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REVIEW

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Chikungunya vaccines in development

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ABSTRACT

Chikungunya virus has become a global health threat, spreading to the industrial world of Europe and the Americas; no treatment or prophylactic vaccine is available. Since the late 1960s much effort has been put into the development of a vaccine, and several heterogeneous strategies have already been explored. Only two candidates have recently qualified to enter clinical phase II trials, a chikungunya virus-like particle-based vaccine and a recombinant live attenuated measles virus-vectored vaccine.

This review focuses on the current status of vaccine development against chikungunya virus in humans and discusses the diversity of immunization strategies, results of recent human trials and promising vaccine candidates.

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Introduction

Chikungunya virus (CHIKV) is an arthritogenic arbovirus belonging to the alphavirus genus of Togaviridae, transmitted to humans by infected female Aedes arthropods. Apart from acute infections causing fever and severe joint pain, CHIKV can cause chronic rheumatism with long-lasting debilitating arthralgia.[1,2] The term Chikungunya (CHIK) means "to be contorted" in the Kimakonde language, describing the crouched physical appearance of anguished patients. Like Dengue virus (DENV), CHIKV is maintained by a primate-mosquito-primate cycle with 2 species of Culicidae (Aedes aegypti/Stegomyia aegypti and Aedes albopictus/Stegomyia albopicta), among others, serving as main arthropod vectors, both of which have been implicated in large CHIKV outbreaks. Infection by mosquito bites occur throughout the day, with highest occurrence at dusk, both in- and outdoors. The distribution of Aedes aegypti is largely restricted to tropical and sub-tropical urban areas. In contrast, Aedes albopictus, the Asian tiger mosquito, has undergone a dramatic global spread beyond its original boundaries, invading temperate climate zones of the industrial world and spreading CHIKV to new geographic regions.[3]

Epidemiology

So far, CHIKV has been identified in over 60 countries in Europe, the Americas, Asia and Africa.[4] The first isolation of CHIKV was recorded in 1952 in present-day Tanzania.[5] While outbreaks in the 20th century in Southern- and Southeast Asia have remained small and localized, an increasing number of endemic outbreaks on all continents have been reported since 2000. The largest outbreaks were recorded in the Democratic Republic of Congo (50,000 reported cases),[6] on Lamu Island in Kenya (13,500 reported cases, which represents 75% of the island's population),[7] and on La Réunion Island (266,000 reported cases)[8]; the biggest outbreak of all was recorded in India (1,300,000 reported cases).[9]

Europe

The establishment of Aedes albopictus in Europe allowed CHIKV to spread beyond (sub)tropical regions.[10] The first ever autochthon CHIKV transmission recorded in Europe was in Northeast Italy in August 2007, probably transmitted by a traveler from Southwest India, resulting in 205 confirmed cases of CHIKV infection.[11] One hundred twenty-six laboratory confirmed imported CHIK cases were recorded in mainland France in the summer of 2014.[12] Chikungunya virus, however, is not endemic in Europe and the risk of infection is mainly associated with traveling.

The Americas

In 2013, a member of the old Asian lineage CHIKV strain vectored by Aedes aegypti was introduced into the French part of the Caribbean Island of St. Martin and spread through Florida and South America. Until January 2015, 1,094,661 suspected CHIKV infections with 26,606 laboratory-confirmed cases were reported in the Americas,[13] yet its spread has probably remained geographically limited;[14] Aedes albopictus is the primary mosquito spreading CHIKV into climate zones. The etiologic Asian lineage strain of the Caribbean outbreak, however, ineffectively infects this arthropod due to a lack of E1-adaptive protein substitution.[15]

In this context, as reported by the CDC, 325 CHIK cases have been documented in 37 U.S. states as of September 2015. All cases were associated with travel and occurred in people returning from outbreak zones.[16]

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Genetic diversity

Chikungunya virus is endemic in tropical and subtropical areas of Africa, Southeast Asia and India. CHIKV circulates in an enzootic cycle of non-human primates (NHP) and arboreal mosquitoes. Transmission of CHIKV into an urban human-mosquito cycle causes regular outbreaks in naïve populations.[14]

CHIKV is an enveloped, single-stranded positive-sense RNA virus. RNA viruses are genetically very diverse,[17] with high mutation rates improving viral fitness and pathogenicity to ensure their survival. The genome of CHIKV is about 11.7 kb and encodes 2 open reading frames (ORF), flanked by 5′ and 3′ un-translated regions. The 5′ ORF is translated from genomic RNA by a cap-dependent mechanism resulting in the formation of 5 structural (envelope proteins E1-E3 forming trimeric spikes on the virions' surface, capsid and 6K/TF) and 4 non-structural proteins (nsP1–4).[18] While glycoprotein (GP) E1 is responsible for fusion within endosomes of target cells and nucleocapsid release, GP E2 interacts with cellular receptors for cell entry.[19] The small GP E3 mediates pH-protection during virus biogenesis and prevents E1 from premature fusion.[20] The capsid protein of alphavirus serves as a serine protease for self-cleavage, is necessary for the interaction with viral spike proteins during virion formation and serves a major function in nucleocapsid formation.[21] The 6K/TF protein is essential for formation and budding of new virions.[22]

Two-thirds of CHIK virus' RNA encodes for non-structural polyprotein precursors nsP1-4 serving RNA helicase, nucleoside triphosphatase and RNA dependent 5′ triphosphatase enzymatic activity.[23]

Three major CHIKV genotypes have been isolated - an Asian, a West African and an East/Central/Southern African subtype (ECSA). Sequencing ECSA and Asian genotype strains obtained during Malaysian outbreaks in 2006 and 2008 revealed 96.8% amino acid similarity.[24] The greatest genetic diversity was found in ns-proteins, the 6K and the E3 epitope. Furthermore, the genome length differs somewhat between genotypes and is longer in West African (11,843 to 11,881 nucleotides) and Asian (11,777 to 11,999 nucleotides) strains than in the ECSA lineage (11,557 to 11,789 nucleotides).[25] The Indian Ocean lineage (IOL), responsible for CHIKV epidemics in Europe in 2007 and 2010, evolved from the ECSA enzootic genotype and was primarily isolated in 2004 during an outbreak in coastal Kenya and the islands of the Indian Ocean. Analysis of the IOL genomic sequence revealed a new viral variant characterized by the substitution of Alanine instead of Valine (A226V) within the E1 protein, the major envelope surface protein. These novel mutations in the envelope glycoproteins suggest adaptive evolution of the virus to local vector abundance and allows CHIKV to use Aedes albopictus in addition to Aedes aegypti as a vector thereby increasing its distribution beyond tropical areas to the Western world. Analysis of A226V showed that the new mutation provided better vector specificity and improved fitness of CHIKV.[26]

Aedes albopictus is considered the most invasive mosquito species, with a high ability to adapt to different environments and strong competitive tendencies. Its global distribution and introduction to moderate climate zones during the past 30 years was facilitated by globalization, increased human travel, climate change, and the mosquitos' high adaptability to cold temperatures.[27]

Disease pathogenesis

Data on the pathophysiology of CHIKV infection are based on *in vitro* experiments, animal models and human studies. However the molecular mechanisms of virus host-cell interactions and the pathogenesis of disease chronification are not fully understood.

