impact

Chikungunya virus (CHIKV) is an arthritogenic arbovirus belonging to the Old World clade of the alphavirus genus *Togaviridae*. As with other clinically impactful arboviruses, acute infection with CHIKV causes fever and severe joint pain. However, while CHIKV is not typically fatal it may lead to chronic and long-lasting arthralgic sequelae that can be debilitating (1, 2). Interhost transmission of CHIKV occurs via the bite of *Aedes* (subgenus *Stegomyia*) mosquito species. In regions where CHIKV is endemic, including tropical and subtropical areas of sub-Saharan Africa and Southeast Asia, two separate maintenance and transmission modes are evident. Enzootic cycles within nonhuman primate (NHP) species such as African green monkeys (*Cercopithecus aethiops*) and baboons (*Papio ursinus*) are believed to represent the evolutionarily ancestral maintenance pattern in forest and savannah (Tsetsarkin et al. 2016). Sporadic spillover of the virus into urban areas is thus likely to be the primary source of human CHIKV outbreaks (Diallo et al. 2012). While the sustained human–mosquito–human transmission is largely attributed to *Aedes aegypti* or *Aedes albopictus* species, the crucial factors that drive emergence into urban venues are not known. Nonetheless, the reality of ongoing enzootic transmission and periodic but unpredictable urban spillover means that in the absence of vector elimination strategies, human outbreaks will occur with regularity. Importantly, this also makes eradication of the virus by way of clinical, human host-administered treatments improbable. However, human populations could be rendered resistant to infection and thus limit spread through the generation of virus-specific immunity elicited by vaccination. While no CHIKV vaccines are currently approved for human use, many promising, safely efficacious, and novel vaccine platforms have been described including some that are currently in clinical trials.

While CHIKV-associated fatality rates are low, the economic impact of disease can be extreme and disproportionate between health systems and tiers of societal development. This includes the direct medical expenses associated with acute illness such as symptomatic treatment (de Brito et al. 2016) but also chronic inflammatory conditions which are more protracted and costly (Cardiel 2012). Perhaps more importantly, large indirect financial impacts result from acute and chronic CHIKV including illness-associated work absence, diminished productivity, and recurrent transportation to health providers. These indirect costs can be particularly high since many countries experiencing CHIKV outbreaks are underdeveloped and their economic capacities rely heavily on manual labor (Seyler et al. 2010). One analysis

estimated that between 2013 and 2015 in the Americas >23 million disability-adjusted life years were lost leading to >\$83 billion in direct medical costs and >\$101 billion in indirect societal costs (Bloch 2017). In addition, regions susceptible to CHIKV transmission often represent destinations popular with tourists. In line with this, the 2005–2006 outbreak on the island of La Reunion led to an estimated loss of \$160 million in this industry (Enserink 2007). An efficacious vaccine would thus confer economic benefits in multiple ways including preventing incapacitation of labor forces, diminishing direct medical costs, and mitigating tourist uncertainty.

2 CHIKV Replication and Immune Reactivity

The CHIKV genome is approximately 11.8 kb single-stranded positive sense RNA that includes 5' and 3' untranslated regions (UTR), a 3' poly-A tail and 5' 7-methylguanosine cap [reviewed in (Vaney et al. 2013)]. Two open reading frames (ORFs) encode polyproteins separately comprised of four nonstructural (nsP1-4) and six structural (capsid [C], 6 K, transframe [TF], envelope [E] 1, E2, E3) proteins. Infectious CHIKV exists as a 70 nm enveloped virion composed of 240 copies of E1 and E2 heterodimers in an icosahedral lattice configuration surrounding a nucleocapsid (Voss et al. 2010). E1 and E2 play essential molecular roles during initiation of replication that involve virion interaction with cellular receptors and internalization by clathrin-mediated endocytosis (Strauss and Strauss 1994). Following the release of the nucleocapsid into the cytoplasm, viral genomic RNA is translated to generate the nsP polyprotein, the proteolytic maturation of which gives rise to the RNA replicase [reviewed in (van Duijl-Richter et al. 2015)]. This is followed by the translation of the structural polyprotein from a subgenomic promoter at the terminus of the nsP coding region. Proteolysis of the structural proteins enables nucleocapsid interaction with genomic RNA, association with envelope glycoproteins at the plasma membrane, and budding of assembled particles.

Humoral and cellular adaptive immune processes are potently induced in response to CHIKV infection. Cell-mediated immunity is triggered and CD8+ and CD4+ T cells reactive to CHIKV antigens are known in humans (Hoarau et al. 2013), nonhuman primates (Messaoudi et al. 2013), and mice (Morrison et al. 2011; Teo et al. 2012a). The role for T cells in viral clearance, however, is not well understood and, in fact, may contribute to disease exacerbation (Teo et al. 2012a). In contrast, numerous studies have demonstrated the importance of antibodies with neutralization capacity for controlling CHIKV infection and tissue damage (Couderc et al. 2009; Sheela and Sumathy 2013; Lum et al. 2013; Hawman et al. 2013). IgM and IgG antibodies reactive to CHIKV appear early after symptom onset and IgG persists at high levels for years (Couderc et al. 2009; Kam et al. 2012a). Prior infection and development of anti-CHIKV neutralizing antibodies in humans associated with protection from re-infection (Yoon et al. 2015; Kam et al. 2012b; Galatas et al. 2016). In addition, passively transferred serum from

convalescent humans protects mice from virus-associated lethality (Couderc et al. 2009). Intriguingly, CHIKV-reactive monoclonal antibodies have been isolated from patients that can also protect mice and even lead to diminished viremia and virus-associated disease in nonhuman primates (Smith et al. 2015; Broeckel et al. 2017). Longitudinal analysis in humans has identified the E2 and E3 glycoproteins as well as capsid and nsP3 as key targets of the antibody response (Kam et al. 2012a, c). However, the E2 protein appears to represent the immunodominant viral protein and antibodies reactive to it can confer broad-based viral neutralization (Kam et al. 2012c; Fox et al. 2015; Weger-Lucarelli et al. 2015). Surprisingly, no established minimum protective antibody titers have been described, although a reciprocal titer of approximately 35 was shown to be required in IFN-deficient mice, which presumably exhibit heightened susceptibility to the virus in comparison to humans (Chu et al. 2013).

