Immunogenicity, safety, and tolerability of the measles-vectored chikungunya virus vaccine MV-CHIK: a double-blind, randomised, placebo-controlled and active-controlled phase 2 trial

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Summary

Background Chikungunya fever is an emerging viral disease and substantial threat to public health. We aimed to assess the safety, tolerability, and immunogenicity of a live-attenuated, measles-vectored chikungunya vaccine (MV-CHIK).

Methods In this double-blind, randomised, placebo-controlled and active-controlled phase 2 trial, we enrolled healthy volunteers aged 18–55 years at four study sites in Austria and Germany. Participants were randomly assigned to receive intramuscular injections with MV-CHIK (5×10^4 or 5×10^5 50% tissue culture infectious dose), control vaccine, or measles prime and MV-CHIK, in two different administration regimens. Randomisation was done by use of three-digit randomisation codes in envelopes provided by a data management service. The participants and investigators were masked to treatment assignment, which was maintained by use of sterile saline as a placebo injection. The primary endpoint was immunogenicity, defined as the presence of neutralising antibodies against chikungunya virus, at day 56, which is 28 days after one or two immunisations. The primary endpoint was assessed in all participants who completed the study without major protocol deviations (per-protocol population) and in all randomised participants who received at least one study treatment (modified intention-to-treat population). The safety analysis included all participants who received at least one study treatment. This trial is registered with ClinicalTrials.gov (NCT02861586) and EudraCT (2015-004037-26) and is completed.

Findings Between Aug 17, 2016, and May 31, 2017, we randomly assigned 263 participants to receive control vaccine (n=34), MV-CHIK (n=195), or measles prime and MV-CHIK (n=34). 247 participants were included in the per-protocol population. Neutralising antibodies against chikungunya virus were detected in all MV-CHIK treatment groups after one or two immunisations, with geometric mean titres ranging from 12·87 (95% CI 8·75–18·93) to 174·80 (119·10–256·50) and seroconversion rates ranging from 50·0% to 95·9% depending on the dose and administration schedule. Adverse events were similar between groups, with solicited adverse events reported in 168 (73%) of 229 participants assigned to MV-CHIK and 24 (71%) of 34 assigned to control vaccine (p=0·84) and unsolicited adverse events in 116 (51%) participants assigned to MV-CHIK and 17 (50%) assigned to control vaccine (p=1·00). No serious adverse events related to the vaccine were reported.

Interpretation MV-CHIK showed excellent safety and tolerability and good immunogenicity, independent of pre-existing immunity against the vector. MV-CHIK is a promising candidate vaccine for the prevention of chikungunya fever, an emerging disease of global concern.

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Introduction

The development of vaccines against emerging infectious diseases is a global public health challenge. 106 countries and territories, including the USA and Europe, have reported autochthonous, endemic, or epidemic chikungunya virus infections,[1] with about 1·3 billion people living in at-risk areas.[2] During the large outbreak of chikungunya virus in the Americas in 2013–15, more than 2·9 million suspected and laboratory-confirmed cases were recorded. Travellers from affected areas in the Americas imported the virus into the USA, leading to locally transmitted infections.[3] Increased travel and global warming are driving the transmission of vector-borne diseases such as chikungunya virus by facilitating the spread of virus-carrying arthropods. Both *Aedes aegypti* and *Aedes albopictus*, the primary vectors of chikungunya virus in urban areas, have already established themselves in the USA and Europe, highlighting the threat of autochthonous chikungunya emergence in these parts of the world.[4,5]

Symptoms of chikungunya virus infection appear within 2–12 days of a mosquito bite[6] and include high

Research in context

Evidence before this study

Chikungunya fever is a severe, debilitating disease that leads to chronic joint pathologies and depression in many patients. No approved vaccine exists for chikungunya virus, and treatments are limited to unspecific, symptomatic interventions. Re-occurring outbreaks, including a large outbreak in the Americas in 2013–15 that caused an estimated 1·4 million suspected cases of chikungunya fever, emphasise the urgent need for a vaccine. We searched PubMed and ClinicalTrials.gov up to July, 2018, for chikungunya vaccine projects using the keywords "chikungunya", "chikungunya virus", and "chikungunya vaccine". No language restrictions were applied. We identified eight chikungunya virus vaccine candidates, five of which are currently being assessed in phase 1 trials: three formalin inactivated, one adenovirus vectored, and one live attenuated. The live-attenuated chikungunya virus vaccine progressed into a phase 2 trial but was not pursued further because of unacceptable adverse events. Additionally, we identified a virus-like particle-based vaccine that is currently being investigated in a phase 2 trial and several experimental vaccines that are in pre-clinical development. Clinical data are publicly available for the virus-like particle vaccine and one of the formalin-inactivated vaccines, with both showing promising safety and immunogenicity in phase 1 trials.