While infected female Aedes mosquitoes are feeding on blood, CHIKV gets inoculated intradermally, along with salivary molecules, including proteins and ribonucleic acid.[28-30] These salivary components alter host hemostasis for blood feeding purposes and enhance pathogen transmission by modifying immune processes[31-34] toward a T(H)2 response, while T(H)1 cells and antiviral cytokines are suppressed.[32,34]

Both hematopoietic and non-hematopoietic cells are involved in the host control of CHIKV infection.[35,36] Local epithelial and endothelial cells, primary fibroblasts as well as monocyte-derived macrophages are susceptible to the virus and allow for its replication.[37] Studies on immunocompetent mice identified dermal fibroblasts and skin macrophages as primary target cells (dermal injection phase).[37,38] An innate immune response mediated through the recognition of pathogen-associated molecular patterns by pattern recognition receptors (toll like receptor and retinoic acid inducible gene I-like receptors)[39] leads to a release of inflammatory cytokines, evoking a pyrogenic reaction. Type I IFN response is critical in the early phase of infection for viral clearance. Accordingly, IFN signaling and chemokine levels (IL-1ß, IL-6, MCP-1) correlate with disease severity, viral load (IL-6, MCP-1)[40] and persistent arthralgia (IL-6).[38,39] Interferons increase the expression of prostaglandins with ensuing nociceptor activation and sensitization, causing CHIK-characteristic joint pain.[41] In this context, high levels of IFNα were more likely to be found in patients with persistent polyarthritis than in those without.[42]

Migration of infected cells into the draining lymph nodes (lymphatic replication phase) and through the lymph circulation into the blood (viremic phase)[39] is followed by viral dissemination throughout the body into peripheral tissue, involving liver cells, muscle cells, joint cells[38,43] and - at least in immunodeficient mice - stromal cells of the central nervous system.[38] Long-term and recurrent arthralgia in CHIKV infection might be related to the persistence of CHIKV in synovial macrophages,[1,44] which provide a protective cellular reservoir. In human synovial fibroblasts, CHIKV induces miRNA 146a expression, inhibiting TRAF6, IRAK1 and IRAK2, which enhances its replication and interferes with pro-inflammatory pathways, i.e. NFkB- signaling.[45] In addition, IL-6 is considered a critical driver of long-lasting joint pain and CHIK-related rheumatic complaints by dysregulating the RANKL (Receptor Activator of NF-κB Ligand)/osteoprotegerin ratio, causing enhanced and sustained activity of osteoclasts and osteoclastogenesis.[46,47]

However, CHIKV infection commonly results in convalescent adaptive immune protection.[41] Adaptive immunity is characterized by CD8+ T cell response during the acute phase

of infection and a CD4+ T cell dominant immune response in advanced stages.[42] Although the mechanisms of the T lymphocyte response to CHIKV infection are not completely understood, it is considered necessary to maintain long-term immunity.[48]

CHIKV specific antibodies are detectable as early as one week after infection.[43] Immunoglobulin synthesis during the acute viremic phase of CHIK disease has been intensively studied, providing the basis for vaccine development and the use of CHIKV antibodies as passive immunization.[49-51]

Immunopathogenesis of DENV compared to CHIKV infection

Dengue virus has been reported to infect the same cell types as CHIKV;[52] a type-I interferon immune response is triggered by toll-like receptors and DExD/H box RNA helicases;[53,54] however, inhibitory mechanisms of the DENV against innate immunity responses are also established.[55,56]

Interferon response factors (IRF) 3 and 7 limit disease severity in CHIKV infection, protecting against haemorrhagic fever and shock.[52] In DENV infection, IRF-7 is described to be crucial in early disease response, together with the transcription factors STAT1-3, IRF9, IRF1, CEBPB, and SP1.[57] Inadequate IFN α/β–signaling is thought to contribute to complications in both CHIKV and DENV infections.[52]

In contrast to CHIKV, DENV infection does not become chronic.[58] Exosomes have been shown to contribute to disease limitation in Dengue Virus-2 (DENV-2) infections, conferring IFN inducible transmembrane protein 3 (IFITM3) to neighboring cells.[59] IFITM 1, 2 and 3 proteins exhibit antiviral properties and IFITM3 is suggested to reduce DENV-2 cell penetration.[60,61] This mechanism might be different from CHIKV infection, although this has yet to be ruled out. Furthermore, it is indicated that monocytes and macrophages, which are of importance in the development of CHIKV chronicity, substantially contribute to DENV infection control.[62]

Clinical presentation

Acute infection

CHIKV infects all age groups and both sexes at an equal rate.[63] It was previously believed that asymptomatic seroconversion was a rare event, occurring in less than 15% of cases.[1] Recent data from a prospective cohort study in the Philippines, however, suggest (at least for the Asian genotype) that asymptomatic infections account for the majority of cases depending on age, with a subclinical-to-symptomatic infection ratio of 2:1 in 6-month to 5 year olds and 12:1 in those 50 years of age and over.[64] The majority of patients present with rapid-onset fever (usually >39°C), indicating acute viremia, headache, myalgia and joint pain after a mean incubation period of 3 days (range 2–12 days).[65] Lymphocytopenia is the main viremia-related laboratory finding.[1] The duration and intensity of acute infection correlates with the viral load (up to one billion viral genome copies per mL blood) until viremia ends (5–7 days after onset of symptoms) and immunoglobulin M (IgM) is detectable.[1,65] Both IgM and Immunoglobulin G (IgG) levels positively correlate with disease severity.[66]

Severe joint pain and arthritis is the primary symptom of CHIKV infection and helps to differentiate it from DENV infection, which is one of the most common causes of fever in travelers returning from the (sub)tropics.

