

TITLE:

Chikungunya as a paradigm for emerging viral diseases: Evaluating disease impact and hurdles to vaccine development

ABSTRACT:

Chikungunya fever (CHIKF) is an emerging infectious disease caused by an alphavirus transmitted by *Aedes* spp. mosquitoes. Because mosquito control programs are not highly efficient for outbreak containment, vaccines are essential to reduce the burden of disease. Although no licensed vaccine against CHIKF is yet available, many highly promising candidates are undergoing preclinical studies, and a few of them have been tested in human trials of phase 1 or 2. Here, we review recent findings regarding the need for a CHIKF vaccine and provide an update on vaccines nearing or having entered clinical trials. We also address needs to tackle bottlenecks to vaccine development—including scientific and financial barriers—and to accelerate the development of vaccines; several actions should be taken: (i) design efficacy trials to be conducted during the course of outbreaks; (ii) evaluate the opportunity for adopting the “animal rule” for demonstration of efficacy for regulatory purposes; (iii) strengthen the collective commitment of nations, international organizations, potential donors and industry; (iv) stimulate public and/or private partnerships to invest in vaccine development and licensure; and (v) identify potential markets for an effective and safe CHIKF vaccine.

Understanding the impact of CHIKF:

Developing a safe and effective vaccine against CHIKF—as well as against other reemerging diseases, such as Ebola, Lassa, or Nipah—is important for several reasons. The impact of CHIKF in terms of burden of disease, work and school absenteeism, and other financial costs is particularly high, especially given its formidable epidemic potential. A paradigmatic example is provided by the 2006 epidemic wave that occurred in India, in which more than 1,500,000 cases of CHIKF were reported (http://www.who.int/denguecontrol/arbo-viral/other_arboviral_chikungunya/en/). Furthermore, the global impact of CHIKV is constantly growing, due to the introduction and spread of the virus into new continents in which it finds optimal conditions for its expansion, including, in some cases, a completely naïve population (Fig 1). The capacity of CHIKV to adapt to a new mosquito vector has been demonstrated during the Indian Ocean epidemic, when a series of mutations increased fitness for transmission by *A. albopictus* [14, 15], a mosquito that can survive at higher latitudes than *A. aegypti* [16]. This may lead to the occurrence of outbreaks in temperate climates, as seen in Europe [8, 9].

Secondly, although the case–fatality rate of CHIKF is relatively low—usually well below 1% [1, 2]—it may be underestimated in small outbreaks and in epidemic waves that occur in resource-poor countries. Excess mortality was investigated during an outbreak in Mauritius, and the case–fatality rate was estimated to be around 2.3 per 1000 [17]. Although “Old World” alphaviruses are not considered neurotropic *sensu strictu* [18], cases of meningoencephalitis have been documented, especially during Indian outbreaks [1], and fatal encephalitis has been reported with CHIKV infection both in Italy [8] and in La Reunion, where it was observed in patients mostly below 1 year or over 65 years of age [19]. Relatively high case–fatality rates (17%) and persistent disability (30% to 45%) were documented among patients with CHIKV-associated encephalitis. An increased incidence of other neurological syndromes, such as Guillain-Barré syndrome, was also reported during a CHIKF outbreak in French Polynesia [20]. These findings provide evidence of severe disease associated with CHIKV infection, which may have a high impact in terms of hospitalization and mortality during large outbreaks.

Thirdly, persistent arthralgia and joint swelling are common long-term manifestations of CHIKF. Unlike other mosquito-borne viruses such as dengue, Zika, and even yellow fever, CHIKV typically causes symptomatic infection, and consequently outbreaks are accompanied by high attack rates. Chronic joint pain, along with asthenia and mood changes, is a common cause of quality-of-life impairment. Ninety-four percent of symptomatic travelers infected in La Reunion complained of joint or bone pain 6 months after the epidemic peak; this pain was continuous in 41% of the cases. The effect of chronic symptoms on the quality of life was defined as totally disabling or important in almost half of the patients, whereas only 16% reported a normal mood [21]. A study conducted in La Reunion on 147 individuals over the age of 16 found that 84 confirmed cases (57%) self-reported rheumatic symptoms; of these, 63% reported permanent pain, whereas 37% had recurrent symptoms. An age of over 45 years (odds ratio [OR] 3.9), severe

initial joint pain (OR 4.8), and the presence of underlying osteoarthritic comorbidity (OR 2.9) were independent predictors of nonrecovery [22]. An assessment of 173 individuals with CHIKF conducted in Mauritius found that 79% reported persisting musculoskeletal symptoms 27.5 months after infection, associated with older age, female gender, and a baseline symmetrical distribution of arthralgia [23]. A study conducted on Italian patients found that over 66% of those with CHIKF developed long-lasting rheumatic disorders, leading to functional impairment affecting daily living activities up to one year after infection [24]. Therefore, the long-term impact of CHIKF is far from negligible in terms of suffering, need for care, impairment of work ability, psychological problems, diminished quality of life, and associated economic costs.