Added value of this study

We showed that the live-attenuated, measles-vectored vaccine, MV-CHIK, is safe, well tolerated, and highly immunogenic in healthy volunteers aged 18–55 years. The vaccine induced functional, neutralising antibodies against chikungunya virus after one administration, and the concentrations of neutralising antibodies increased after a second immunisation. Pre-existing measles immunity did not affect the vaccine potency, further validating this vector platform. To our knowledge, MV-CHIK is the most advanced vaccine for an infectious disease to be based on a measles vector platform, suggesting that broader use of this technology, potentially for other outbreak threats, could be warranted. On the basis of these results, the European Medicines Agency granted MV-CHIK a priority medicines status, which might facilitate the rapid licensure of this urgently needed vaccine.

Implications of all the available evidence

Our data support further development of MV-CHIK towards licensure. Vaccination of people at risk of acquiring this emerging disease will prevent its spread to non-endemic areas with populations previously unexposed to chikungunya virus. A phase 3 clinical trial of MV-CHIK is currently being planned.

fever, severe myalgia and arthralgia of multiple joints, headache, exanthema, conjunctivitis, and fatigue.[6] Joint pain is typically polyarticular and symmetrical and affects mainly the extremities, wrists, ankles, and fingers but also large joints, including the shoulders and knees.[7] The symptoms usually disappear within 1–2 weeks, but musculoskeletal and joint pain, fatigue, and depression can relapse or persist for several months in up to 60% of cases and for 5 years in up to 12% of cases.[8] Risk factors for the chronic course of disease and higher-intensity symptoms during the acute phase include age older than 45 years and pre-existing rheumatological disorders.[9,10] Serious complications are not common, but in older people with comorbidities, the disease can contribute to death.[11,12] Mother-to-child transmission occurs at a high rate during the intrapartum period, which can be related to neonatal death and long-term disabilities. However, the frequency of such events is low compared with that of chikungunya virus outbreaks in general.[13]

Differential diagnoses of chikungunya fever include rheumatoid arthritis, dengue fever, and infections with other alphaviruses, including o'nyong-nyong virus and Ross river virus. Diagnosis is usually based on clinical observations and confirmed by detection of serum IgM or IgG antibodies or viral RNA by RT-PCR. Treatment is symptomatic with non-steroidal anti-inflammatory drugs. No specific antiviral therapy is available. The case fatality rate in some studies exceeds one in 1000.[14] Given that vaccination with a single chikungunya virus strain can elicit cross-protective neutralising antibodies against all four circulating genetic lineages of chikungunya virus,[15,16] an efficient vaccine against just one strain could contribute to a reduction in the burden and spread of the disease and be beneficial to many people living in endemic areas, and to international travellers.

The MV-CHIK vaccine is a live-attenuated, recombinant, measles-vectored vaccine that is based on the Schwarz vaccine strain. The measles vector was modified to harbour genetic information for chikungunya virus structural genes derived from a clinical isolate that was obtained in La Réunion, France, in 2006.[17] The recombinant vaccine is fully replication competent. In 2015, we did a first-in-man phase 1 study[18] to assess the optimum dose and schedule for MV-CHIK vaccine in healthy adults. We found that the vaccine had promising immunogenicity following one or two immunisations and an acceptable safety and tolerability profile. We also showed that pre-existing immunity to the vaccine's vector component (ie, measles) did not impair its immunogenicity.[18]

In this phase 2 trial, we aimed to further investigate the immunogenicity, safety, and tolerability of MV-CHIK in healthy volunteers. Other aims of the study were to select a vaccine dose and schedule for a phase 3 clinical trial and to investigate the effect of pre-existing antibodies to the measles vector on the immunogenicity of MV-CHIK.