CHIKV-related arthritis usually coincidences with the onset of myalgia and fever and presents as severe symmetric (60%) polyarthralgia (positive predictive value for CHIK virema >80%)[1] manifesting in distal joints involving the knees, ankles, wrists and hands.[67] The axial skeleton is affected in up to 50% of cases.[68,69] Three-fourths of patients experience a diffuse maculopapular rash lasting about one week, usually on the trunk and resembling rash-pattern of DENV infection.[69] Infrequently, less specific symptoms seen in CHIKV infection include throat discomfort, abdominal pain, constipation, conjunctivitis, pruritus and lymphadenopathy.[70]

CHIKV infection is commonly a self-limiting disease. During recent outbreaks, however, complicated cases occurred, necessitating hospitalization. The enhanced disease severity observed likely has multiple causes including a more comprehensive recording of CHIK cases, larger-scale outbreaks, better viral adaptability to arthropod vectors, and new mutations of circulating CHIKV - increasing viral fitness and pathogenicity. This is supported by recent data from a neonatal mouse model, which indicate that increased CHIKV virulence may be based on a strains' ability to infect the host's myofibers.[71] Josseran et al published a case fatality rate of 1/1000 patients during the 2006 outbreak on La Réunion Island.[72] Severe complications including encephalitis, myocarditis, hepatitis and multi-organ failure mainly occured in multimorbid patients with chronic heart, kidney or neurological disorders, patients with diabetes, neonates, young children and elderly over 65 years of age. During the La Réunion outbreak, the mortality of hospitalized patients (17 per 10^5) with a severely complicated form of the disease was approximately 35%, highlighting the potential fatality of CHIKV infection.[73]

Persistent symptoms

Persistent pain and chronic musculoskeletal complaints are critical sequelae of CHIKV infection. A study of 180 patients with CHIKV infection found chronic symptom persistence, mostly musculoskeletal complaints, in 60% during a follow-up period of 36 months.[74]

According to Simon and colleagues, however, 2 distinct patterns of disease chronicity must be distinguished.[75] While the vast majority of patients with pain persisting beyond 3 months of infection suffer from heterogeneous musculoskeletal complaints (but do not have polyarthritis), about 5% of patients develop chronic inflammatory rheumatism (including spondyloarthritis, rheumatoid arthritis, or polyarthritis). Rapid differentiation between these 2 entities is important from both a diagnostic and a therapeutic point of view. While the former likely responds well to prolonged therapy with non-steroidal anti inflammatory drugs (NSAIDs), the latter may need early treatment with a disease-modifying antirheumatic drug (DMARD) to timely counteract the potentially destructive process of rheumatic inflammation.

In this context, patients with diabetes mellitus and those with pre-existing traumatic or rheumatic joint disorders have an increased risk of persisting joint pain.[74] It is not fully understood why previously injured joints are more severely affected and more susceptible to chronic infection. Similarities in immune responses and inflammatory pathways, including activation of synovial macrophages and increased osteoclastogenesis as the crucial event inducing bone loss, however, suggest a pathogenic relationship between classic rheumatoid arthritis and post-CHIK arthritis.[76] Alternatively, the reaction to CHIKV may simply aggravate or drive pre-existing inflammation. Although chronic viremia does not seem to occur in CHIKV infection, analysis of muscle biopsies in a patient suffering from long-term myalgia revealed persistence of the virus within the muscle tissue.[77] Moreover, CHIKV can reside and replicate in synovial macrophages.[78] This is in contrast to most other arboviruses and is presumed to be the underlying cause of chronic joint pain. Analyzing data from the La Réunion outbreak, Schilte and colleagues estimated the costs of long-term arthralgia at 250€ per patient per year, which, although probably overestimated due to selection bias, underlines the economic burden on the health care system.[74]

Diagnosis

Given the high predictive value of debilitating arthralgia accompanied by high fever in a CHIK endemic region, diagnosis is mainly clinical. However, different laboratory methods may be used to confirm diagnosis.

Viral culturing based on serum inoculation of mosquito cell cultures, mosquitoes, mammalian cell cultures or mice remains the gold standard, allowing further virus characterization,[79-81] but is not used routinely. During the first 8 days of the appearance of symptoms, reverse-transcriptase polymerase chain reaction (RT-PCR) detects serum CHIKV RNA with variable sensitivity. While viral culturing is limited by its restricted availability and temporary extra effort, RT-PCR additionally provides the possibility of genotyping to compare different samples and to detect other arboviruses in a multiplex format.[82] PCR assays have been performed on synovial tissue samples and fluids to confirm viral persistence within joints, but this is not recommended as a routine diagnostic test. Foissac and colleagues suggest that post-CHIK chronic rheumatism should be considered in patients unresponsive or dependent on steroid therapy beyond 3 months of disease onset.[83]

CHIKV serum IgM, assessed by enzyme-linked immunosorbent assay (ELISA), normally presents at the end of the first week of symptoms (days 3-8), indicating disappearance of viremia, reaches peak levels 3-5 weeks after the onset of acute illness, and wanes over a one- to 3-month period. IgG levels, primarily IgG3 isotype,[84] are usually detectable as of day 4-10 and persist for years.[85,86] A 4-fold increase in IgG levels indicates seroconversion. Continuing high IgM antibody titers are thought to result from limited antibody clearance. Unfortunately, no specific assay exists for the assessment of chronicity of CHIK disease.[1] Studies investigating persistent arthralgia following CHIKV infection assessed chronic joint symptoms on a clinical basis by medical examination and by interviewing patients using different questionnaires, including questions about the frequency and location of symptoms, intensity and quality of their pain, and its impact on their everyday activites.[87,88]

Treatment and prevention

No specific therapy or preventive treatment for CHIK disease in humans is currently available. According to the guidelines on the clinical management of CHIK fever by WHO, therapy is entirely supportive and limited to administering fluids, rest, physiotherapy and the administration of NSAIDs, chloroquine (in the case of refractory arthralgia) and short-term steroids for the management of osteoarticular and ocular manifestations.[68,89] In particular, there is no consensus on how to treat and when to initiate therapy in post-CHIK rheumatoid arthritis.

However, re-emerging CHIKV outbreaks during past years sparked research on new strategies for the prevention and treatment of CHIKV infection.

Several antiviral agents including ribavirin and IFNα inhibit CHIKV replication *in vitro*.[90] Doxycycline combined with ribavirin significantly reduces the viral load and the extent of inflammation in ICR mice.[91] Chloroquine sulfate failed to be effective in a placebo-controlled, double-blind, randomized clinical trial embarked during the 2006 CHIKV outbreak on La Réunion Island.[92] Further DMARDs have been tested only in a few patients. Ganu and colleague[93] assessed the efficacy of methotrexate (MTX) in 16 patients with poor to moderate response to hydroxychloroquine and sulfasalazine combination therapy after 3 months. At a 2 year-follow up, out of 14 patients started on MTX all but one experienced a moderate (21%) to good (71%) response. Beneficial use of MTX in the treatment of post-CHIK arthritis is supported by several other reports.[94,95] Very recently, Javelle et al published their results of a 6-year case series retrospective study in Réunion Island of patients suffering from CHIK arthritis, in whom treatment with MTX achieved a positive response in 75% (n = 54) of the patients, and provided the first diagnostic and therapeutic algorithm on how to treat rheumatic disorders that persist after an acute CHIKV infection.[96] However, DMARDs other than chloroquine have not been evaluated in large-scale clinical trials and have not yet been implemented in WHO's guidelines on the clinical management of CHIK fever.[89]

Newly invented antivirals include polymerase- and protease-inhibitors. Favipiravir, a small molecule inhibitor with broad-spectrum antiviral activity currently approved in Japan for the treatment of influenza virus infection, which protected mice from lethal CHIKV infection,[97] is one promising candidate.

