Vaccines and Related Biological Products Advisory Committee Meeting
08 November 2019

Company Briefing Document

MV-CHIK - Themis Bioscience GmbH

Active substance:	MV-CHIK vaccine is the recombinant MV-CHIK construct delivering the genes encoding CHIK virus surface structure.
Intended indication(s):	Active immunization for the prevention of disease caused by chikungunya virus (CHIKV) infection.
Sponsor:	THEMIS Bioscience GmbH, Muthgasse 11/2, 1190 Vienna, Austria
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Themis Executive Summary – MV-CHIK

Themis Bioscience has completed the phase 2 development of MV-CHIK, a vaccine candidate for the prevention of chikungunya disease for people at risk of infection. Chikungunya disease is caused by chikungunya virus (CHIKV), which is transmitted by mosquitoes of the genus *Aedes*. Once restricted to tropical areas with endemic self-limiting outbreaks, CHIKV has evolved into a global threat with continuous geographic expansion from African and Asian countries to more temperate climates of the industrialized world (Lindsey et al. 2015; Morens and Fauci 2014; Rezza 2014). The disease is characterized by a sudden onset of unspecific fever accompanied by joint and muscle pain, headache, nausea, fatigue and rash (Burt et al. 2017; Rudolph et al. 2014). In the chronic stage of the disease the joint pain often becomes disabling and up to 60% of patients develop a chronic polyarthritis, that can incapacitate the patient for several months or even years (Rudolph et al. 2014; Staples, Breiman, and Powers 2009; Weaver et al. 2012). In rare cases, chikungunya leads to death with a mortality rate of 1-2.3 in 1,000 patients (Rezza and Weaver, 2019). Due to the growing threat of epidemics, the lack of a vaccine or a specific antiviral drug treatment for chikungunya as well as the impact on affected individuals and economic factors, there is a significant need for an effective vaccine to protect against outbreaks worldwide.

Themis vaccine vector platform is based on the modification of the measles vaccine Schwarz strain, a very well-established childhood vaccine used since the 1960s. The vaccine shows a remarkable safety and efficacy profile in all age groups. The vector was modified to express chikungunya structural genes, resulting in the so called MV-CHIK vaccine. Since program inception in 2011 the company has followed a rigorous clinical development program for its MV-CHIK vaccine candidate to confirm its safety in over 1,000 administered doses and demonstrate high levels of seroconversion and immunogenicity. Building on positive safety and seroconversion data from a large-scale Phase 2 clinical trial in healthy participants completed in 2018 (Reisinger et al. 2018) a Phase 3 clinical program was initiated.

The licensure of a vaccine is classically based on randomized controlled trials (RCTs) that demonstrate efficacy by prevention of disease in endemic areas. However, the epidemiology of the vector-borne chikungunya virus is not predictable: outbreaks are short-lived and spatially restricted in endemic areas. Themis has explored the feasibility of conducting a randomized controlled clinical trial for demonstrating vaccine efficacy of MV-CHIK. However, the unpredictability of outbreaks and the uncertain number of cases as well as the regulatory and operational preparation of efficacy trials in outbreak- and endemic situations has complications to the extent that make performance of an RCT impossible. For this reason, alternative pathways to licensure like the Accelerated Approval (21 CFR Subpart E. 601,41) shall be considered as a viable option.

Based on these facts, Themis is continuing a development program consisting of the following components:

- Clinical trials to be conducted world-wide, in endemic and non-endemic areas. Here, safety, immunogenicity and lot-to-lot consistency will be crucial aspects.
- Passive transfer experiments in non-human-primates (NHP) demonstrating the protective capacity of neutralizing antibodies raised by MV-CHIK in clinical trial participants, followed by the establishment of an Immune Correlate of Protection (ICP).
- Post-licensure efforts to confirm vaccine effectiveness and clinical benefit in humans.

The economic burden for the societies and the healthcare systems in "low- and middle-income countries" most affected by chikungunya is devastating. The economic costs of chikungunya infection are difficult to calculate and are not only linked to costs of medication and hospitalization, but also have to take into account a societal perspective. Chikungunya is estimated to cause a burden of 24 million disability life

years (DALYs) lost and about US$185 billion in societal cost for a total of about 40 million cases in the Americas. Based on its long-term experience in developing a safe and effective protection against chikungunya, Themis is committed to develop a licensure strategy with FDA and other regulatory agencies to enable licensure of an urgently needed vaccine.

I. Background on the Disease

I.1. Chikungunya Pathogenesis and Diagnosis

Chikungunya is a disease caused by chikungunya virus (CHIKV), which is transmitted by mosquitoes of the genus *Aedes*. CHIKV was first isolated in 1952 during an outbreak in southern Tanzania. The name 'chikungunya' derives from a root verb in the Kimakonde language, meaning "to become contorted" and describes the stooped appearance of sufferers with joint pain. Once restricted to tropical areas with endemic self-limiting outbreaks, CHIKV has evolved into a global threat with progressing geographic expansion from African and Asian countries to more temperate climates of the industrialized world (Lindsey et al. 2015; Morens and Fauci 2014; Rezza 2014). Increased travelling activities and global warming critically drive the transmission of vector-borne diseases by facilitating the spreading and establishment of virus carrying arthropods. Both *Aedes aegypti* and *Aedes albopictus*, which are currently known to be the primary vectors transmitting CHIKV in urban areas, have already established themselves in the USA and Europe highlighting the menace of emerging autochthonous chikungunya cases in these parts of the world (Vega-Rua et al. 2013; Vega-Rua et al. 2014).