Methods

Study design and participants

This double-blind, randomised, placebo-controlled and active-controlled phase 2 study was done in healthy volunteers at four sites in Austria and Germany: Department of Clinical Pharmacology and Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria; Hansa Sanatorium Graz, Graz, Austria; and Department of Tropical Medicine and Infectious Diseases, Rostock University Medical Center, Rostock, Germany. Healthy volunteers aged 18–55 years were included if they used reliable methods of contraception; had medical history, physical examination, and laboratory test findings that were considered normal or clinically irrelevant by the investigator; understood the risks and benefits of the study; and were available for the duration of the trial.

Exclusion criteria were history of immunodeficiency (eg, infection with HIV or hepatitis B or C viruses); close contact with individuals who are immunocompromised, children younger than 15 months, or pregnant women; drug or alcohol misuse disorders; pregnancy, lactation, or use of unreliable contraception in women of child-bearing potential; history of neoplastic disease within the past 5 years; history of a haematological malignancy or autoimmune disease; a case history, physical findings, or routine laboratory parameters indicative of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, inflammatory, neurological, or psychiatric disease that, in the opinion of the investigator, might create ethical conflicts or interfere with the aim of the study; history of severe adverse reactions to vaccine administration or to any component of the vaccine; previous vaccination against chikungunya virus; measles vaccination within the past 5 years; non-study vaccinations or systemic use of immunosuppressive drugs (starting 4 weeks before entry to this study); history of moderate or severe arthritis or arthralgia; receipt of blood products or immunoglobulins within 3 months before study entry; expected blood plasma donations during the study; use of medication (except hormonal contraception) within 2 weeks before the initial treatment visit, which the investigator considered to affect the validity of the study; and any infection within 1 week before study entry.

This study was performed in accordance with Good Clinical Practice guidelines, the Declaration of Helsinki, and all applicable national laws. The protocol (appendix) was approved by the lead ethics committees of Vienna (approval number 1957/2015) and the state of Berlin (approval number 15/0502-Ek15), and by the local ethics committees of each study centre. Written informed consent was obtained from all participants before entry into the study. An independent data safety monitoring board was established by the study sponsor to monitor the risk–benefit profile of the vaccine during the study on the basis of safety and immunogenicity data provided by the data management service (Assign Data Management and Biostatistics, Innsbruck, Austria).

Randomisation and masking

Study participants were randomly assigned to receive MV-CHIK (groups A and B: 5×10^4 50% tissue culture infectious dose [TCID50]; groups C and D: 5×10^5 TCID50), control vaccine (control group 1 or 2), or measles prime (measles prime group 1 or 2) with three-digit randomisation codes in envelopes provided by the data management service. Study personnel who were not masked to treatment assignment performed randomisation, prepared the study treatments, and were otherwise not involved in the trial. Study monitors, both masked and unmasked to treatment assignment, regularly confirmed the integrity of masking. Investigators at the study sites were masked to treatment assignment (syringes were taped up to conceal injection volume) and injected MV-CHIK, control vaccine, or placebo into the deltoid muscles of the non-dominant arms of participants. Study participants and investigators were masked to group allocation.

Procedures

MV-CHIK was provided by the study sponsor as a lyophilised formulation in two different concentrations: 5×10^4 TCID50 and 5×10^5 TCID50 (within 0·5 log) per dose in an injectable volume of 0·3 mL water. The control vaccine, Priorix (GlaxoSmithKline Biologicals, Vienna, Austria), is a live virus vaccine for immunisation against measles, mumps, and rubella that contains the attenuated Schwarz measles virus strain that was also used as the vector backbone of MV-CHIK. Information about preparation of MV-CHIK, control vaccines, and placebo is provided in the study protocol.

Participants in groups A to D and both control groups received three intramuscular injections: one each on days 0, 28, and 196. Participants in groups A, C, and control group 1 received MV-CHIK or control vaccine on days 0 and 28 and placebo on day 196, whereas participants in groups B, D, and control group 2 received placebo on day 0 and MV-CHIK or control vaccine on days 28 and 196. Participants in the measles prime groups received a total of five intramuscular injections (one 28 days before baseline and then one each on days 0, 28, 168, and 196). Both measles prime groups received control vaccine on day –28; participants in measles prime group 1 then received MV-CHIK (high or low dose) on days 0 and 28 and placebo on days 168 and 196, whereas participants in measles prime group 2 received placebo on days 0 and 28 and MV-CHIK (high or low dose) on days 168 and 196 (appendix).