A further, quite novel, approach is the use of small, interfering RNA sequences and short hairpin RNAs to inhibit CHIKV protein synthesis by post-transcriptional gene silencing.[98,99] Although the data surrounding these new agents are promising, their efficacy has yet to be proven in human trials and their indications must be clearly defined.

Passive immunization against CHIKV

Table 1 gives an overview of different monoclonal antibodies tested against CHIKV.

Human protection from CHIKV infection is considered primarily mediated by humoral memory host response and the presence of neutralizing antibodies targeting the virions' outer

surfaces of envelope glycoproteins.[100] Similarily, several studies support the efficacy of monoclonal antibodies (mAbs) as post-exposure therapy against alpha virus infections. Two recombinant IgG1 human mAbs, 5F10 and 8B10, directed against the CHIKV E1/E2 trimer significantly delayed CHIKV-related mortality in mice and conferred a 100% protection rate upon lethal CHIKV challenge.[101-103]

Several further CHIKV-neutralizing mAbs (targeting either the GP E1,[104] E2[105,106] or the caspid protein[107]) have been satisfyingly tested *in vitro* and in animal models,[50,105,106] but need to be evaluated in human clinical trials.

Likewise, no data exist on the optimal therapeutic window within which mAbs should be administered in humans. However, a timely infusion seems reasonable. Screening a panel of 230 mouse antibodies, Pal and colleagues recently identified 4 neutralizing mAbs (CHK-102, CHK-152, CHK-166, CHK-263), protecting immunocompromised mice from lethal CHIKV infection. However, no survival benefit from mAb injection was observed in the presence of overt disease 72 hours after infection. In contrast, combined mAb-therapy protected mice when given, at the latest, 24 hours before CHIKV induced death.[50] The authors suggested that combining mAbs may also confer synergistic efficacy in humans. Not only the time of mAb administration but other factors including viral burden and virulence, the mAb-dosage, the neutralizing potency of the particular mAb, the synergistic effects of combination therapies, and the possible emergence of viral escape mutants are likely to influence the success of this intervention. Future clinical trials in humans have to answer these questions and need to identify the optimal therapeutic window.

Theoretical risks of passive immunization may include the occurrence of adverse immune reaction (IgE-mediated anaphylaxis and anaphylactoid reactions), acquired immunodeficiency, mAb-specific adverse reactions like thrombotic disorders and cardiotoxicity and the selection of resistant virus variants.[108]

In view of the high costs, the use of immunotherpeutics against CHIKV infection will not be widely affordable in developing countries. Its use will be restricted to high risk populations including pregnant women, neonates and patients with a complicated form of the disease.[51]

In addition to their prophylactic and therapeutic potentials, however, mAbs provide a tool to understand host-CHIKV interactions, facilitating the development of active immunization strategies.[101]

Active immunization against CHIKV

Table 2 summarizes the most researched vaccine types over the past 4 decades.

Vector control through the use of larvicides and adulticides, the removal of larval habitats, limitation of human-vector contact and public education is critical to further control CHIKV outbreaks.[109]

However active immunization is still considered to be the most cost-effective preventive health intervention. Due to relatively low antigenetic diversity, the development of a CHIK vaccine is a viable goal. Funding of studies on orphan vaccines for low-prevalence infections, however, is limited by

Table 1. Monoclonal antibodies against CHIKV.

ORIGIN	NAME	TARGET	EFFECT	DOSAGE	REFERENCES
Human	5F10; 8B10	E2 (5F10), E2 and/or E1 (8B10)	Prophylactic: 100% protection Therapeutic: Delay in lethality but did not fully protect	250μg	Warter et al. 2011[101] Fric et al. 2013[103]
	C9	E2	Prophylactic: 100% protection Therapeutic: 100% protection	100μg	Selvarajah et al. 2013[106]
Mouse	CHK-102; CHK-152; CHK-166; CHK-263	E1 and E2	Prophylactic: 100% protection in immunocompromised mice Therapeutic: Mouse model: 58% (CHK-152) & 63% (CHK-166) protection from death Rhesus macaques: Significant reduction of viral load and spread (combination of CHK-152&CHK-166)	100μg 15mg/kg	Pal et al. 2013[50] Pal et al. 2014[172]
	1.3A2; 4.6F5	E2	Prophylactic: 100% protection against CHIKV arthritis, significant suppression of viremia	400μg	Goh et al. 2013[105]
	CK47	E1	*In vitro*: Inhibits virus release from infected cells		Masrinoul et al. 2014[104]
	1.7B2; 4.1H11; 4.8E2; 4.10A11; 5.1B12; 5.2F8, 5.2H7; 5.4G8; 5.5A11; 5.5D11; 5.5G9	Capsid Protein	*In vitro*: Reactivity was demonstrated in ELISA, western blot and IFA		Goh et al. 2015[107]

comprehensible disincentives. Limited global economic demand, low market potential, and high costs of vaccine development, coupled with unsafe investment returns (in light of unpredictable dynamics in vector distribution and the limited purchasing power of low-income countries) are factors that keep pharmaceutical companies from investing in the resource-consuming process of vaccine development, marketing and distribution. The absence of patent protection in some developing countries further impedes the willingness of pharma companies to invest in vaccine development.[110]

The ongoing geographic vector spread beyond tropical areas, as well as the genetic plasticity of CHIKV, however, raise concerns about the spread of the disease, the possibility of further epidemics, and highlight the need for effective and comprehensive preventive measures against human CHIKV infection.

Viral epidemics may have a severe long-term impact on a country's economy due to increased medical costs and a decline in tourism - a critical source of economic prosperity in many affected countries.[111,112] The CHIKV outbreak on La Réunion, which infected about 300,000 people in 2006, diminished tourism by 60% and the associated economic burden was estimated to be as high as 44 million Euro.[113] The economic burden related to such an outbreak is estimated to be more than 300 times greater than the costs associated with preventive measures.[114]

Though various types of vaccinations against CHIKV infection have been introduced and tested during the past decades, only 3 of them have reached clinical trials phase I/II testing.

Attempts to develop vaccinations against CHIKV are manifold and include live-attenuated vaccines, chimeric alpha-virus candidates, adenovirus-, poxvirus- and DNA-based vaccines, subunit formulations based on recombinant envelope proteins of CHIKV and inactivated Virus-like particles (VLPs).[22,115-127] Extensive research on immunization against CHIKV was done in animal models mainly in mice and NHP (Table 2).