Unfortunately, little information is available on the economic costs of CHIKV infection. Healthcare costs during the La Reunion in 2005 to 2006 epidemic were estimated: the medical management of CHIKF was associated with a major economic burden, with 60% of the CHIKF-related expenditure attributable to direct medical costs, such as medical consultation (47%), hospitalization (32%), and drug consumption (19%). The cost of analgesics accounted for 80% of the CHIKF-related drug expenditures. Loss of productivity, measured as absenteeism costs, was also high [25]. Studies conducted in 2006 in Gujarat, India, estimated an immediate cost to household of CHIKF and dengue around 3.8 billion Indian rupees (ca. US\$55 million) per annum, whereas another study conducted in Ahmedabad, a city of 3.5 million people in the State of Gujarat, found that the disease affected primarily working-age adults, with an immediate cost of the outbreak due to lost wages and treatment of approximately US\$1.7 million based only on officially reported cases; these figures are probably underestimated, because only 23% of the cases seek treatment within public facilities [26]. Another study estimated a total of about 40 million cases in the Americas, with a burden of 24 million disability life years (DALYs) lost and about US\$185 billion in societal cost [27]. Therefore, although CHIKF is in most cases a relatively mild illness, its burden during epidemic waves may be impressive. Finally, when CHIKF outbreaks occur in tourist destinations, economic loss due to decreased numbers of visitors may cause further economic impacts. The under-recognition of the potential impact of CHIKF led to undesired political consequences during the epidemic waves on the tourist destination island of La Reunion, with the French government being accused of negligence and delays in outbreak response [65].

Therefore, for the reasons reported above, the development of a safe and effective vaccine against CHIKF would have a significant impact on the global burden of this disease with important health, economic, and ethical implications: (i) because of the high infection rates during epidemic waves, the disease burden may be relatively high. An effective vaccine would reduce the number of cases and hospitalizations worldwide, producing economic benefits through the reduction of absenteeism, lower costs for care and hospitalizations, and reduced loss of income associated with tourism; (ii) countries outside the tropics might also benefit from the development of a CHIKF vaccine, because the virus may be introduced from endemic and/or epidemic areas or enzootic circulation and eventually spread to regions where competent urban vectors are present; (iii) protection of travelers and military personnel may be another positive outcome of vaccine development; (iv) there are clear ethical implications in the development of a vaccine against an emerging neglected disease that primarily affects resource-limited parts of the globe.

Formalin-inactivated vaccines :: CHIKF vaccine candidates: State of development:

Initially developed in the 1970s, these induce neutralizing antibodies in animal models and humans [28, 29]. However, most development of these vaccines was discontinued decades ago because of high-production costs and risks associated with handling large quantities of virulent, wild-type, virus prior to inactivation. Therefore, although newer inactivated candidates have been tested with promising results [30, 31], most subsequent vaccine research was directed towards live-attenuated candidates.

Live-attenuated vaccines :: CHIKF vaccine candidates: State of development:

A serially passaged, empirically live-attenuated candidate vaccine called strain 181/clone25 [32] was eventually evaluated in a Phase 2 clinical study [33]: all vaccinated individuals developed neutralizing antibodies but 8% of them experienced mild, transient joint pain, a common symptom of CHIKF. The occurrence of arthralgia suggested insufficient and/or unstable attenuation; later studies indicated that the 181/clone25 attenuation is mediated by only two point mutations, which can revert following vaccination [34]. In other studies, seroconversion rates were low among those who had been previously vaccinated with other live-attenuated alphavirus vaccines (i.e., Venezuelan equine encephalitis virus), suggesting immunological interference [35].

Ultimately, development of this live-attenuated vaccine was discontinued not only because of reactogenicity and the absence of widespread and well-documented outbreaks but also due to the scarcity of funding and concerns regarding marketing [36, 37].

To design better live-attenuated candidates, genetically engineered complementary DNA (cDNA) clone-based, rationally attenuated CHIKV strains were more recently developed and successfully tested in animal models [38, 39], including NHP [40]. These vaccines contain very specific and stable mutations, providing better safety profiles compared with earlier live-attenuated vaccines such as 181/clone25, and retaining strong immunogenicity [12].

The first of these rationally attenuated CHIKF vaccines to undergo extensive preclinical testing employed a picornavirus-derived internal ribosome entry site to replace the CHIKV subgenomic promoter [41]. This replacement is highly stable after serial cell culture and mouse brain passages [42], and a single dose of the vaccine elicits robust neutralizing antibody responses and protects mice and NHPs against virulent CHIKV challenge, including weight change, fever, and viremia in cynomolgus macaques [40]. Protection after a single dose in mice lasts over 9 months, and protection of both mice and NHPs from this Indian Ocean lineage-derived strain also extends to Asian/American lineage CHIKV strains [43].