Following the bite of an infected mosquito, CHIKV replicates in the skin and then disseminates to the liver and joints. The incubation period is 2 to 12 days, typically followed by a sudden onset of unspecific clinical symptoms with fever accompanied by joint pain. Other common signs and symptoms include muscle pain, headache, nausea, fatigue and rash. The joint pain is often debilitating, but usually ends within a few days (Burt et al. 2017; Rudolph et al. 2014). Most patients recover fully, but up to 60% develop a chronic polyarthritis, that can incapacitate the patient for several months, or even years after the acute stage (Schilte et al. 2013).

The diagnosis of CHIKV requires laboratory confirmation from blood samples ((Tanabe et al. 2018); Figure 1), as the clinical manifestations are relatively unspecific. During the initial viremic phase of the disease (day 0 - 7), CHIKV may be detected in blood samples by real-time polymerase chain reaction (RT-PCR) analysis. Following the viremic phase, serological tests may be performed, because antibody of the isotypes anti-chikungunya IgM and IgG are detectable starting from day 7 up to day 21. Levels of IgG persist over long periods of time and confer lifelong protection against re-infection.

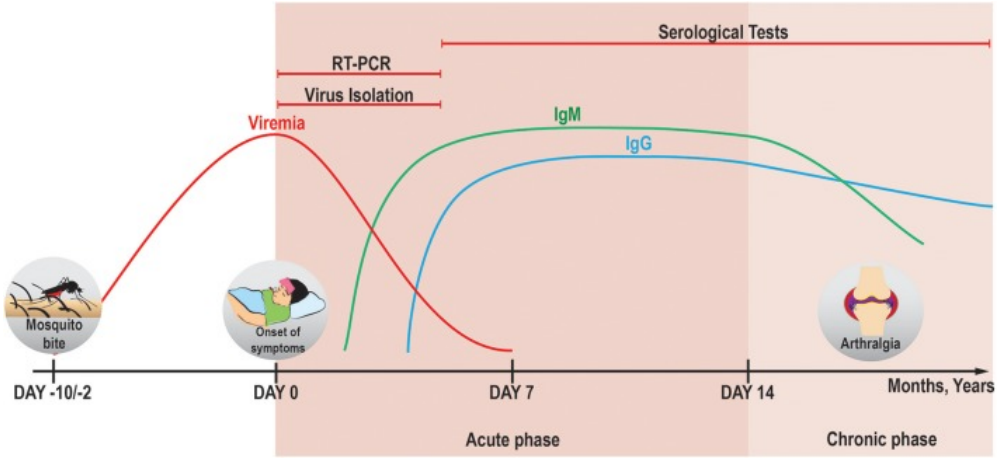

Figure 1 Applicability of different diagnostic methods in the course of CHIKV infection

I.2. Prevention and Treatment of Chikungunya

Currently, there is no vaccine or specific antiviral drug treatment for chikungunya infections available. Over-the-counter pain medication is used to reduce fever and relieve some symptoms. In severe cases, patients often need to be hospitalized to receive supportive treatment, such as intravenous fluids, pain medication, and nursing care.

II. MV-CHIK – A Measles-Vectored Vaccine to Prevent Chikungunya

Since 1963 attenuated measles strains are used in licensed vaccines in the US, EU, Japan, or by the WHO in vaccination campaigns worldwide, and confer high efficacy accompanied by a remarkable safety profile. Indeed, the measles vaccine is even recommended for infants and for compromised populations, such as HIV-infected patients, as long as immune suppression is not severe (McLean et al. 2013). Therefore, measles virus (MV) vaccine strains can be regarded as an ideal vector platform to design recombinant vaccines that present antigens of choice to the immune system in a highly favourable immune stimulatory context (Muhlebach and Hutzler 2017).

The MV-CHIK vaccine is a recombinant, measles-vectored vaccine that is based on the Schwarz vaccine strain. The measles vector was modified to harbour genetic information of chikungunya virus structural genes derived from a clinical isolate that was obtained in La Réunion, France, in 2006 (Brandler et al. 2013). Nucleotide sequences encoding the CHIKV structural proteins were chemically synthesized and inserted into the measles vector (Figure 2). The virus surface glycoproteins E1 and E2 that are encoded in the vaccine construct contain the main epitopes that elicit neutralizing antibody responses. The MV-CHIK vaccine lacks the non-structural genes, hence no fully functional chikungunya virus can be assembled. This potentially reduces the risk for Adverse Events (AEs), including joint symptoms.

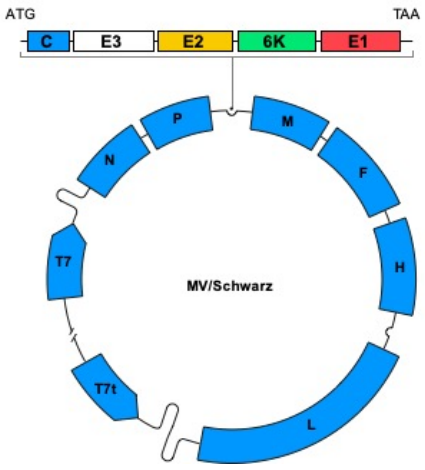

Figure 2 Schematic representation of CHIKV C-E3-E2-6K-E1 construct of recombinant MV vector

II.1. MV-CHIK Vaccine Mechanism of Action

Upon vaccination with MV-CHIK, the measles virus delivers the CHIKV antigens directly to antigen presenting cells susceptible to measles virus, including dendritic cells and monocytes, allowing for the mounting of CHIKV-specific cellular and humoral immune responses without the need for an additional adjuvant (Brandler et al. 2013).