See Online for appendix

We did case histories and physical examinations at screening; routine laboratory tests (haematology, serum chemistry, and coagulation) and urinalysis at screening, day 56, and day 224; and urine pregnancy tests at all study visits. Local tolerability and systemic adverse events were

recorded over the 7 days after each treatment by participants in diaries. Unsolicited adverse events were assessed throughout the study up to day 224.

Serum samples collected on days 0, 28, 56, 196, and 224 for all groups (and on day 168 for measles prime groups) were tested for neutralising antibodies against chikungunya virus with the plaque reduction neutralisation test, as described previously.[16] Briefly, two-fold serial dilutions of serum were pre-incubated with chikungunya virus (LR-2006-OPY) for 1 h, after which the mixture of serum and virus was added to monolayers of Vero cells (CCL-81; American Type Culture Collection, Manassas, VA, USA) grown in six-well plates. Infection occurred for 1 h, followed by immobilisation of cells via agarose overlay. After a 48 h incubation, cell monolayers were stained with crystal violet overnight, followed by plaque counting. The plaque reduction neutralisation test PRNT50 value was defined as the serum dilution required to reduce viral plaques by 50% of the control value (ie, cells infected with virus only). Seroconversion was defined as the participant having a neutralising antibody titre of 10 or greater (ie, a 1/10 or greater dilution of the participant's serum giving a positive PRNT50 result).

We also measured concentrations of chikungunya-specific and measles-specific IgG antibodies in serum samples using commercially available ELISA kits (Euroimmun, Lübeck, Germany), according to the manufacturer's instructions. We collected peripheral blood mononuclear cells (PBMCs) from a subset of participants to assess cell-mediated immunity. Additionally, we assessed shedding of live recombinant virus using real-time PCR in urine and saliva samples from a subset of participants.

Outcomes

The primary endpoint was immunogenicity (presence of neutralising antibodies against chikungunya virus, as assessed with plaque reduction neutralisation tests) on day 56. Secondary endpoints were immunogenicity (PRNT50 values) and concentrations of anti-chikungunya virus antibodies, as measured with an ELISA, on study days 0, 28, 56, 196, and 224 for all groups (and on day 168 for the measles prime groups); concentrations of measles-specific antibodies on days 0, 28, and 56 for all groups (and on day –28 for the measles prime groups); solicited local and systemic adverse events; serious and severe adverse events; adverse events up to 28 days after last vaccination; safety laboratory parameters; and shedding of live recombinant virus on days 0, 7, 10, 14, 28, and 196 in participants from one site (Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria). We also assessed cell-mediated immunity in a subset of participants, although the data for this analysis will be reported elsewhere. Severity was defined as mild (transient symptoms, no interference