Four main phylogenetic clades of CHIKV are recognized and include East/Central/South African (ECSA), West African, Asian, and Indian Ocean lineages (IOL) that collectively share 84.5–97.8% amino acid identity (Sahadeo et al. 2017; Volk et al. 2010). ECSA and Asian lineages are considered to be older and having evolved for transmission via *A. aegypti*. In recent years, however, adaptive mutations arose in the viral E1/E2 proteins of the IOL that likely facilitated interhost transmission by the more anthropophilic *A. albopictus*, a factor potentially playing a role in the virus' explosive global emergence (Tsetsarkin et al. 2007; Tsetsarkin and Weaver 2011). However, virus derived from the Asian lineage appears to be responsible for the emergence and local transmission in the Americas, which was first observed in 2013 (Lanciotti and Valadere 2014; Leparc-Goffart et al. 2014). While differences in virulence have been observed between viral lineages in mice (Wei Chiam et al. 2015; Teo et al. 2015) [especially those deficient in the type I interferon response with WA strains appearing more lethal (Langsjoen et al. 2018)] whether these or other differences are manifest in humans is not known. Interestingly, high CHIKV levels were detected in blood from healthy donors in Puerto Rico during an epidemic in 2014 that involved Asian/American lineages, suggesting that these strains may associate with higher rates of asymptomatic infection (Simmons et al. 2016). Importantly, from the standpoint of vaccination and control efforts, all CHIKV lineages comprise one serotype and cross-protection between strains is both observable and enduring in humans and other species (Langsjoen et al. 2018; Auerswald et al. 2018). Furthermore, antibodies reactive to CHIKV antigens may even confer protection against related Alphaviruses such as Ross River (Gardner et al. 2010) and O'nyong'nyong viruses (Partidos et al. 2012). However, despite this, variation has been reported in the neutralization efficiency of convalescent human sera across ECSA and Asian clades (Chua et al. 2016) suggesting that evaluation of such issues in vaccination efficacy studies is worthwhile. Nevertheless, vaccines that are, in general, antigenically focused are likely to protect against circulating lineages from all clinically relevant clades.

3 Animal Models of CHIKV Infection and Immunity

Fortunately for the purposes of vaccine and therapeutic development, tractable animal models that faithfully recapitulate many aspects of human infection are available for examination of CHIKV replication, immunity, and pathogenesis [reviewed in (Haese et al. 2016)]. These have been instrumental for both investigating the safety and efficacy of novel vaccination platforms as well as characterizing the associated immune-mediated mechanisms. The most widely employed are wild-type and type I interferon (IFN) deficient mouse models (Taylor et al. 2015; Teo et al. 2012b). As experimental systems, these exhibit many desirable attributes including relatively low cost, ease of handling and care, ability to control environmental and genetic variables, and availability of reagents. They can also be utilized for studies of acute, chronic, and lethal CHIKV infection as well as virus-associated inflammatory dysfunction (Morrison et al. 2011; Hawman et al. 2013; Ziegler et al. 2008; Couderc et al. 2008a; Poo et al. 2014). In particular, lack of type I IFN-dependent antiviral phenotypes renders animals fully susceptible to CHIKV-induced mortality (Couderc et al. 2008b; Rudd et al. 2012; Gardner et al. 2012), and, as such, this represents a highly stringent model for exploring the potency of antibodies and T cells generated by vaccination or following inter-animal transfer (Smith et al. 2015; Plante et al. 2011; Pal et al. 2013). Furthermore, many fundamental innate and adaptive immunologic processes involved in CHIKV protection and clearance are shared between humans and mice. As such they represent a powerful and cost-effective first-pass testing platform for characterization and mechanistic understanding of novel vaccine technologies. Informative nonhuman primate (NHP) models of CHIKV infection and immunity have also been developed and are commonly used, including Cynomolgus (Chen et al. 2010; Messaoudi et al. 2013) and Rhesus (Chen et al. 2010; Messaoudi et al. 2013) macaque species. NHP models exhibit advantages over murine models that make them more translatable to humans such as increased susceptibility to infection, out-bred genetic structure, and maternal/fetal transmission. In addition, NHP models are considered more representative of human vaccine responses owing to their size, distribution of immune cell subsets, innate immune receptor expression, and the morphology of injection site and lymphatic systems [reviewed in (Thompson and Loré 2017)]. However, their expense and requirement for highly specialized housing render NHP models more applicable to studies examining vaccine technologies for which a more comprehensive proof of principle has been established.