In humans, the induction of neutralizing antibodies after vaccination, mostly directed against the viral structural proteins, was found to correlate with protection against chikungunya fever (Rezza and Weaver 2019). Chikungunya virus circulates in four genetic lineages that comprise a single serotype. MV-CHIK induced neutralizing antibodies are cross-protective against all circulating CHIKV lineages, as demonstrated with serum from immunized NHPs and serum pools from human clinical trial participants (Rossi et al. 2019). Further, passive transfer of pre-immune sera mediates protection against chikungunya fever in mice (Brandler et al. 2013; Chu et al. 2013) and passive transfer of serum from trial participants vaccinated with MV-CHIK protects NHP from CHIKV induced disease.

In addition to immune responses against chikungunya virus structural proteins, the recombinant vaccine also induced a significant increase in measles-specific IgG titres at both doses evaluated, indicating that the measles backbone of MV-CHIK also elicits an immune response. This enhanced immunity against measles could be a major benefit of MV-CHIK.

II.2. Intended Indication and Target Population

The intended indication of MV-CHIK is the active immunization for the prevention of disease caused by chikungunya virus infection.

Target populations are adults in non-endemic countries (i.e. travellers and military personnel serving in endemic countries) and individuals in endemic countries. Vulnerable populations, such as the pediatric population, are considered as well.

II.3. Preclinical Development

The safety, immunogenicity and efficacy of the MV-CHIK vaccine was assessed in small and large animal models: in CD46/IFNAR mice, a mouse strain that is susceptible to measles virus (Couderc et al. 2008), and in non-human primates (NHP) (Brandler et al. 2013; Rossi et al. 2019).

GLP compliant pharmaco-toxicological studies were performed in cynomolgus macaques (*Macaca fascicularis*). The vaccine was well tolerated in all animals, and there were no systemic or local adverse reactions up to the highest dose. A biodistribution and shedding study showed that the transgene expression did not affect the tissue tropism of the recombinant virus (Themis unpublished data).

CD46/IFNAR mice were vaccinated with MV-CHIK followed by lethal challenge with homologous and heterologous CHIKV strains. The MV-CHIK vaccine induced substantial immune responses and animals were protected from lethal doses after a single immunization. Also, transfer of sera from immunized mice conferred protection from lethal CHIKV challenge. In addition, immunogenicity of MV-CHIK was not impaired by pre-existing immunity against measles virus.

Similarly, MV-CHIK was found to be effective in cynomolgus macaques. All vaccinated animals showed high levels of chikungunya virus-specific neutralizing antibodies as determined by a virus neutralisation assay. In line with these data, all MV-CHIK vaccinated animals were fully protected against chikungunya viremia post-challenge with the La Réunion challenge strain (Rossi et al. 2019).

In summary, the non-clinical studies demonstrate an excellent immunogenicity, efficacy and safety profile of the vaccine candidate, which justified further development and its application in human clinical trials.

II.4. Phase 1 and 2 Clinical Development

The MV-CHIK vaccine was tested in clinical trials in different geographic regions of the world. A phase 1 and a phase 2 study were completed in Europe (Austria/Germany). Clinical trials ongoing in the US mainland and in a previously endemic area in Puerto Rico will give further insight on the safety in the potentially more heterogenic US population and participants living in endemic areas, some of which were pre-exposed to CHIKV. So far, the collective data from these studies showed the safety and immunogenicity of over 1,000 doses of the vaccine.

Clinical Assessment of Final Dose and Schedule

The phase 1 first-in-human clinical trial was conducted to investigate the immunogenicity, safety and tolerability of MV-CHIK (EudraCT: 2013-001084-23). Increasing doses of MV-CHIK ($1.5x10^4$ to $3x10^5$ TCID50 per individual) were injected *i.m.* in three groups of volunteers (42 healthy adults). The control group received Priorix® (a licensed measles, mumps, rubella vaccine), containing the parental measles virus Schwarz strain. MV-CHIK induced a robust immune response as determined by induction of neutralizing antibodies against CHIKV after one or two immunizations. In addition, the vaccine was well tolerated and safe (Ramsauer et al. 2015).

The immunogenicity and safety of the MV-CHIK vaccine was verified in a double-blind, randomized, placebo- and active comparator-controlled Phase 2 clinical trial (NCT02861586), conducted in Germany and Austria (Reisinger et al. 2018). Healthy, eligible adults aged 18 - 55 years were randomized to receive *i.m.* injections of either $5x10^4$ or $5x10^5$ TCID50 per dose of MV-CHIK in two different administration-regimens, or to receive Priorix® as the active comparator. In addition, the impact of pre-vaccination against the vector was assessed in two groups that received Priorix® prior to MV-CHIK administration.

Analysis of vaccine safety revealed a profile for MV-CHIK very similar to the licensed control vaccine Priorix®. Adverse events (AE) frequencies were highly similar between MV-CHIK and the Priorix® control group. No serious AEs related to the vaccine were identified.

A single immunization induced significant levels of neutralizing antibodies. A second immunization on day 28 substantially increased the titers (Figure 3).