Clinical trials investigating CHIK vaccines

Back in 1967, early attempts at immunization used a formalin-inactivated tissue culture CHIKV, prepared in bank-frozen green monkey kidney cells.[128-130] The vaccine was derived from a clinical CHIKV isolate (strain 15561) obtained during an outbreak in Thailand in 1962. Nine years later, in 1971, Harrison et al tested this live vaccine in 2 cohorts (n=8 each) of 16 healthy volunteers aged 21-25 years, assigned to receive two 0.5mL or 1mL doses twice in a 28-day sequence. The vaccine induced robust immunity (100% seroconversion rate 2 weeks after the second vaccination) and had an excellent safety profile without the occurrence of any local or systemic adverse events. Its development was stopped due to high manufacturing costs.

Based on a lot from the 15561 strain-vaccine, the United States Army Medical Research Institute of Infectious Diseases developed another live virus vaccine (TSI-GSD-218) by serial passage in MRC-5 cells.[131] After successful evaluation in a small phase I study, the TSI-GSD-218 vaccine, produced at the Salk Institute, Swiftwater entered phase II in 2000. Seventy-three healthy adults were vaccinated in a double-blind, placebo-controlled manner.[131,132] The vaccine was highly immunogenic after one-time immunization and showed an overall acceptable safety profile. However 8% of subjects reported arthralgia, generating concerns about incomplete viral inactivation. Lack of funding and higher priority development efforts led to a halt in further development.[116]

Recent vaccines in development

To date 3 experimental vaccines have advanced to the stage of human testing. Two candidates (the VRC-CHKV[133] and the MV-CHIK[127] vaccine) finished phase I, in 2014/15. The third candidate (CHIKV/IRES[115] vaccine) yielded promising efficacy and safety results in mice and macaques and plans are in place for a phase I trial. Table 3 summarizes the advantages and disadvantages of different types of vaccines currently under development.

CHIKV/IRESv1+v2 vaccines

IRES-based attenuated live vaccines derived from the CHIKV-La Réunion strain.

Preclinical development efforts

Just recently, Roy and colleagues published their results of an animal trial testing 2 live-attenuated vaccines (CHIKV/IRESv1+v2) in 3 cohorts of cynomolgus macaques (n = 4 per cohort).[115] Seven animals served as sham-controls. The candidate-CHIKV was attenuated by inserting a picornavirus (encephalomyocarditis virus) internal ribosome entry site (IRES) element into the genome of the 2006 La Réunion outbreak strain.[134] IRES sequence insertion down-regulates the expression of CHIKV structural genes and impedes infection of vector mosquitoes due to inefficient IRES translation in insects. The precursor vaccine (IRESv1) was previously shown to induce robust immunogenicity in mice[135,136] and cross-protected from the closely related (85% homology) African O' nyong'nyong alphavirus.[137] Single-vaccination of NHP with IRESv2 was highly immunogenic without any signs of disease. Antibody titers were first detected 15 days after immunization. Neutralizing antibodies assessment was done using 50% and 80% plaque reduction neutralization tests (PRNTs).

In vaccinated cynomolgus macaques no viremia occurred within 3 days upon subcutaneous challenge with wild-type CHIKV La Réunion strain 13 weeks after immunization.

The IRES vaccines were developed to overcome considerable safety issues associated with other attenuated live-virus vaccines and have 2 advantages: First of all, IRES sequence insertion is considered more stable compared to traditional attenuating single-point mutations, which may harbor the risk of mutation-reversion and recovery of pathogenicity *in vivo*; Secondly, the IRES element disables the vaccine strain to infect insect cells,[115] making a vaccine derived in this way particularly suitable for safe use in CHIKV-endemic regions with high vector density and frequent mosquito exposure.

The CHIKV/IRES vaccines are encouraging candidates, but have yet to demonstrate their efficacy in human trials.

VRC-CHKVLP059-00-VP

Virus-like particles containing envelope proteins from the West African CHIKV strain – The VRC-311 trial.

Table 2. Studies evaluating heterogeneous vaccine types within the past 44 years. IRES, Internal Ribosome Entry Site; NHP, non-human primates; nsP, non-structural protein.

VACCINE TYPE	STUDY TYPE	MODE	REFERENCE
LIVE ATTENUATED	Mice Primates	Subgenomic promoter replaced by IRES from encephalomyocarditis virus	Plante et al. 2011[135] Roy et al. 2014[115]
	Mice Humans: Phase I Humans: Phase II	Passages in green monkey kidney cells and MRC 5 cells	Levitt et al. 1986[163] McClain et al. 1998[131] Edelman et al. 2000[132]
	Mice	Deleting E2 and passages in baby hamster cells and mosquito cell lines	Piper et al. 2013[164]
	Mice	Passage in Chinese hamster ovarian fibroblasts and mosquito cells	Gardner et al. 2014[173]
	Mice	Deleting nsP	Hallengärd et al. 2014[22]
	Mice	Deleting 6K	Hallengärd et al. 2014[22]
INACTIVATED	Humans: Phase I	Formalin inactivated	Harrison et al. 1971[128]
	Primates	Formalin or UV-light inactivated	Nakao and Hotta 1973[165]
	Mice	Formalin inactivated	Tiwari et al. 2009[125]
	Mice	Formalin inactivated	Kumar et al. 2012[124]
	Mice	BPL inactivated	Kumar et al. 2012[124]
SUBUNITS	Mice	Bacterially produced E1	Khan et al. 2012[123]
	Mice	Bacterially produced E2	Khan et al. 2012[123]
	Mice	Bacterially produced E2	Kumar et al. 2012[124]
DNA	Mice	Expression of E1, E2, E3	Muthumani et al. 2008[166]
	Mice	Expression of C, E1, E2	Mallilankaraman et al. 2011[167] Bao et al. 2013[168]
	Mice	Expression of nsP3	Hällengard et al. 2014[22]
	Mice	Expression of 6K	Hällengard et al. 2014[22]
	Mice	Immunization DNA encoding the full length infectious genome	Tretyakova et al. 2014[122]
	Mice	Expression of 6K, E1, E2, E3	Hällengard et al. 2014[22]
VIRUS-LIKE PARTICLES/SUBUNITS	Mice Primates Humans: Phase I	Transfection of human embryonic kidney cells with plasmid DNA encoding C, 6K, E1, E2, E3	Akahata et al. 2010[139] Akahata and Nabel 2012[169] Chang et al. 2014[133]
	Mice	Production in insect cells (Baculovirus)	Metz et al. 2013[138]
RECOMBINANT VECTOR	Mice	Eastern equine encephalitis virus as vector	Wang et al. 2008[117]
	Mice	Adenovirus as vector	Wang et al. 2011[118]
	Mice	Vesicular stomatitis virus as vector	Chattopadhyay et al. 2013[119]
	Mice	Poxvirus as vector	Garcia-Arriaza et al. 2014[120]
	Mice Humans: Phase I	Measles virus as vector	Brandler et al. 2013[48] Ramsauer et al. 2015[127]