4 Vaccine Technology Classes

Given the importance of humoral immunity for controlling infection, ongoing vaccine development efforts are primarily focused on eliciting antibody-dependent responses for protection against CHIKV. To accomplish this, five general technological platforms have been or are being explored. These include live attenuated

chikungunya viral (LAV) strains, chimeric viral vectors, inactivated virions or virus-like particles (VLP), subunit vaccines, and DNA plasmid. Each has displayed efficacy in animal models and some are even immunogenic in humans. A LAV for CHIKV involves limited intrahost replication and has proven particularly capable of eliciting neutralizing antibody production in humans (Edelman et al. 2000). However, as a class, such vaccines are often associated with suboptimal safety profiles owing mostly to their unlikely but demonstrated the potential for reversion to a pathogenic viral state. As such they are contraindicated in hosts with weakened or immature immune systems including the elderly, neonates, and those undergoing strategic immunosuppression. This has necessitated the development of novel immunogen delivery platforms that do not involve viral growth within vaccinees. Moreover, while direct administration of inert viral antigen or molecular platforms that involve the synthesis of such antigen in vivo are safer, these often lead to weaker, shorter lived immunity since they stimulate fewer facets of the immune response in a localized, rather than disseminated manner. As such they often require co-delivery of nonantigenic molecules termed adjuvants that act by stimulating localized innate immune processes to enhance immunogenicity [reviewed in (Reed et al. 2013; Coffman et al. 2010)]. Alternatively, recombinant heterologous viral platforms that encode and express CHIKV antigen but are incapable of growth in vivo represent another safe and powerful method for eliciting protective immunity [reviewed in (Ramsauer and Tangy 2016)]. Diverse efforts pursuing these and other technologies are currently the subject of substantial research investment to identify an optimal method of immune protection against CHIKV.

4.1 Live Attenuated Viral Vaccines

The earliest CHIKV vaccine to successfully demonstrate protective immunostimulation in humans involved a clinical strain from Thailand that was sequentially passaged multiple times on human MRC5 human lung fibroblasts and designated 181/clone 25 (Levitt et al. 1986). In clinical trials, the vaccine was highly immunogenic with all but one out of 59 adults generating neutralizing antibody titers (Edelman et al. 2000). However, 10% of recipients experienced mild arthralgia. It was subsequently determined that only two amino acid changes were responsible for the pathogenic differences between the clinical and the attenuated vaccine strain, making the likelihood of reversion unacceptably high for clinical use (Gorchakov et al. 2012). Other efforts to construct LAV have involved modifying targeted genomic regions to impair viral growth. This is greatly facilitated by utilizing infectious cDNA clones for viral reconstitution, the mutation of which is straightforward by standard molecular methods (Vanlandingham et al. 2005; Tsetsarkin et al. 2006). For instance, CHIKV-containing deletions in open reading frames that encode nsP3, 6 K, or E2 have each been shown to be replication incompetent in vivo yet capable of eliciting protective antibody responses in mice (Hallengård et al. 2014; Piper et al. 2013; Gardner et al. 2014). The most promising

implementation of this approach is the $\Delta 5\text{nsP3}$ -attenuated virus from the laboratory of Peter Liljestrom that contains a 60 amino acid deletion of the nsP3 protein (Hallengård et al. 2014). This attenuation strategy allows for sufficient in vitro replication but no viremia after inoculation of mice with a dose sufficient to elicit neutralizing antibody titers and antiviral T cell responses and block detectable viremia. This vaccine has proved to be similarly efficacious in a cynomolgus macaque viral immunogenicity and challenge model (Roques et al. 2017). Importantly, a phase I clinical trial using this platform (identifier NCT03382964) that involved 120 participants was very recently completed by Valneva and showed a superior safety profile and seroconversion in all vaccinees at day 14 after a single dose.

A rational attenuation method devised by Scott Weaver and colleagues involves replacing the endogenous subgenomic viral promoter region responsible for driving normal expression of antigenic structural proteins with a picornavirus internal ribosomal entry site (IRES), leading to diminished protein synthesis (Chu et al. 2013; Langsjoen et al. 2018; Partidos et al. 2012; Plante et al. 2011, 2015; Roy et al. 2014; Kim et al. 2011). The resulting virus grows slowly in vitro, is fully attenuated in vivo, and does not replicate in mosquito cells so it cannot be conventionally transmitted between hosts. Importantly, given the size and complexity of the modified genomic region, reversion mutations are quite improbable making it a presumably safe treatment. This vaccine effectively generates protective antibody responses in both wild-type and IFN-deficient mice as well as NHP without adverse effects. While conventional T cell activation appears to be triggered by the vaccine, adoptive transfer of CD4+ or CD8+ cells to mice following vaccination did not protect against challenge; an additional indication that cellular immunity is of limited relevance during CHIKV infection. Moreover, the vaccine's efficacy in immunocompromised animals further predicts its safety for immune-insufficient patients. This vaccine was also found to elicit protective immunity against the antigenically related Alphavirus O'nyong'nyong virus (ONNV) in IFN-deficient mice and dams receiving the vaccine vertically transferred antibody-mediated protection to their offspring. This platform is currently being developed commercially by a partnership between Takeda Pharmaceuticals and Zydus Cadila.

4.2 Chimeric Viral Vector Platforms

Another impactful vaccination approach being actively explored involves the delivery of CHIKV antigen using heterologous viral vectors [reviewed in (Ramsauer and Tangy 2016)]. This includes the construction of chimeric virus particles containing CHIKV genomic regions encoding antigenic proteins that enter cells in vivo but either do not generate progeny or only undergo a single round of replication. Their limited viability renders them unlikely to confer pathogenic or reactogenic effects. Virus types co-opted for this purpose include both RNA- and DNA-based agents, including related Alphaviruses. Moreover, depending on the

platform they are also able to elicit both humoral and cell-mediated immune responses especially since, by utilizing cellular protein synthesis and processing pathways, they lead to antigen presentation via MHC class I and subsequent T cell activation (Liu 2010). Being structurally complete viruses, these vectors are also likely to be comprised of molecular patterns that activate pattern recognition receptors and thus enhance immunogenicity through innate immune stimulation (Hu and Shu 2018; Iwasaki and Medzhitov 2015). While preexisting immunity against some common human viruses can lead to diminished efficacy of vectors on which they are based (Saxena et al. 2013), solutions appear to be obtainable including utilizing nonhuman virus types or antigenically modifying human viruses. As with LAV, chimeric viruses must be maintained and administered in a manner that permits viability as determined by cellular fusion and genome delivery. As such, both technologies can require specialized manufacturing, storage, and transport procedures that are not always feasible with application in underdeveloped areas (Ulmer et al. 2006; Lloyd and Cheyne 2017).