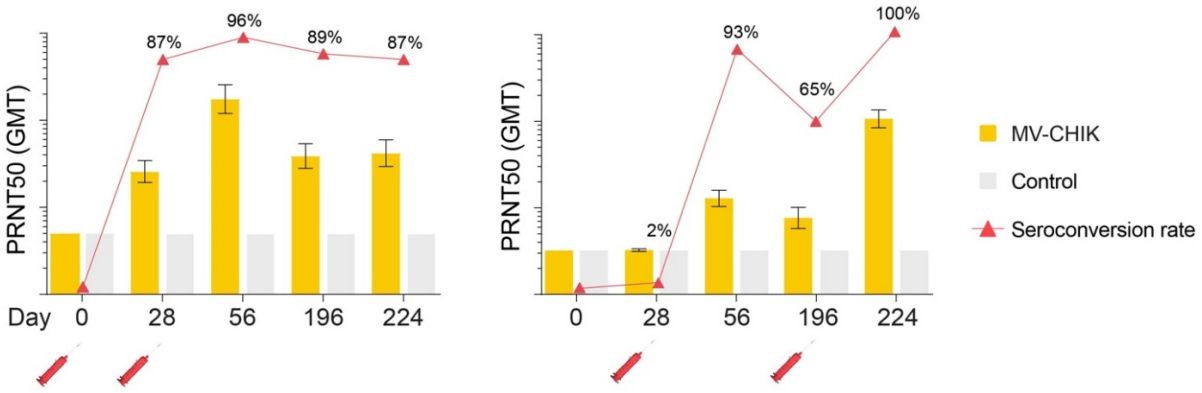

Figure 3 Neutralizing antibody titers and seroconversion rates upon MV-CHIK administration

The seroconversion rate (SCR), defined as a PRNT value of ≥10, ranged from 50 - 96% after a single dose and increased to 86 - 100% after two doses. While the overall neutralizing antibody titers decreased

over a period of 6 months of follow up, the seroconversion rate remained high. Pre-existing immunity against measles virus did not affect the immune response to MV-CHIK.

Supportive Clinical Trials

A clinical trial under US IND was sponsored by the NIH/NIAID (NCT03028441). Recruitment is completed and data analysis is ongoing. The study objective was the assessment of vaccine safety and immunogenicity in the US population. Two dose levels and three immunization schedules (1, 3, and 6 months) were evaluated. Each subject received two injections using one of the three immunization schedules. Subjects were followed up for approximately six months following the second dose.

Another clinical trial was funded by the US Department of Defence (DoD). The study objectives were the assessments of safety of the vaccine in a previously endemic area and the occurrence of adverse events of special interest (AESI), defined as pathologies of the joints (NCT03101111). 50% of the study population was previously exposed to chikungunya as confirmed by presence of CHIKV specific IgG. Recruitment is completed and data analysis is ongoing.

A follow-up study funded by the DoD was recently initiated (NCT03807843). The study objective is to assess the safety in pre-exposed subjects up to 65 years. The data will add important information to further expand the safety database for subsequent use of the vaccine in a pre-exposed population.

II.4.1. MV-CHIK Safety

Measles vaccine strains have been used for decades, therefore the available safety database is extensive. Possible risks that are frequently associated with measles vaccination are local reactions at the injection site as well as mild to moderate headache, myalgia, flu-like symptoms or fatigue.

MV-CHIK shows a good safety and tolerability profile. The vast majority of observed AEs was mild to moderate, transient, and resolved within a couple of days, comparable to marketed measles vaccines.

In the completed phase 2 clinical trial adverse events were highly similar between the MV-CHIK and control vaccine groups, reporting solicited and unsolicited AEs. No serious AEs related to the vaccine were recorded. All reported related AEs and solicited AEs are known from approved measles vaccines.

In contrast, a live attenuated chikungunya virus vaccine caused virus specific side-effects, including transient arthralgias, in a phase 2 study in 73 healthy adult volunteers (Edelman et al. 2000). A concise safety analysis of chikungunya-like symptoms related to arthritis following MV-CHIK treatment did not reveal increased frequencies compared with immunisation with the comparator vaccine (Reisinger et al. 2018).

In summary, the safety and tolerability profile of MV-CHIK is highly favorable and very similar to the measles control vaccine.

III. Pathway to Licensure

III.1. Introduction

The licensure of a vaccine is traditionally based on randomized controlled trials that demonstrate efficacy by prevention of disease in endemic areas. However, the unpredictability and the currently short duration of a typical chikungunya outbreak (2-8 months) compared to the challenging timelines for regulatory

approval and operational setup of an efficacy trial (6-12 months) has complications that make performance of an RCT impossible. For such situations the US Code of Federal Regulation (CFR) allows alternative pathways for licensure (Accelerated Approval 21 CFR Subpart E. 601,41), i.e. "approval of a drug that demonstrates an effect on a surrogate endpoint that is reasonably likely (…) to predict clinical benefit (…)". Therefore, Themis is currently preparing a data package for licensure consisting of:

- A Phase 3 clinical program in endemic and non-endemic adult populations to demonstrate safety and immunogenicity;
- A Non-human primate (NHP) model to demonstrate efficacy / clinical benefit;
- Bridging from NHP to humans, i.e. evaluation of an immune correlate of protection;
- Post-licensure effectiveness confirmation in humans, such as an observational/case control study and/or an outbreak intervention protocol.

III.2. Chikungunya Virus Epidemiology

III.2.1. Dynamic Epidemiology – Past and Present

Chikungunya virus outbreaks were recorded since the 1950s. The outbreaks were locally constrained and self-limiting. The epidemiology of chikungunya virus has changed dramatically in the 21st century. Since the 2000s, after a re-emergence in Kenya and the Indian Ocean region, CHIKV has been regularly detected across the global tropics.