Preclinical development efforts

Enveloped VLPs (eVLPs), containing outer-structure proteins, expressed on a host cell-derived membrane without viral nucleic acid, are considered safe and induce robust immunity by eliciting high titer virus neutralizing antibodies. The VRC-CHKV vaccine is composed of CHIK-VLPs manufactured at the VRC, National Institute of Allergy and Infectious Diseases (Vaccine Pilot Plant operated by Leidos Biomedical Research) by plasmid-transfected embryo kidney cells. The VLPs used comprise capsid and envelope glycoproteins E1 and E2 from the West African CHIKV strain 37997. Several previous studies proving efficacy and safety of eVLPs in mice[138] and NHP[139] advanced the development status of CHIK-VLP vaccines,[140] warranting their advancement into human trials.

Clinical development efforts in humans

Chang and colleagues recently tested an adjuvans-free non-replicating CHIK VLP-based vaccine (Vaccine Research Center (VRC) CHIK virus candidate vaccine VRC-CHKVLP059-00-VP) in a 3 dose escalation (10μg, n = 5; 20μg, n = 10; 40μg, n = 10) phase I trial, funded by the National Institute of Health (NIH) intramural research program.[133] Vaccinations were administered intramuscularly at weeks 0, 4 and 20 and the CHIKV specific immune response was assessed by neutralizing antibody assays and ELISA.

This single center trial enrolled 25 healthy adults aged 18-50 years of largely (76%) Caucasian origin from December 2011 to March 2012. Overall, the VRC-CHKV vaccine was well tolerated, without the occurrence of any serious adverse events. No patient experienced arthralgia after immunization with VRC-CHKV. It was highly immunogenic which was reflected by a 100% seroconversion rate in all dose cohorts after a booster immunization. Injection site-related tenderness occurred in 36% of participants but was mild, as were systemic reactions (40% of vaccinees) including myalgia, malaise, headache and nausea. Although no neutralization geometric mean titers (GMT)-threshold

Table 3. Potential assets and drawbacks of CHIK vaccines that have reached human clinical trial testing.

VACCINE	CHIKV STRAIN	ADVANTAGE	DISADVANTAGE	CURRENT STATUS
VRC-CHKVLP059-00-VP[133]	West African CHIKV strain 37997	• VLPs are effective immunogens[153] eliciting high titer neutralizing antibodies[139] • High safety: Contains no live or replicating virus • No infection of insect cells (favorable for use in endemic regions) • No live-virus production needed • Plant-based or insect cell-based development possible[140] • Phase I:[133] No serious adverse events, vaccine-induced antibodies were detected in all participants up to 6 months after vaccination	• Multiple doses required; • Adjuvant required for dose-reduction • Expensive manufacturing of *in vitro* VLPs[48] • Large-scale DNA transfection needed[153] • Lack of strategies for cost-effective production	Finished Phase 1 in 2014
CHIKV/IRESv2[115]	La Réunion LR2006 OPY1 (ECSA lineage)	• High immunogenicity and long-term protection by single immunization • More stable attenuation by IRES sequence insertion compared to single point mutations • No transmission to insects/vectors • Comparably lower manufacturing costs	• Safety concerns regarding reversion of mutation and recovering to wild type pathogenicity • Safety concerns regarding immunosuppressed conditions	Projected for Phase 1
MV-CHIK[127]	La Réunion 06-46 (ECSA lineage)	• Live attenuated Schwarz strain elicits a robust humoral and cellular immune response • Long-term experience in safety of MV as vaccine vector; contains no replicating CHIKV; replication of RNA virus is limited to cytoplasm • MV optimal vector for reverse genetics[170] • No adjuvant required: comparably low effective doses • Measles vaccine can be easily produced on a large scale in most countries and distributed at low cost[171]	• Pre-existing immunity to measles may impede or prevent immunogenicity of a recombinant MV vaccine • Safety concerns about use in immunocompromised patients • Phase 1: Adverse event rate 17% (n=6)	Finished Phase 1 in early 2015, Projected for Phase II in late 2015
TSI-GSD-218[132]	Clone 25/181 (SE Asian isolated strain AF15561)	• Highly immunogenic upon one-time immunization	• Concerns about insufficient or unstable attenuation by single-point mutation • Phase 2: 8% rate of arthralgia	Development stopped due to lack of funding

protecting against CHIKV infection is known, the one month-titer levels achieved after 3-time vaccination with VRC-CHKV were comparable to convalescent titers after natural CHIKV infection and are considered to be protective. This compares to the rather low $PRNT_{50}$ of <200 detectable 11 months after (albeit single-) immunization with the live attenuated vaccine TSI-GSD-218.[132] More importantly, neutralizing antibody titers were still detectable in all dose cohorts 6 months after immunization with VRC-CHKV, suggesting robust immunogenicity. The results of this trial confirm the findings of the preclinical study on VRC-CHKV in NHP.[139] The human trial, however, was limited by its small sample size.

MV-CHIK

Live attenuated measles virus (MV)-based vaccine expressing surface proteins from the La Réunion ECSA CHIKV strain – The MV-CHIK trial

Preclinical development efforts

Originally developed as a vector to express heterologous viral antigens at the Institut Pasteur (Paris, France), the live attenuated Schwarz strain has proven to elicit robust humoral and cellular immune responses, yielding protective immunity against various arthropod-borne diseases (including DENV and West

Nile virus) in mice and monkeys.[141-143] Measles virus vaccines have been mass-produced at low cost for about 50 years, are well established, safe and provide good boosterability with a humoral and cellular mediated long-term memory effect.[144]

In 2013, Brandler et al introduced the CHIKV La Réunion strain-06-46 (obtained from viremic patients) subgenomic open reading frame encoding for the structural genes C-E3-E2-6K-E1 into the MV vector.[48] The La Réunion strain belongs to the ECSA lineage of CHIKV, which was responsible for most Indian Ocean epidemics in the past. Preclinical studies on the efficacy and safety of MV-CHIK were done in CD46 expressing transgenic mice lacking IFNα/β receptors (CD46-IFNAR). The live attenuated MV-vaccine expressing CHIKV envelope and capsid proteins induced a robust neutralizing immune response and completely protected the mice from a lethal CHIKV challenge after one or 2 immunizations. The passive transfer of MV-CHIK pre-immune sera containing neutralizing antibodies conferred full protection to mice.[48] The immunogenicity of the recombinant vaccine was also demonstrated in NHP (data not published; Dr. Ramsauer K., personal communication, August 25th 2015).