The earliest chimeric vaccines against CHIKV involved vectors that encode the nonstructural proteins of other related alphavirus species including Sindbis virus (SINV), Eastern equine encephalitis virus (EEEV), or an attenuated strain (TC-83) of Venezuelan equine encephalitis virus (VEEV) with the structural proteins of CHIKV (Wang et al. 2008, 2011a). This allowed generation of virus particles that grow to high titer in cell culture but do not replicate *in vivo*, indicative of appropriate safety. Intriguingly, the VEEV- and EEEV-based vectors also elicited neutralizing antibody titers in vaccinated mice and led to protection from viremia and virus-associated mortality and disease in these animals. VEEV-based vectors were additionally modified by the introduction of IRES elements to drive structural protein synthesis, further improving their safety, rendering them effective in IFN-deficient mice, and preventing their mosquito transmissibility.

Vesicular stomatitis virus (VSV) represents another RNA-based virus that can be engineered using simple methods and exploited for heterologous vaccination purposes (Roberts et al. 1999). VSV is a negative-stranded RNA member of the Rhabdoviridae family whose envelope can be pseudotyped with glycoproteins derived from unrelated viruses to enhance immunogenicity and alter cellular tropism. In addition, VSV-based vectors are able to elicit potent CD8⁺ T cell responses against target antigens (Haglund et al. 2002) and have been successfully employed in the context of diverse viral and bacterial pathogens. Chattopadhyay and colleagues have constructed chimeric VSV in which the endogenous glycoprotein G coding region is replaced by CHIKV structural proteins (Chattopadhyay et al. 2012). This yields virions into which antigenic CHIKV envelope glycoproteins are incorporated but that also enables cellular entry and new intracellular synthesis of these antigens following inoculation. The vector was shown in mice to elicit both neutralizing antibody and cellular immune responses against CHIKV and protection against viremia and CHIKV-associated disease. Whether CHIKV-directed vaccines that incorporate this platform operate safely in NHP models or humans still requires examination but currently a VSV-based vaccine against Ebola virus is undergoing phase III clinical trials (Coller et al. 2017).

One particularly novel chimeric virus vaccine was recently described by Erasmus and colleagues that involves Eilat virus (EILV), an alphavirus specific to insect species (Erasmus et al. 2015, 2016). The virus is incapable of replication in mammals due to an inability to enter cells or form nonstructural protein-mediated replication complexes (Nasar et al. 2015). Based on this, it was predicted that chimeric genomes consisting of the EILV 5'UTR and nonstructural polyprotein and CHIKV structural polyprotein would yield antigenic virus particles when introduced into mosquito cells. Furthermore, despite containing intact genomes and CHIKV virion proteins, the virus should not be capable of generating a replication competent, and thus pathogenic, particles *in vivo* since the nsPs do not function in mammalian cells. Moreover, the lack of viral RNA transcription also precludes the emergence of replication-viable mutants. Remarkably, a single dose of the vaccine was shown to elicit neutralizing antibodies and protection against viral challenge in WT and IFN-deficient mice as well as NHP. While the vaccine's efficacy has not been comparatively evaluated relative to other viral-vectored or VLP-based platforms, it may involve an enhanced mechanism of antigen presentation by entering cells via the endocytic pathway (Erasmus et al. 2016). In addition, the presence of genomic RNA in the viral particle may provoke innate immune reactivity and thus adjuvant-like properties (Vasilakos and Tomai 2013). As such, EILV may represent a powerful vaccine platform for CHIKV and other viruses that warrants thorough exploration.

DNA-based viruses have also been shown to be effective as vectors for vaccine antigen delivery and immunostimulation. This includes adenoviruses and the poxvirus modified vaccinia virus Ankara (MVA) (Volz and Sutter 2017; Majhen et al. 2014). Non-replicating adenovirus vectors have been well-studied for use in both gene therapy and vaccination and this includes hundreds of clinical trials. As such, ample evidence of their safety and efficacy has been amassed. The complex adenovirus 5 (Ad5) vaccine vectors contain deletions of regions essential for replication that can be complemented in transgenic cell lines to allow particle formation. These are amenable to accepting large user-defined insertions and have been effectively employed as vaccines against viral pathogens including influenza, Ebola, Dengue, West Nile, and Rift Valley fever viruses. Wang and colleagues used this platform to deliver CHIKV envelope glycoproteins and capsid to CD-1 and C57BL/6 mice (Wang et al. 2011b). They show that the vaccine elicits antibody titers as high as those generated by natural infection and confers complete protection against viral challenge. Additionally, adenoviruses of nonhuman species also represent potentially safe and efficacious vaccine vectors that circumvent any impacts of preexisting immunity in recipients. This is currently being employed in clinical trials using Chimpanzee adenovirus expressing CHIKV structural proteins (identifier NCT03590392) (Ewer et al. 2017).