In 2006 an explosive outbreak hit a completely susceptible population on La Réunion island in the Indian Ocean near Madagascar. This outbreak resulted in more than 260,000 cases, which represented approximately two-thirds of the population. After smaller outbreaks in the Philippines and Easter Island, CHIKV was introduced into the Americas in 2013, which led to an epidemic with more than two million clinically suspected cases reported across approximately 50 countries in a period of two years. The explosive nature of this CHIKV outbreak was due to the high susceptibility of the human population and the presence of the established vectors (*Aedes* mosquitoes). CHIKV was introduced and spread rapidly through a wholly naïve population in the 2014-2016 epidemic in the Americas (Figure 4). Approximately two million cases were reported during this explosive outbreak.

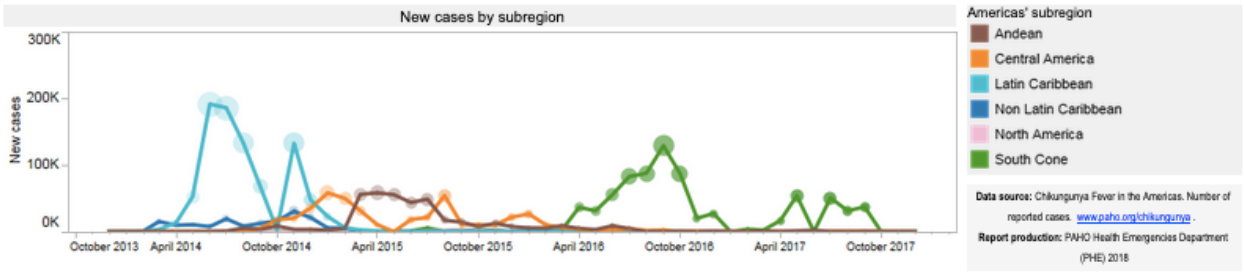

Figure 4 CHIKV epidemic on the American Continent 2014-2017 (source: PAHO, http://ais.paho.org/phip/viz/ed_chikungunya_amro.asp)

To date, chikungunya has been diagnosed in more than 110 countries and territories worldwide, with particularly high frequencies in "Low-and-Middle-Income Countries (LMIC)" of the tropical regions. Autochthonous transmissions in Europe or the USA have so far occurred only at small local levels, being diagnosed in several hundred cases in 2016-2017 (Figure 5).

Figure 5 Current activities of CHIKV transmission (status: May 2019; source: ECDC)

Widespread transmission has ceased, and incidence rates have receded significantly in most areas previously affected (Figure 5). In most cases, outbreaks are focal, with very low incidence rates, and of short duration, with the number of cases declining 10 to 15 weeks after onset of the outbreak.

The dynamics of CHIKV post-emergence have changed, as the number of susceptible humans has decreased. Nevertheless, recent data from Brazil show recurring high numbers of cases from 2016 to 2018 and corresponding proportional number of chikungunya-associated deaths, which previously had not been reported. In 2017, 165 chikungunya related deaths were confirmed in approximately 170,000 confirmed cases, which represented a mortality rate of 1 in 1,000 patients (Secretaria de Vigilância em Saúde Ago. 2018).

Currently, an outbreak is ongoing in a tourist region in the south of Thailand that has caused approximately 7,000 cases this year. While this number of cases suggests a major event, it only corresponds to 22 cases / 100,000 population, which reflects an attack rate of 0.02%. Similarly, transmission is currently reported in the State of Rio de Janeiro with more than 2,800 people affected. This number translates into an attack rate of 0.04% (42 cases/100,000 population). Data from the Asia-Pacific region showed that the overall occurrence of outbreaks has increased since the 1980s and transmission is currently ongoing at low levels, but that these recurrent outbreaks are similarly small and short-lived.

In some geographic areas, low level endemic transmission with attack rates of approximately 0.005% is occurring, occasionally interrupted by small local outbreaks. The mechanisms that drive or suppress outbreaks are poorly understood, making it impossible to predict when and where they will occur. Close-meshed international surveillance efforts would be required to identify areas with accumulating cases, in which an outbreak may occur. Current surveillance data of infected human subjects or of mosquito populations are insufficient to provide predictive maps of future CHIKV transmission activity in endemic regions.

A further complication of chikungunya epidemiology is the highly variable time span between two major outbreaks in an affected country: large intervals between CHIKV outbreaks were observed in Sri Lanka (41 years), India (32 years), Philippines (28 years: 1968–1996, 15 years: 1996–2011), Myanmar (14 years), Thailand (13 years), Malaysia (7 years), and Indonesia (6–8 years) (Wimalasiri-Yapa et al. 2019).

III.2.2. Implications of Epidemiology on Clinical Trial Design

CHIKV epidemiology impacts the size and the design of clinical trials in two major ways:

1. The low attack rate in endemic situations requires a sample size for a randomized controlled clinical trial (RCT) that would render the performance of such a trial unfeasible;
2. CHIKV outbreaks are unpredictable and of short duration, whereas the lead time for start-up of a clinical trial particularly in LMIC is challenging due to regulatory approval timelines, negotiation of contracts, logistical, ethical, and analytical components. Thus, it is likely that by the time a trial can commence, the outbreak is waning or has subsided.

To estimate the number of CHIKV cases required to power a traditional randomized controlled trial (RCT) assessing the efficacy of MV-CHIK, different potential attack rates were considered and the resulting total sample sizes calculated (Table 1).