Other clone-derived, rationally attenuated CHIKF vaccine candidates include strains with deletions of a large part of the nonstructural protein 3 (nsP3) gene or the entire 6K/TF gene [38]. These vaccines, either administered as viral particles or infectious genomes launched from DNA, are highly immunogenic and efficacious in mice [38]. The nsP3 deletion vaccine, Δ nsP3 (VLA1553), is now in the recruitment stage for a Phase 1 clinical trial (Table 1).

Viral-vectored vaccines ::: CHIKF vaccine candidates: State of development:

A CHIKF vaccine in advanced stages of clinical development employs an attenuated measles virus strain as a vector to express the CHIKV structural proteins [44]. In a Phase 1 trial, this vaccine was well tolerated and induced neutralizing antibodies in 44% of volunteers receiving a single low-dose, 92% receiving a medium-dose group, and 90% receiving a high-dose. A booster raised seroconversion to 100%, and immunogenicity was not affected by preexisting anti-measles immunity. This vaccine is now in Phase 2 trials (Table 1).

Another unique viral-vectored vaccine, EILV/CHIKV, was developed based on the insect-specific alphavirus, Eilat (EILV), which is completely defective for replication in vertebrate cells [45, 46]. When chimerized to replace the Eilat structural polyprotein open reading frame (ORF) with that of CHIKV, the host restriction remains, as indicated by a complete lack of RNA genome replication and virus production in several vertebrate cell lines as well as in serial passages in infant, immunodeficient mouse brains, the most permissive vertebrate environment for most alphaviruses [47, 48]. A single dose of this replication-deficient vaccine produced in mosquito cells protects mice and NHPs from all measures of disease and viremia, with murine protection extending beyond 9 months. This remarkable immunogenicity (single dose of a replication-defective vaccine) is mediated, at least in part, by the normal endocytic pathway of the vaccine particles, which are structurally identical to wild-type CHIKV.

Virus-like particle vaccines ::: CHIKF vaccine candidates: State of development:

In addition to inactivated and viral vectored CHIKF vaccines, virus-like particles (VLPs) have also been developed and advanced to clinical trials. Expression via electroporation of mammalian cells of the CHIKV structural polyprotein ORF in a DNA plasmid form produces VLPs with identical protein structure to CHIKV. These VLPs elicit neutralizing antibodies against envelope proteins from diverse CHIKV strains, and immunized NHPs produce high-titer neutralizing antibodies that passively protect immunodeficient mice against lethal infection [49]. In a Phase 1 trial, this VLP vaccine was well tolerated and induced neutralizing antibodies in all dose groups after two vaccinations; a significant boost occurred after a third vaccination [50]. This vaccine is now in Phase 2 clinical trials (Table 1).

Nucleic acid-based vaccines ::: CHIKF vaccine candidates: State of development:

Finally, nucleic acid-based vaccines against CHIKV are under development. These vaccines have clear advantages, from ease of production, to safety, to the ability to induce both humoral and cell-mediated immunity; however many have shown a relatively low immunogenicity, requiring large doses, repeated boosters, and the use of adjuvants [12]. One RNA-based vaccine, VAL-181388, is recruiting for a Phase 1 clinical trial (Table 1).

Overall, nearly 30 vaccine candidates against CHIKF have been reported, but only four of these have entered Phase 1 or 2 trials [51–53] (Table 1). Whether any of these vaccine candidates will

advance to Phase 3 trials cannot be easily predicted and will be based on the perceived market as well as the identification of a suitable site with adequate incidence of CHIKF to demonstrate efficacy.

Bottlenecks for chikungunya vaccine development: Which obstacles should be removed? :::

CHIKF vaccine candidates: State of development:

CHIKF outbreak control is hampered by the lack of licensed vaccines that can be used in preventive immunization programs and for emergency response. Although the technical challenges of developing a CHIKF vaccine are not as great as those for some other viral diseases such as dengue, in which multiple serotypes must be targeted and partial immunity can lead to disease enhancement [54], there are several critical obstacles and financial constraints that need to be overcome in order to make available an affordable and effective vaccine.