MVA is derived from vaccinia virus smallpox vaccine following 570 passages in avian tissue culture, which caused large genomic deletions that abrogate productive replication in many species including humans (Moss et al. 1996). MVA also supports stable introduction and expression of heterologous antigenic proteins for which it has been effectively utilized to stimulate immunity against viral, bacterial,

and parasitic pathogens. Importantly, MVA also leads to the generation of both humoral and cell-mediated protective immunity (Draper et al. 2013). In 2014, three separate groups reported the synthesis and assessment of MVA-vectored vaccines against CHIKV. Van den Doel and colleagues constructed a vector that expresses CHIKV 6K-E1-E3-E2 polyprotein and fully protects IFN-deficient A129 mice from CHIKV-induced lethality without adverse effects (van den Doel et al. 2014). Weger-Lucarelli and colleagues similarly generated MVA that expresses only E2E3 (Weger-Lucarelli et al. 2014). This also protected A129 mice from lethal CHIKV challenge. Interestingly, however, sera from mice vaccinated with this vector failed to protect nonvaccinated A129 mice from CHIKV mortality following the passive transfer. Moreover, depletion of CD4⁺, but not CD8⁺ cells from vaccinated mice prevented MVA-mediated protection, suggestive of a role for cell-mediated immunity in this outcome. Finally, Garcia-Arriaza and colleagues inserted the entire CHIKV structural polyprotein (C-E3-E2-6K-E1) into MVA and demonstrated strong innate immune induction following the vector's administration, consistent with that observed during conventional viral infection or injection of adjuvanted vaccines (discussed below) (García-Arriaza et al. 2014). This vector clearly elicited a broad, polyfunctional T cell response as well as neutralizing antibodies and protection against viremia in C57BL/6 mice. Currently, both adenovirus and MVA-based CHIKV vaccines are in a preclinical stage of development and NHP studies would greatly enhance our evaluation of their translatability to humans. Importantly, however, these platforms also represent important research tools that can lead to useful mechanistic insight regarding the respective roles of humoral and cell-mediated immune processes for protection against CHIKV and the development of CHIKV-associated disease.

The most clinically advanced chimeric platform for CHIKV immunization involves the Measles virus (MV) Schwarz attenuated vaccine strain as a delivery vector [reviewed in (Tangy and Naim 2005)]. MV is a negative-stranded RNA virus for which a cDNA plasmid-based reverse genetic system has been optimized that allows expression of heterologous protein antigens (Combredet et al. 2003). MV/Schwarz is an extremely safe, efficacious, and historically utilized vaccine that confers lifelong protective immunity following a single immunization. Furthermore, it has been effectively adapted for the generation of protective responses against viral pathogens such as West Nile, dengue, HIV, and SARS coronavirus [reviewed in (Mühlebach 2017)]. For purposes of practical investigation and clinical translatability, the MV platform exhibits important advantages. First, transgenic mouse models have been constructed to be susceptible to MV infection through the introduction of the viral receptor CD46 in the presence and absence of a functional IFN response for added stringency (Combredet et al. 2003). Next, NHP are also infectible with MV and immune reactivity to encoded antigens occurs in the following exposure (de Swart and DeSwart 2009). Finally, preexisting immunity to MV in vaccines does not appear to impact heterologous immunogenicity elicited by the vector in animals or humans (Reisinger et al. 2019).

Frederic Tangy's research group has elegantly leveraged this technology to synthesize a safe and immunogenic CHIKV vaccine (Brandler et al. 2013;

Ramsauer et al. 2015). Specifically, MV/Schwarz genomes have been constructed that contain the full CHIKV structural polyprotein that replicates to high titer in vitro and confers potent antibody-mediated protective immunity against multiple clades of CHIKV in IFN-deficient mice as well as cynomolgus macaques (Rossi et al. 2019). Importantly, this vaccine has also completed phase II clinical trials (identifiers NCT03101111, NCT02861586) clinical trials to demonstrate safety, tolerability, and immunogenicity (Reisinger et al. 2019). In the first trial, 36 subjects received one of three MV-CHIKV doses and were boosted after either 1 or 3 months. Single immunization led to detectable neutralizing antibody titers for all doses. However, full seroconversion was achieved in all vaccine groups after the booster immunization, preexisting anti-MV antibodies did not affect the immune response, and the vaccine displayed acceptable tolerability. The second trial involved 236 participants that received control MV (n = 34), MV-CHIK (n = 195), or MV control and MV-CHIK (n = 34). This study demonstrated seroconversion of 50–93% of participants after a single MV-CHIK dose and 86–100% of participants after MV-CHIK boost. Moreover, antibody titers were positively correlated with vaccine dose and preexisting immunity to MV did not affect reactivity to MV-CHIK. It is important to note that the precise MV-CHIKV mechanism of action is not defined. However, cells infected in vitro with the virus are known to synthesize and release virus-like particles (VLP) that are likely to be immunogenic (Brandler et al. 2013) (discussed in more detail below). It is possible that these are taken up and trafficked to lymph nodes by antigen presenting cells to initiate immune responses. Currently, this vaccine is being commercially developed by Themis Bioscience.