The sample size calculations were based on a vaccine efficacy of 80%, assuming a relatively low loss of study participants to follow-up (10%). Post-outbreak attack rates recently reported from Colombia and Brazil are generally closest to the lowest attack rate of 0.005% (Table 1; yellow), with few local exceptions of attack rates increased to 0.05% (Table 1; orange). Attack rates of 0.5% or above (representing 500 cases / 100,000 population or more) are typically only seen during intense outbreaks that are spatially confined and short-lived.

Thus, it is likely that a future clinical trial would need to be conducted under low endemic attack rates of approximately 0.005% to 0.05%.

Table 1: Sample size calculations under changing epidemiological conditions
Assumptions: Vaccine Efficacy of 80% and 10% loss to follow-up. Seroprevalence is not factored in, as it does not affect the number of enrolled participants. Of note, the numbers of volunteers that need to be screened is heavily dependent on seroprevalence.

Attack Rate (AR%) in placebo arm	Vaccine Efficacy	# CHIKV events Required	Sample size - placebo	Sample size - vaccine	Sample size - Total
0.005%	80%	26	464,286	928,572	**1,392,858**
0.05%	80%	26	46,429	92,858	**139,287**
0.5%	80%	26	4,643	9,286	**13,929**
5%	80%	26	2,322	4,643	**6,965**

Vaccine clinical trials within the confines of the unique epidemiology of CHIKV post-outbreak may require a *de novo* design, which should be undertaken carefully to consider the regulatory, logistical, ethical, and analytical components. Thus, an alternative approach should be envisaged, combining aspects of various trial design approaches, in order to have sufficient flexibility to adopt to the dynamic CHIKV epidemiology, most prominently to fluctuating or rapidly decreasing attack rates.

III.3. Proposed Development Strategy

III.3.1. Phase 3 Safety and Immunogenicity Assessment

As described above, a randomized controlled clinical trial that demonstrates vaccine efficacy, i.e. prevention of disease in endemic areas, is not feasible. Therefore, Themis is currently preparing a phase 3 clinical program focusing on safety, tolerability and immunogenicity of MV-CHIK in adults 18 years or older. The study will be conducted in endemic and non-endemic areas. Immunogenicity will be determined by the induction of neutralizing antibodies. In addition, clinical lot-to-lot consistency will be confirmed.

The strategy for clinical trials in the pediatric population will be determined in alignment with FDA and EMA.

III.3.2. Assessment of Clinical Benefit in NHP

III.3.2.1. Relevance of NHP Challenge Model

Non-human primates (NHP) represent an excellent model organism for CHIKV infection, as they are a natural amplification hosts for CHIKV in sylvatic transmission cycles and are genetically and physiologically similar to humans. Initial studies performed in the 1950s and 1960s demonstrated that *Rhesus macaques* infected with CHIKV develop viremia 2 to 4 days post infections (dpi), mount a neutralizing antibody response and are protected from reinfection (Broeckel et al. 2015).

Recent work characterized CHIKV pathogenesis in *Rhesus* and *Cynomolgus* monkeys in comparison to humans. Labadie et al. investigated the infection of Cynomolgus monkeys with a wild-type CHIKV isolate (LR-2006-OPY1) via two different routes of administration (*i.v.* and *i.d.*) and dose levels ranging from 10^1 to 10^7 PFU. The study concludes that the acute phase of the chikungunya disease in humans can be mimicked in NHP, particularly if the challenge dose is similar to the amount of virus secreted from the salivary glands of mosquitoes and thus closely resembles natural infection (Vazeille et al. 2007; Vega-Rua et al. 2014). As shown by Labadie et al., 10^3 PFU induced viremia from 1 dpi, with a peak at 2 dpi and persisting until 6 to 7 dpi, with copy numbers ranging from $7x10^7$ to $5x10^9$ copies/mL. These observations were independent of the route of administration (Labadie et al. 2010). In a separate study evaluating intradermal challenge, viremia was not significantly different in animals challenged with 10^3, 10^5 or 10^7 PFU, with the highest titers observed after challenge with the lowest dose (Cirimotich et al. 2017).

The kinetics of viremia are similar in humans, where viral RNAs are also observed only within the first week in most patients. The amount of viremia in human patients is also comparable, with $8.2x10^8$ to $1.6x10^9$ copies/mL described during the Indian Ocean region outbreak in 2006 (Panning et al. 2008) and $1x10^{6.92}$ described in patients in Nicaragua (Waggoner et al. 2016).

One of the hallmark features of chikungunya disease in humans is the development of fever, usually starting at 2 dpi and lasting for approximately one week. Fever usually coincides with skin manifestations, macular and maculopapular exanthemas, diffuse erythema, with or without pruritus, and facial oedema are the most common presentations (Cunha and Trinta 2017). These manifestations are largely mirrored in NHP models (Broeckel et al. 2015). Interestingly, the development of fever and morbilliform skin rashes in Cynomolgus macaques from 2 dpi was, again, independent of the route of administration (*i.v.* vs *i.d.*) (Labadie et al. 2010)).

Infected NHP also present changes in blood chemistry, including elevated AST and ALT levels, similar to observations made in CHIKV-infected patients (Ng et al. 2009). In addition, several pro-inflammatory cytokines are induced by CHIKV challenge both in humans and NHP. Type 1 interferons have been found to be essential in the control of CHIKV infection in humans and are also rapidly induced in infected *Cynomolgus* and *Rhesus* macaques. In addition, IL6, IL1-RA as well as the chemokine MCP-1 (CCL-2) are similarly found at increased levels and follow the kinetics of viremia (Broeckel et al. 2015). Other laboratory abnormalities commonly found in CHIKV infected patients are leukopenia, lymphopenia and thrombocytopenia (Staples, Breiman, and Powers 2009). This is also observed in macaques, with the biggest decreases occurring during peak viremia (Labadie et al. 2010).