Based on these results, the MV-CHIK single center trial[127] was conducted from November 2013 to June 2014 at the Medical University of Vienna, Austria.

Clinical development efforts in humans

Ramsauer et al recently investigated the immunogenicity, safety and tolerability of a live attenuated recombinant viral-vectored vaccine based on the MV-Schwarz strain, MV-CHIK. This first-in-man trial tested the vaccine's safety and immunogenicity in the presence of pre-existing measles immunity in a randomized, double-blind, controlled dose escalation design (1.5×10^4 - 3×10^5 50% tissue culture infection dose ($TCID_{50}$) per individual) in 42 healthy adults aged 18-45 years.

In the per protocol analysis (n = 36), MV-CHIK raised neutralizing antibodies, as assessed by $PRNT_{50}$, in a dose-dependent manner and yielded a 100% seroconversion rate after the second immunization in all 3 dose cohorts. The GMT persisted over a 3-month period in the high dose group. As with the VRC-CHKV vaccine, no MV-CHIK-related serious adverse events were observed. Transient musculoskeletal pain was reported in 12% of subjects at the first follow-up visit and, overall, 6 participants experienced local pain or erythema, headache and pyrexia. Local reactions, however, were considered related to the 1mL-inoculation volume along with the vaccination's salt buffer content rather than the active ingredient.

The MV-CHIK trial enrolled 42 volunteers (compared to 25 subjects in the VRC 311 trial), mostly of Caucasian origin (98%; compared to 76% in VRC 311 trial). MV-CHIK participants were block randomized to receive booster immunization 4 or 13 weeks after the initial injection. This allowed for the assessment of both short-term immunogenicity/booster ability, a vaccine characteristic that could be relevant for travelers and tourists, as well as single-dose-related, 3-month persistence of neutralizing antibody titers. In contrast, antibody titers yielded by the VRC-CHKV vaccine were assessed and detectable up to 180 days after vaccination.[133] Due to inter-assay differences, however, neutralizing antibody titers cannot be directly compared.

Discussion

MV-based vaccines may be considered suitable for long-term protective mass immunization, given the long-lasting neutralizing antibody response achieved by the measles vaccine. It has been hypothesized that immunity by previous MV infection or vaccination might interfere with the protective efficacy and immunogenicity of a recombinant MV-based vaccine. This is a particular concern for this type of vaccine. However, large-scale clinical trials in humans have demonstrated a boostered anti-measles antibody production upon revaccination of previously MV-immunized individuals.[145,146] A preclinical study tended in the same direction: Immunity to MV did not impair the protective capacity of MV-CHIKV in transgenic CD46/IFNAR mice.[48] Likewise, in healthy adults, the immunogenicity of MV-CHIK was independent from the baseline immunity.[127]

However, given the high measles vaccination-coverage rate (>80% worldwide), pre-existing immunity against MV remains an interesting challenge that must be addressed in future human trials.

An advantage of both the VLP- and the MV-based vaccine could be the lack of a live and replicating CHIKV, rendering both candidates unlikely to induce CHIK-like adverse events including arthralgia and chronic rheumatism. Accordingly, no single subject vaccinated with VRC-CHKV complained of arthralgia, underlining the vaccine's favorable tolerability profile. In addition, VLP based vaccines can be considered safe for immunization of immunosuppressed people. However, the establishment of a chronic infection by replication-efficient CHIK virions may constitute a threat to patients with immunodeficiency. In this context, Seymour and colleagues very recently established a rodent model of chronic CHIKV infection in lymphocyte-deficient RAG1 knock-out mice lacking adaptive immunity for safety evaluation of the live-attenuated CHIK/IRES vaccine candidate under immunocompromised conditions.[147] This is a critical safety issue when using live virus vaccines, given the high rates of HIV infection and malnourishment in several developing countries. As already demonstrated for a type-1-IFN deficient state, CHIK/IRES vaccination was safe and did not cause virus persistence despite a lack of adaptive immunity. These findings warrant further evaluation of the CHIK/IRES vaccine in a clinical trial, including patients with compromised host-immune defense. Likewise, preclinical studies on the efficacy of MV-CHIK were conducted in IFN receptor-deficient mice. However, as live vaccines may lead to uncontrolled viral replication under immune-compromised conditions, this safety aspect needs to be addressed in adequately designed trials.

In contrast to a VLP-based vaccine, a live-attenuated vaccine does not require the use of an adjuvant for sufficient long-term protection. VLP-based vaccines are both safe and strongly immunogenic, but, may need several adjuvanted administrations in order to induce complete immunity.[139] Although other studies investigating the efficacy of adjuvant-free eVLP-vaccines suggest that single-immunization might be sufficient,[133,140] this must be confirmed for recombinant CHIKV VLPs in (further) human trials.

Adjuvants are added to a vaccine to enhance its immunogenicity and to reduce the number of immunizations needed to achieve

a sufficient antibody titer. However, adjuvanting may enhance reactogenicity and impair a vaccine's tolerability profile, depending on the adjuvant used.[148] A large meta-analysis comparing different influenza vaccines found that local adverse reactions were significantly more common with oil-in-water adjuvants than with adjuvant-free and aluminum-salt based vaccines.[149] Furthermore, adjuvants are thought to be implicated in the initiation or exacerbation of autoimmune disorders, a phenomenon known as Autoimmune/inflammatory Syndrome Induced by Adjuvant (ASIA) syndrome.[150] Currently, however, there is insufficient evidence supporting a definitive relationship between the use of adjuvants and the occurrence of autoimmune disorders. Because autoimmunity likely depends on several factors including the individual's genetic predisposition, this is an issue that should be considered when developing a human vaccine.

The lack of an adjuvant, however, may not only translate into a better tolerability profile but may also reduce the costs of manufacturing a vaccine, because relatively lower doses might be effective. This would be an important factor in the realization of large-scale use in low-income countries, considering the cost-intensive manufacturing process of VLPs *in vitro*. In this respect, plant "biofarming" technology is a promising, cost-effective approach to produce VLP-based vaccines,[151] characterized by high scalability, low production costs, lack of human and animal pathogens and the potential to produce oral and parenteral vaccine types. Several plant-based vaccines have already been investigated - including those for swine influenza, rabies and hepatitis B,[152] - but, have not yet been marketed. Another promising possible tool for the mass-production of eVLP-based vaccines is the baculovirus expression vector/insect cell (BEVS/IC) system.[154] A concise review on the current status of the (e)VLP vaccines development was recently published by Pijlman.[153] The use of insect cells as an expression system offers several advantages, including: A high rate of cell division; Growth in the absence of serum; And a high degree of complexity of generated VLPs.[155] Using baculovirus as an expression vector allows for short turnaround times, which provides product development flexibility especially needed for a quick response to pandemic outbreaks.