4.3 *Inert Antigen Vaccines*

In contrast to vaccine platforms that involve molecular synthetic processes during virus-based antigen delivery, others exist that are comprised of inactivated or genome-deficient viral particles or individual purified viral proteins. Given the spectrum of immunocompetence present in the human population, these are generally considered safer than LAV and chimeric virus vectors since they do not undergo any in vivo molecular replication. However, the antigenicity of these may be suboptimal since conformation of native epitopes can be susceptible to damage in response to the inactivation and purification processes. In addition, these are administered locally and without regional amplification or systemic immune activation. As such, inert virus particle-based vaccines exhibit lower immunogenicity and can require booster immunizations as well as the inclusion of adjuvants to stimulate innate immune processes and enhance antigen processing and delivery. Multiple methods have been utilized to inactivate live CHIKV particles in a manner that aims to maintain their immunogenic capacity. This includes treatment with chemicals such as binary ethyleneimine (Gardner et al. 2010), β -propiolactone (Kumar et al. 2012), Tween 80/ether (Eckels et al. 1970), and formalin

(Tiwari et al. 2009) or exposure to UV-irradiation (Nakao and Hotta 1973). These have been examined in preclinical mouse or NHP models and proved to generate protective virus-directed antibody responses, in some cases even without adjuvant. Formalin-inactivated CHIKV has also been used in human volunteers without adjuvants and shown to tolerably elicit antibodies with neutralization capacity (Harrison et al. 1971). Unfortunately, inactivation of live clinical strains of CHIKV requires biosafety level 3 conditions for production. This renders the process costly and difficult to scale-up and thus only feasible for experimental work.

Antigenic fidelity of CHIKV proteins for efficacious vaccine-mediated immunostimulation can also be readily achieved through synthesis and purification of virus-like particles (VLP). These are comprised of self-assembling viral structural proteins that conformationally resemble wild-type virions but contain no genomic material and thus cannot initiate replication [reviewed in (Mohsen et al. 2017)]. The safety profile of VLPs is thus heightened over inactivated, live attenuated, or chimeric viruses since viral growth is not possible in any step of the production or vaccination process. In addition, biosafety requirements for manufacturing are also less stringent and thus scale-up is more technically feasible and cost-effective. Generation of virion-based antigens on mammalian or insect (Metz et al. 2013a) cells additionally benefits from proper protein folding, glycosylation, and post-translational modifications important for epitope structure. Currently, several licensed VLP-based vaccines are in widespread clinical use against human papillomavirus, hepatitis B, and hepatitis E viruses. In the case of CHIKV, ectopic translation of the viral envelope polyprotein (E2-E2-6K-E1) in cultured cell lines following transfection or transduction of expression vectors leads to the secretion of particles that are similar in size, structure, and protein configuration to the native virus (Akahata et al. 2010; Sun et al. 2013). These can then be isolated, purified, and concentrated from cell culture media by ultracentrifugation or filtration for direct in vivo administration. Importantly, human cell lines amenable to CHIKV VLP synthesis have been approved for vaccine production by regulatory agencies and thus tractable methods of clinic-ready scale-up are available (Genzel 2015).

Studies have demonstrated that immunization with VLP synthesized using mammalian or insect cells elicit protective antibody responses in wild-type and an IFN-deficient mouse models as well as in NHP (Metz et al. 2013a, b; Akahata et al. 2010). Importantly, a VLP-based vaccine manufactured using characterized and FDA-approved HEK293 cells by the NIH Vaccine Research Center has completed a phase I clinical trial (identifier NCT01489358) (Chang et al. 2014) and phase II trials in the Caribbean (identifier NCT03483961, NCT02562482) are currently ongoing. The completed phase I study involved 23 participants receiving one of three doses at 0, 4, and 20 days by intramuscular injection. The vaccine was well tolerated and, crucially, led to the production of neutralizing antibodies after the first boost in a manner weakly associated with antigen dose for all participants. Titers were maintained for at least 6 months and were at levels likely to be protective based on observations from convalescent sera (Kam et al. 2012a, b). Moreover, the neutralization capacity of sera collected from participants was shown to exist against nine viral strains comprising three clades, suggesting that vaccination against one strain can elicit cross-protection

(Goo et al. 2016). Interestingly, while published animal studies have employed CHIK VLP admixed with innate stimulating adjuvants, the clinical trial actually observed neutralizing antibodies in their absence. Whether adjuvants are not necessary or, rather, could actually enhance protective immune responses to a degree that allows dose sparing or single administration will necessitate further exploration. The duration that protective antibody titers are measurable following vaccination also requires investigation and this may also warrant the inclusion of adjuvants.

Individual recombinant CHIKV envelope proteins have also been effectively utilized as a vaccine antigen. Immunization with single proteins offers advantages such as ease and simplicity of synthesis and adaptability to strain variation if needed. Bacterial expression systems were used to synthesize and purify E1 and E2 proteins (Kumar et al. 2012; Khan et al. 2012). Administration of these proteins to WT mice elicited neutralizing antibodies in WT mice as well as protection from viremia, viral dissemination, and virus-mediated disease. However, recombinant peptides alone appear to be poorly immunogenic and require adjuvant admixture. Multiple classes of adjuvants were shown to enhance the E1- and E2-associated immune response. In addition, vaccination with E1/E2 subunits is also capable of generating balanced Th1/Th2 polarization as indicated by high titers of IgG1, IgG2c, and IgG3 antibody subclasses. The efficacy of subunit vaccination has yet to be examined in IFN-deficient mice or non-murine models.