A subset of frequent symptoms of acute chikungunya fever, namely arthralgia, myalgia and headache, are more difficult to assess in NHP and only limited data is available. In fact, joint pathologies were only described after challenge with doses much higher than those transmitted during natural infection. In *Cynomolgus* macaques, viral RNA could be detected in knee and phalanx joints 2 to 8 dpi after infection with 10^7 PFU, concomitant with joint swelling. Infiltrating mononuclear cells were identified in ankle joints at 7 dpi in animals treated with 10^8 PFU. In the latter case, animals also showed clinical signs of meningoencephalitis (hunching and wobbling, asthenia and ataxia), and mortality was observed (Labadie et al. 2010). In a study evaluating the efficacy of monoclonal anti-CHIKV antibodies for the prevention of disease, control *Rhesus* macaques similarly showed viral RNA in joints after challenge with 10^7 PFU distributed over 10 sites (Pal et al. 2014). Antibodies in this study were protective, highlighting the previously described importance of humoral immunity in CHIKV control.

In summary, NHP represent the best available model for CHIKV infection, as these animals share many key features of human disease after challenge. As neutralizing antibodies appear to be strongly associated with protective immunity, Themis has adopted an experimental approach combining the passive transfer of serum or plasma collected from clinical trial participants to NHP with subsequent challenge with wildtype CHIKV to demonstrate efficacy of the candidate vaccine MV-CHIK.

III.3.2.2. NHP Passive Transfer Model

For chikungunya Themis proposes to define neutralizing antibodies as a marker that correlates with protection against disease and to demonstrate clinical benefit (i.e., protection from disease symptoms) using human neutralizing antibodies in the proposed NHP model.

As outlined above, clinical benefit cannot be demonstrated in humans because a controlled efficacy study is not possible within reasonable logistic boundaries and an acceptable timeframe. Another option to show efficacy for infectious diseases, like Malaria and Dengue, is the "human challenge model". However, given the potential of CHIKV infections to cause long term sequelae for which no adequate and specific treatments are available such a model is ethically highly questionable for chikungunya vaccine evaluation. Moreover, the population that would be included in such study is typically not representative of the affected age group and population. Along these lines, clinical assessment of an attenuated CHIKV strain revealed that even an attenuated virus can cause arthralgia in a subset of participants (Edelman et al. 2000).

As described above, the NHP chikungunya challenge model mimics many aspects of human disease. To assess if the immune response induced by MV-CHIK in clinical trial participants is indeed protective against chikungunya infection and disease, Themis combines passive transfer of human serum or plasma to NHP, followed by challenge of the animals with wild-type virus. A pilot study assessing the feasibility of this approach was recently concluded.

The aim of this study was to demonstrate the general suitability of the model and to gain initial data on the protective capacity of the humoral response raised in humans by administration of MV-CHIK. Animals were divided into three groups, receiving plasma collected from clinical trial participants that were either "naïve" (i.e., had never experienced natural infection with chikungunya and were not yet vaccinated - the negative control), convalescent (i.e., had previously undergone natural infection but not yet received the vaccine - the positive control) or were MV-CHIK vaccine recipients (that had never undergone natural chikungunya infection). The primary study endpoints were viremia and fever, the most consistent symptoms in NHP challenge experiments found in the literature (Broeckel et al. 2015). In addition, potential joint involvement was assessed by evaluating CHIKV RNA content, infectious virus as well as immune infiltrates in selected joints.

Briefly, animals that received CHIKV antibodies (convalescent or vaccine recipient plasma) were completely protected from viremia and all aspects of CHIKV induced disease. Selected joints were assessed for the presence of viral RNA and signs of inflammation. While there were no immune infiltrates or histological signs of inflammation in the selected joints, viral RNA was detected in the joints of animals in the naïve group. In contrast, no CHIKV RNA was found in any joints of animals that had received convalescent or vaccine recipient plasma. These results indicate that MV-CHIK induced antibodies can control and prevent virus infiltration of the joints. Several studies suggest that joint symptoms are correlated with the presence of virus in the joint (Suhrbier 2019). This implies that either the virus itself, the local innate and adaptive immune response against the virus (cytokine production, infiltration with immune cells), or – most likely – a combination of both is the cause of joint symptoms. The lack of viral RNA in joints of animals receiving vaccine recipient serum in the pilot study thus suggests that arthritis-related symptoms should be prevented just like other assessed clinical parameters. Still, efforts will be undertaken to further address this point in upcoming studies (Themis, unpublished data).

The level of neutralizing antibodies in NHP was assessed in serum collected immediately before virus challenge. Interestingly, in animals that received plasma from MV-CHIK vaccine recipients, full protection against chikungunya symptoms was conferred even at low levels of neutralizing antibodies. Moreover, at the end of study the vaccine recipient group had no detectable CHIKV antibody titer, suggesting that the sterilizing immunity conferred by treatment rendered a humoral response of the NHP B cell compartment unnecessary. In contrast, animals of the naïve control group developed high neutralizing antibody level against CHIKV representing the NHP humoral response (Themis, unpublished data).

In summary, these results show that the NHP challenge model is well suited to assess the protective capacity of antibodies raised in clinical trial participants receiving MV-CHIK, and that the dose and administration route are acceptable. Importantly, the data demonstrates that vaccine-induced antibodies are indeed protective even at very low levels comparable to titers described in humans (Yoon et al. 2015).