Active immunizations against CHIKV need to produce protective immunity against all CHIKV genotypes. Immunity to only one strain could possibly increase disease severity upon reinfection with another viral strain, as seen in secondary wild-type DENV infection. Antibody-dependent disease enhancement (ADE) has not been described in CHIKV infection yet, but it has been suspected to play a role in infections by other arboviruses,[156] including the closely-related alpha virus Ross River virus.[157] In this context, Hallengärd and colleagues recently observed increased disease severity in vaccinated mice with low anti-CHIKV-IgG titers upon subsequent re-infection with a heterogenic CHIKV strain.[121] Although no human data exist supporting the hypothesis of vaccination-related ADE, this issue should be addressed when conducting a clinical trial enrolling subjects at risk of CHIKV infection.

Though MV-CHIK is derived from the La Réunion strain, virus neutralization assays used an attenuated CHIKV strain (clone 25/strain 181) based on an Asian lineage virus isolate. Thus, the MV-CHIK vaccine presumably elicits a cross-neutralizing immune response *in vivo*.[127]

This is in agreement with results from preclinical mice-studies, where MV-CHIK conferred protection against the homologous (06-49) and heterologous (India, Congo, Thailand) CHIKV strains.[48]

In comparison, the amount of cross-reactive neutralizing activity against the ECSA outbreak strain OPY1 induced by the VRC-CHKV vaccine suggests that cross-protection could also be achieved for several strains, including the type circulating in the Americas.[133]

Efficacy trials in humans

No vaccine against CHIK disease has yet undergone efficacy testing in humans. Vaccine-efficacy against virologically confirmed CHIKV infection can only be determined in large scaled clinical trials including people at risk of CHIKV infection in affected countries.

This, however, may be a difficult task to undertake considering the unpredictability of sporadic CHIKV outbreaks and the high rate of asymptomatic infections (at least with the Asian genotype, as recently reported). Alternatively, levels of neutralizing antibodies could serve as surrogate parameter for vaccine efficacy.[158] This is an established concept for several licensed vaccines including those against influenza[159] and measles,[160] where antibody titers correlate well with human protection, which would dramatically facilitate the marketing approval for a vaccine against CHIK disease.

Future perspectives

Treatment: Long-term administration of NSAIDs continues to be the mainstay of therapy for patients with post-CHIK chronic pain and musculoskeletal disorders. However, no consensus exists on the proper diagnosis and treatment of CHIK-related chronic inflammatory rheumatism. There is an obvious need for randomized clinical trials testing the efficacy of different DMARDs in the treatment of post-CHIK rheumatoid arthritis, as well as the implementation of valid regulating guidelines.

Prophylaxis: No CHIK vaccine is currently under clinical investigation. Searching European clinical trials databases (clinicaltrials.gov; clinicaltrialsregister.eu) and the NIH Clinical Research Studies registry (clinicalstudies.info.nih.gov; researchmatch.org), we found only one trial that is currently testing the efficacy and safety of an anti-CHIKV hyperimmune intravenous immunoglobulin for passive immunization against CHIK disease in high-risk neonates born to mothers with presumed active CHIKV infection (clinicaltrials.gov Identifier: NCT02230163).

MV-CHIK: The evaluation of MV-CHIK in a randomized controlled multicenter phase II trial addressing safety and the persistence of functional anti-CHIKV antibodies over an extended period of time in a larger and more heterogeneous population will start in late 2015 (Dr. Ramsauer K., personal communication, June 25th 2015).

VRC-CHKVLP059-00-VP: Likewise, the VRC 311 study team plans to initiate a phase II trial in late 2015 (Dr. Ledgerwood J., personal communication, July 7th 2015).

CHIK/IRES: The CHIK/IRES vaccine, which has been successfully tested in (immunosuppressed) mice and NHP, is projected for a phase I clinical trial by Takeda Inc.,[161] but there is

no date set for the IND submission (Dr. Weaver S.C., personal communication, June 29th, 2015).

Conclusions

Recent dynamics in the geographic distribution of CHIKV with 2.5 million people infected over the past years[162] emphasize the vital necessity of a sufficient immunization coverage rate and demand further vaccine research to limit spreading of the virus, as well as to reduce the economic burden of increased health-care costs.

Substantial progress was achieved in the research and development of vaccinations against CHIK within past years. Varied approaches yielded encouraging possiblities, yet, substantial challenges remain. An ideal vaccine should be highly immunogenic, safe, adjuvant-free, have minimal side effects, and should confer long-term immunity after a single vaccination – and at a low cost. Mass immunization necessitates affordable vaccines.

Virus-like particle-based vaccines combine high safety with strong efficacy but may be constrained by their comparably lower long-term protective capacity, the need for multiple-dosing and higher manufacturing costs thereby limiting their use in low-income countries. Optimal strategies for cost-effective VLP production must be implemented in order to provide high scalability, sufficient quality and good immunogenic potency of VLPs. Live-virus vaccines elicit strong immune responses and long-term protection without the need for an adjuvant. However, there are numerous safety concerns that limit their usability in CHIKV-endemic regions - particularly for immunosuppressed patients: Unstable attenuation; Viral recovery to wild type virulence; Chronic infection; and transmission to vector insects.

Further vaccination strategies have been explored with promising preclinical results including a chimeric alphavirus[117] and a DNA replicon vaccine encoding the CHIKV envelope (tDREP-Env),[121] p62-E1 protein vaccines,[121] GP E2 protein subunits,[124] adenovirus-based[118] vaccine and the recombinant-modified vaccinia virus Ankara vaccine expressing CHIKV antigens (MVA-CHIKV).[121] Future studies will define their role in the ongoing fight against CHIKV.

Abbreviations

CHIK	Chikungunya
CHIKV	Chikungunya Virus
DENV	Dengue Virus
ECSA	East/Central/Southern African subtype
ELISA	Enzyme-linked Immunosorbent-assay
GMT	Geometric Mean Titers
GP	Glycoprotein
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN(s)	Interferon(s)
IOL	Indian Ocean Lineage
IRES	Internal Ribosome Entry Site
mAbs	Monoclonal Antibodies
MTX	Methotrexat
MV	Measles Virus
NHP	Non-Human Primate
nsP	Non Structural Proteins
PRNT	Plaque Reduction Neutralization Tests
RT-PCR	Reverse-Transcriptase Polymerase Chain Reaction
(e)VLP	(enveloped) Virus-Like Particles
VRC	Vaccine Research Center

Disclosure of potential conflicts of interest

MS was an investigator of the MV-CHIK trial (PMID: 25739878). BJ served as a regulatory advisor to Themis GmbH. NB, PPW and CS declare no competing interests.

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