4.4 Nucleic Acid Based Vaccines

Another approach to CHIKV vaccination involves the utilization of plasmid DNA that encodes immunogenic or protective factors that prevent infection. Direct delivery, often involving intracellular electroporation, into receptive tissues of DNA vectors can lead to in situ synthesis of user-specified proteins. This technology exhibits many logistical and biological advantages including stimulation of both humoral and cell-mediated immune responses, low production cost, high vaccine stability (cold chain independence), ease of manufacturing, capacity for co-delivery of multiple antigens, and capacity for rapid customization in the event of spontaneous strain emergence. Moreover, only basic molecular cloning methods are needed for synthesizing and adapting vectors of interest, greatly facilitating the experimental investigation. Muthumani and colleagues described the first DNA-based vaccine vector for CHIKV (Muthumani et al. 2008). The authors designed consensus E1, E2, and capsid proteins based on multiple sequences derived from clinical isolates across years of outbreaks and cloned the respective coding regions into a DNA expression vector. Intramuscular electroporation was used to deliver the constructs to mice and shown to elicit both CHIKV-reactive antibodies and IFN γ -producing T cells in response to CHIKV antigen. Follow up work by this group included the construction of an E1, E2, and E3 expressing vector that led to similar immune induction as well as protection against viral

challenge in mice (Mallilankaraman et al. 2011). Additionally, this vector was also able to induce neutralizing antibody and T cell responses following administration to NHP indicating that it is operational across species. A similar approach that has also been used involves the introduction to DNA vectors of CHIKV-derived genomic regions that allow the *in vivo* synthesis and secretion of immunogenic VLP (Hallengård et al. 2014; Hallengard et al. 2014) or the LAV strain 181/25 (Tretyakova et al. 2014). Both methods led to the production of neutralizing antibody responses in mice and while protection against CHIKV challenge was not checked for the LAV, it was observed for the VLP as indicated by the absence of viremia and CHIKV-associated disease. An antigen-independent DNA vector-based approach has also been used to successfully elicit prophylactic immunity to CHIKV infection in a manner that is more rapid than traditional vaccination. This involves the DNA encoding of an envelope-reactive monoclonal antibody sequence with broadly neutralizing capacity (Muthumani et al. 2016). *In vivo* delivery of this vector led to the synthesis of detectable CHIKV-reactive antibodies after three days and peak titers were observed by day 15–21. Remarkably, this vector conferred protection against CHIKV when administered as early as two or as late as thirty days pre-challenge. While DNA-based vaccines hold much promise, additional mechanistic and efficacy studies will be needed to fully elucidate their potential clinical applicability.

In addition to antigenic CHIKV proteins being encoded by DNA, they may also be expressed *in vivo* directly from messenger RNA (mRNA). The susceptibility of RNA to degradation leads to more stringent delivery requirements that protect the molecule such as lipid nanoparticle formulations. This approach has been used to deliver liposomal mRNA that encodes a highly potent neutralizing antibody identified from a B cell clone of a convalescent patient. This has led to vaccine dose-associated protection of IFN-deficient mice from lethal CHIKV challenge (Kose et al. 2019). Furthermore, administration of this platform in WT mice also led to substantial reduction or prevention of CHIKV viremia and viral RNA tissue loads and its use in cynomolgus macaques led to detectable serum levels of encoded IgG. Finally, mRNA encoding CHIKV antigens has proven capable of eliciting neutralizing antibodies in human subjects (Shaw et al. 2019). A phase I clinical trial using this vaccine is currently ongoing under the direction of Moderna, Inc. (identifier NCT03325075).

5 Priorities for and Barriers to Clinical CHIKV Vaccine Development

As outlined above and summarized in Table 1, numerous vaccine technologies have been shown to safely and efficaciously elicit protection in animal and human models against CHIKV infection and disease. A number of these platforms have even obtained regulatory approval for use against other pathogens. In addition,

Table 1 Vaccine list

Platform	Name	Description	Models	Development	References
LAV	181/25 (TSI-GSD-218)	Clinical strain attenuated by serial passage	Mouse, NHP, Human	Phase II (halted)	Edelman et al. (2000), Levitt et al. (1986)
LAV	LR-E2-E79 K	Clinical strain LR attenuated by mutations in heparin sulfate binding domains	WT, IFN-deficient mice	Preclinical	Gardner et al. (2014)
LAV	Δ5nsP3, Δ6 K	Clinical strain attenuated by targeted mutations in nsP3, 6 K	WT Mice, NHP	Phase I	Hallengård et al. (2014), Roques et al. (2017)
LAV	CHIKV TM17-2	Clinical strain attenuated by mutations in E2 transmembrane domain	WT Mice	Preclinical	Piper et al. (2013)
LAV	CHIKV/IRES	Subgenomic promoter replaced with IRES	WT, IFN-deficient mice, NHP	Preclinical	Chu et al. (2013), Langsjoen et al. (2018), Partidos et al. (2012), Plante et al. (2011), Plante et al. (2015), Roy et al. (2014), Kim et al. (2011)
Chimeric Virus	VSVdelG-CHIKV	Glycoprotein-deficient VSV encoding CHIKV structural proteins	WT Mice	Preclinical	Chattopadhyay et al. (2012)
Chimeric Virus	VEE/CHIKV; VEE/IRES-C/CHIKV	Chimera consisting of VEEV nsP and CHIKV structural polyproteins	WT, IFN-deficient mice	Preclinical	Wang et al. (2011a, 2008)
Chimeric Virus	EILV/CHIKV	Viral genome encodes Eilat virus nonstructural and CHIKV structural proteins	Mouse, NHP	Preclinical	Erasmus et al. (2015, 2016)
Chimeric Virus	CAdVax-CHIK	Replication-deficient Ad5 encoding CHIKV structural polyprotein	WT Mice	Preclinical	Wang et al. (2011b)

(continued)

Table 1 (continued)