First of all, barriers to the acquisition of human efficacy data for vaccine candidates due to the unpredictable nature of CHIKF epidemics may delay the development of a vaccine, even though promising candidates are available. For example, randomized controlled trials, which are considered the gold-standard for evaluating vaccine efficacy, may not be feasible during interepidemic periods because of the low expected number of cases; for this reason, epidemic events with a large number of cases may represent unique opportunities to ensure study power for testing vaccine candidates in efficacy trials. Approaches to overcoming these barriers include the development of platform technologies in which the critical antigens of a newly emerging viral strain can be rapidly incorporated into DNA or RNA platforms with proven safety records. However, based on initial results with DNA Zika vaccines that were generated within a few months of the recognition of congenital Zika Syndrome, these vaccines require multiple doses and immunity is not long lived; they are therefore far from ideal for intervening during an explosive epidemic or for long-term protection in endemic locations. Viral-vectored platforms such as vesicular stomatitis or measles can also be rapidly adapted for new viral targets and may offer more rapid and durable immunity.

Another approach to overcoming challenges of unpredictably emerging viral diseases is acceleration of clinical testing of new vaccine candidates and providing a robust rationale for particular trial designs and regulatory pathways. Therefore, vaccine trials should be designed very carefully to implement quickly and maximize their results during outbreaks. Potential opportunities for CHIKF vaccine testing may include cities with histories of recurrent dengue outbreaks (dengue and CHIKV share the same human-amplified transmission cycles, so regions susceptible to one should eventually have outbreaks of the other); for example, Sao Paulo State, Brazil, and Iquitos, Peru, have still not experienced major CHIKF outbreaks. However, ethical concerns with placebo-immunizing at-risk populations during an epidemic may need to be overcome with nontraditional designs, such as that used during the Ebola vaccine trials in West Africa [55].

There may also be opportunities to perform efficacy trials in regions endemic for CHIKF, but the typical misdiagnosis of CHIKF as dengue fever [56] will need to be overcome with improved surveillance and diagnostics to identify such opportunities. Finally, CHIKF will continue to occur mainly in poor-resource countries located in tropical areas, where the presence of trained and well-equipped clinical sites, which are essential for the implementation of clinical trials, can be challenging. However, there may be opportunities to capitalize on sites already developed for dengue vaccine trials, which are generally in locations endemic for both viruses.

In case these challenges to clinical efficacy trials cannot be overcome, alternative strategies should be considered. For example, it will be important to obtain reliable information on correlates of immune protection, which are essential in order to apply the so-called “animal rule.” This entails the use of surrogate end-points derived from animal data instead of the results of human trials.

This approach could be considered as an alternative option when large efficacy studies on humans, which are usually requested for traditional regulatory approval, are virtually impossible to realize [57]. For example, if human antibodies against CHIKV developed from individuals vaccinated in phase 1 and 2 trials are transferred to NHPs and are demonstrated to confer protection, this could provide surrogate evidence of vaccine efficacy, leading to a provisional license [58]. In this regard, the level of neutralizing antibodies has been already proposed for use as a surrogate marker of vaccine-induced protection [10, 36, 49, 58]. Although there is strong evidence that neutralizing antibodies against CHIKV protect against infection and disease, the lack of complete understanding of chronic arthritis, and its determinants, could limit the ability to relate animal efficacy to human protection. Further work to model arthralgia and arthritis in NHPs could greatly enhance the value of preclinical CHIKF vaccine testing. Other regulatory

considerations, including “traditional approval,” “accelerated approval,” and the “animal rule,” have been reviewed extensively in another article focused on CHIKF vaccines [13]. Obstacles to the provision of scientific evidence are not only represented by the barriers described above to vaccine development. In fact, most research and development (R&D) projects do not deliver a licensed vaccine for routine or targeted immunization—not because of methodological problems, but due to political and economic obstacles [59]. In fact, neglected diseases disproportionately affect poor and marginalized populations, and vaccines may have low returns on investment, so commercial firms may be reluctant to commit themselves to the expensive development and licensure of vaccine candidates, which typically totals hundreds-of-millions of US dollars [60]. To overcome this problem, several strategies may be implemented, including the creation of public and/or private partnerships, the identification of target population groups for vaccination to ensure a potential market, such as the military market, travelers and tourists, and the commitment of donor agencies and affected and/or donor countries [61]. Combination private and public consortia should address those vaccine development projects that are not considered highly profitable by industry in the absence of support from the governments of industrial countries. A recent example is the Coalition for Epidemic Preparedness Innovations (CEPI), funded by both government entities and private foundations, and include partners from the pharmaceutical industry, which is funding late preclinical and clinical development of vaccines for infections by Lassa, Nipah, and MERS coronavirus [62].

Conclusion:

In summary, the burden of disease caused by CHIKV is very high, due to the expanding geographic range of virus activity, increasing numbers of cases worldwide, and to the severe and long-lasting arthralgic sequelae of the disease. Developing an effective vaccine is crucial to contain outbreaks and to reduce the clinical and financial impact of CHIKF at the global level. However, as for other neglected and sporadically emerging diseases, barriers to traditional vaccine development and licensure need to be overcome by investing appropriate resources, which may require novel strategies to bring together diverse stakeholders.