In summary, Themis has performed a NHP passive transfer pilot study demonstrating that neutralizing antibodies induced in clinical trial participants vaccinated with MV-CHIK protect from chikungunya disease. These results substantiate cumulative evidence in the literature suggesting that neutralizing antibodies represent a suitable Immune Correlate of Protection (ICP). A follow-up study is currently under preparation, that aims to define minimal titer levels necessary for protection by passive transfer as ICP and demonstrate likely clinical benefit by assessing a broad set of disease parameters in the NHP model which reflect human disease.

III.3.3. Immune Correlate of Protection

An immune correlate of protection is defined as a type and amount of immunological response that correlates with vaccine-induced protection against an infectious disease and that is considered predictive

of clinical efficacy. Currently, there is no formal ICP established for CHIKV. However, there is significant consensus within the scientific community that the induction of antibodies in general and neutralizing antibodies in particular correlates with protection against symptomatic chikungunya infection and that animal models could be used to establish an immunological correlate (Milligan et al. 2018; Yang et al. 2017).

Recent epidemiological studies conducted in the Philippines (Yoon et al. 2015) and Cambodia (Auerswald et al. 2018; Galatas et al. 2016) have confirmed that:

(1) a positive baseline CHIKV plaque reduction neutralization titer is associated with 100% (95% CI 46.1-100.0) protection from symptomatic infection;

(2) broad cross-neutralization among CHIKV lineages, i.e. ECSA, WAf, Asian, IOL, exists; and that

(3) it is highly likely that the elicitation of a neutralizing antibody response will provide very long-lasting (if not lifelong) immunity across all CHIKV genotypes.

Similarly, in a study of serum antibodies from a 2008 outbreak in Singapore, the early induction of neutralizing antibodies correlated with rapid clearance of virus from the periphery and clinical protection against arthralgia (Kam, Simarmata, et al. 2012). Similar findings regarding the importance of early neutralizing antibody responses in protection against arthralgia have also been documented recently in a prospective cohort in India.

This collective evidence in humans that experienced a CHIKV infection suggests that the induction of neutralizing antibodies correlates with clearance of the virus from the system and prevention of severe disease (Milligan et al. 2018).

These observations are well replicated in animal models. Passive transfer of IgG antibodies isolated from plasma of convalescent patients can efficiently prevent and cure CHIKV infection in mice (Couderc et al. 2009; Kam, Lum, et al. 2012). Additionally, treatment with neutralizing monoclonal antibodies specific for CHIKV E1 and E2 proteins protected IFNAR1$^{-/-}$ mice against lethal infection and prevented development of chronic infection in Rag-1$^{-/-}$ mice. Similarly, passive transfer of monoclonal antibodies against CHIKV also protected NHPs from CHIKV challenge (Pal et al. 2014).

To establish a protective threshold titer and define an immune correlate of protection Themis is proposing a passive transfer model in NHP as described above.

III.3.4. Effectiveness of MV-CHIK - Post-Licensure Efforts

Chikungunya outbreaks are unpredictable, focal and short. Thus, due to the lack of a sufficiently large CHIKV outbreak and the long timelines for setting up a clinical trial in an outbreak situation, a randomized, controlled efficacy trial is not possible before vaccine licensure.

It is important to note that even after approval of the vaccine, the implementation of a randomized, controlled clinical trial remains a challenge under the current unpredictable epidemiology for chikungunya virus. However, it is essential to show effectiveness and eventually clinical benefit of the vaccine in humans and the use of a licenced vaccine expands the possibilities with regards to study design.

Vaccine effectiveness trials within the confines of the dynamic epidemiology of CHIKV in the post-outbreak phase may require a design, which should be undertaken carefully to consider the fluctuating or rapidly decreasing attack rates, the logistical, ethical, analytical and statistical aspects. An alternative approach should be envisaged to have sufficient flexibility to adopt to the changing CHIKV epidemiology.

Thus, Themis evaluates the following components for post-licensure activities:

1. **Observational study** - Clinical trial protocols will be designed that could show vaccine effectiveness.
2. **Outbreak Response** - Themis is carefully following CHIKV epidemiology to be able to respond to larger outbreaks should they occur. An interventional outbreak protocol will be designed.
3. **Affected Country Engagement** - To successfully implement clinical trial protocols rapidly, Themis is extending relationships with affected countries that would allow for conduct of post-licensure studies. Potential clinical trial protocols will be presented to affected countries and, if possible, submitted for pre-approval.

Additionally, WHO pre-qualification is planned to eventually allow access to the vaccine in affected countries, particularly in an outbreak situation.

III.4. Summary

The traditional licensure of a vaccine is based on clinical studies that demonstrate efficacy by prevention of disease in endemic areas. However, the epidemiology of the vector-borne chikungunya virus renders this approach impossible, necessitating an alternative approach.

The US Code of Federal Regulations allows approval of a drug that demonstrates an effect on a "surrogate endpoint" that is reasonably likely, based on epidemiologic, therapeutic, pathophysiologic, or other evidence, to predict clinical benefit (Accelerated Approval, 21 CFR Subpart E, 601.41).

Themis is currently preparing data package for licensure consisting of:

- A Phase 3 clinical program in endemic and non-endemic adult populations to demonstrate safety and immunogenicity
- Non-human primates (NHP) model to demonstrate efficacy / clinical benefit
- Bridging from NHP to humans, i.e. evaluation of an immune correlate of protection
- Post-licensure effectiveness confirmation in humans, such as observational/case control studies and/or an outbreak intervention protocol.

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