Platform	Name	Description	Models	Development	References
Chimeric Virus	MVA-6KE1E3E2	MVA encoding CHIKV structural polyprotein	IFN-deficient Mice	Preclinical	van den Doel et al. (2014)
Chimeric Virus	MVA-E2E3	MVA encoding CHIKV E2E3	IFN-deficient Mice	Preclinical	Weger-Lucarelli et al. (2014)
Chimeric Virus	MVA-CHIKV	MVA encoding CHIKV structural polyprotein	WT Mice	Preclinical	García-Arriaza et al. (2014)
Chimeric Virus	MV/Schwarz	Measles virus encoding CHIKV structural proteins	Mouse, NHP, Human	Phase II	Reisinger et al. (2019), Brandler et al. (2013), Ramsauer et al. (2015), Rossi et al. (2019)
Chimeric Virus	CHIK001	Chimpanzee adenovirus expressing CHIKV structural proteins	Mouse	Phase I	Ewer et al. (2017)
Inactivated Particle	UV-CHIKV	CHIKV particle inactivated by UV-irradiation	NHP	Preclinical	Nakao and Hotta (1973)
Inactivated Particle	BEI-CHIKV	CHIKV particle inactivated by binary ethyleneimine	WT Mouse	Preclinical	Gardner et al. (2010)
Inactivated Particle	Formalin-inactivated CHIKV	CHIKV particle inactivated by formalin	Mouse, Human	Preclinical	Tiwari et al. (2009)
Inactivated Particle	Tween/Ether-CHIKV	CHIKV particle inactivated by Tween 80 and ether	WT mice	Preclinical	Eckels et al. (1970)
Subunit	rCHIKVE1/E2	E. Coli synthesized recombinant E1 and E2 proteins	WT mice	Preclinical	Kumar et al. (2012)
Subunit	rE2p	E. Coli synthesized recombinant E2 proteins	WT mice	Preclinical	Khan et al. (2012)

(continued)

Table 1 (continued)

Platform	Name	Description	Models	Development	References
VLP	VRC-CHKVLP059-00-VP	Virus-like particle made on human HEK293 cells	Mouse, NHP, Human	Phase II	Kam et al. (2012a, b), Sun et al. (2013), Chang et al. (2014), Goo et al. (2016)
VLP	SF21-VLP/PXVX0317	Virus-like particle made using baculovirus and SF21 insect cells	WT mice	Phase II	Metz et al. (2013a)
DNA	p181/25-7	DNA vector expressing the full genomic sequence of attenuated 181/25 CHIKV strain	WT mice	Preclinical	Tretyakova et al. (2014)
DNA	CVM1-IgG	DNA vector expressing E2-reactive monoclonal antibody	WT mice	Preclinical	Muthumani et al. (2016)
DNA	pVax1-MCE321	DNA vector expressing CHIKV E1, E2, E3 proteins	Mouse, NHP	Preclinical	Muthumani et al. (2008, 2016), Mallilankaraman et al. (2011)
DNA	DREP-Env	DNA replicon encoding nsP and E1, E2, E3	WT mice	Preclinical	Hallengård et al. (2014a, b)
mRNA	mRNA-1944	Liposome-encapsulated mRNA encoding CHIKV-directed monoclonal antibody	WT, IFN-deficient mice, NHP	Preclinical	Kose et al. (2019)
mRNA	mRNA-1388	Liposome-encapsulated mRNA encoding CHIKV structural antigens	Mouse, Human	Phase I	Shaw et al. (2019)

clinical trials have also been completed or are currently underway for some technologies. However, despite this, regulatory bodies are yet to approve the widespread commercial utilization of any vaccine against CHIKV in humans. Unfortunately, the financial cost of developing a vaccine ready for distribution in human populations is on the order of hundreds of millions of dollars and thus requires a substantial commitment from private, nonprofit, or public institutions. As such, it will be necessary that threshold levels of either commercial potential or public need are crossed and associated with suitable risk criteria in order to attract the resources necessary for bringing a CHIKV vaccine into clinical use. Given that the burden of the virus is disproportionately incurred by economically developing nations, market forces may not forecast a return on investment necessary to incentivize for-profit entities in this endeavor. Moreover, the focal and episodic nature of CHIKV outbreaks, as well as establishment of herd immunity, renders phase III efficacy trials logistically problematic. However, spontaneous and unforeseen factors that drive arbovirus emergence and spread may fluctuate in a manner that results in outbreaks of magnitude or that shift geographically such that expanded market potential is revealed. Additionally, a market may also exist among travelers from developed countries interested in obtaining immune protection prior to the visitation of afflicted areas. Military needs may also drive vaccine development and thus government(s) may fund clinical advancement in the absence of commercial demand. The Global Alliance for Vaccines and Immunization (GAVI) also financially supports efforts in low-to middle-income countries to develop vaccines based primarily on disease burden and overall program sustainability.

Priority populations in which enhanced susceptibility to or severity of CHIKV-induced disease are evident include neonates (Charlier et al. 2017) and the aged (Lang et al. 2017; Godaert et al. 2017). As such, vaccine platforms pursued clinical use need to account for the immature or senescent immune status of these groups. Specifically, this includes an enhanced safety profile (including reactogenicity, adverse effects, and vaccine-induced disease) and appropriate immunostimulatory capacity. As discussed above, LAV are considered less safe due to the possibility of reversion or enhanced replication in the absence of robust immunity. Subunit and VLP-based vaccines exhibit improved safety profiles but are clearly less immunogenic. However, adjuvant classes and combinations exist and are being actively pursued that are capable of addressing this (Bonam et al. 2017; Aspinall et al. 2007; Levy et al. 2013; Borriello et al. 2017). Importantly, clinical trials examining vaccine safety and efficacy should be designed and executed in accordance with these goals. In addition, the establishment of a standard antibody neutralization protocol and criteria that can be applied to allow comparison across studies will also be crucial for further development. Finally, prioritization of a CHIKV vaccine will also depend on the emergence and outbreaks of other viral diseases such as Zika and Ebola or unknown zoonotic agents. These impose their own unique burdens on human populations and independently attract attention, effort, and investment.

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