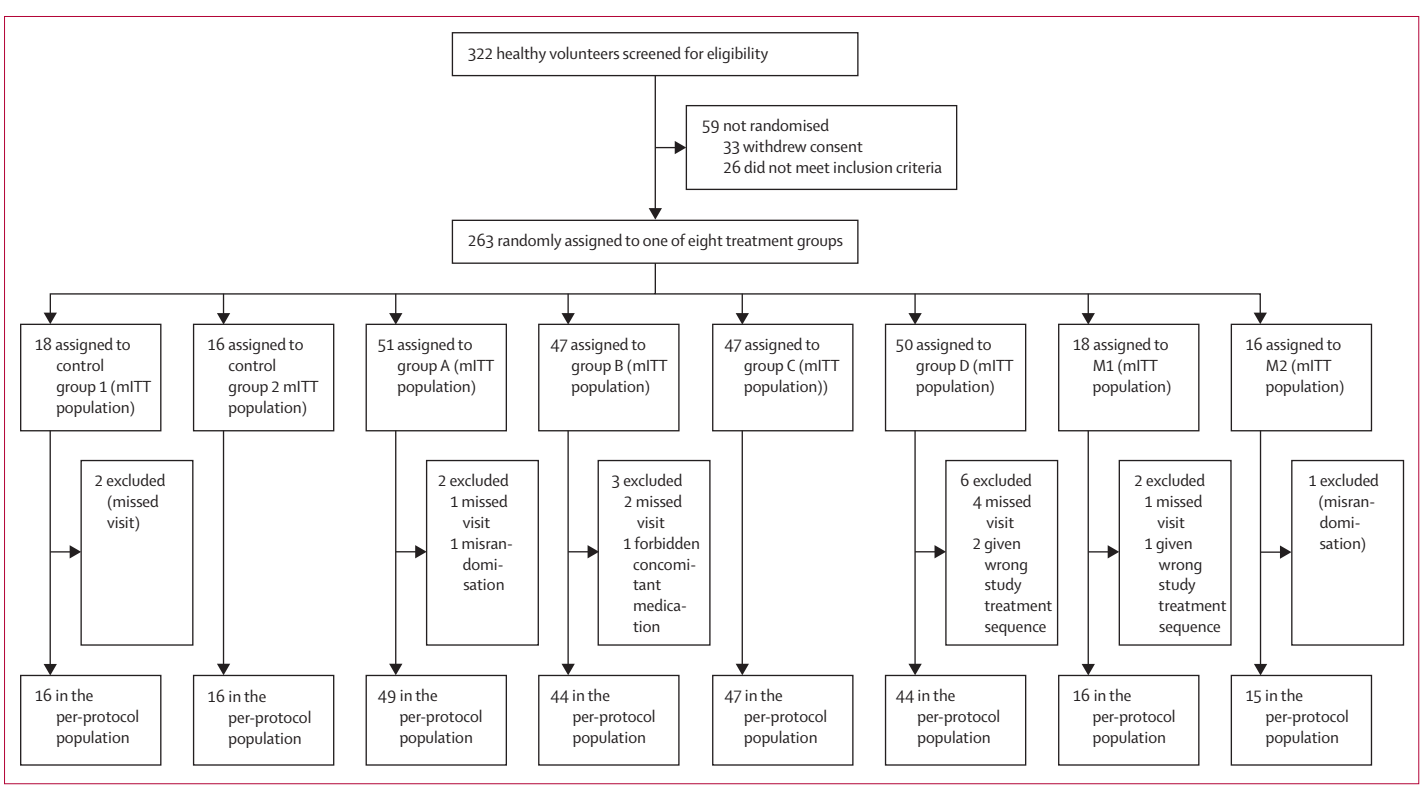

Figure 1: **Trial profile**
mITT=modified intention to treat. M1=measles prime group 1. M2=measles prime group 2.

with daily activities), moderate (marked symptoms, moderate interference with daily activities), or severe (considerable interference with daily activities).

Statistical analysis

The sample size of this study was not based on the results of a formal hypothesis test but decided on the basis of feasibility and common practice in similar trials.

The primary endpoint was assessed in the per-protocol and in the modified intention-to-treat (mITT) populations. The per-protocol population was defined as all participants who completing the study without major protocol deviations and the mITT population as all randomised participants who received at least one study treatment. Protocol deviations were classified as major if they had the potential to affect the primary endpoint. The safety analysis included all participants who received at least one study treatment.

Continuous variables related to immunogenicity are presented as geometric means with 95% CIs. To generate geometric mean titres of neutralising antibodies, we took the anti-log of log_{10}-transformed least squares mean PRNT50 values. We then used ANOVA to compare geometric mean titres between groups. Pairwise comparisons in these ANOVAs were adjusted for multiple tests according to the Tukey-Kramer test. The same ANOVA model was also used to compare geometric mean titres 28 days after the first and second vaccinations between treatment groups A and B, as well as between groups C and D, and to compare treatment groups A to D 28 days after first vaccination according to baseline percentiles of anti-measles antibodies: 0–24%, 25–49%, 50–74%, and 75–100%.

We compared the proportions of participants with solicited and unsolicited adverse events across all treatment groups using the Fisher-Freeman-Halton test and between groups using Fisher's exact test. A two-sided significance level of 5% was used for all statistical tests. Statistical analyses were done with SAS version 9.3.

This trial is registered with ClinicalTrials.gov, number NCT02861586, and EudraCT, number 2015-004037-26.

	Control groups (n=34)	MV-CHIK treatment groups (n=229)
Sex		
Female	17 (50%)	106 (46%)
Male	17 (50%)	123 (54%)
Age (years)	32·0 (24·0–38·0)	29·0 (24·0–41·0)
Ethnicity		
White	33 (97%)	224 (98%)
African	0	2 (1%)
Asian	1 (3%)	0
Other	0	3 (1%)

Data are n (%) or median (IQR).

Table 1: **Baseline characteristics of the safety population**

Role of the funding source

The sponsor of the study supported the data management via an independent contract research organisation and sponsored independent statistical analysis. The sponsor also made financial contributions to the study sites to undertake the trial. The sponsor designed the study,

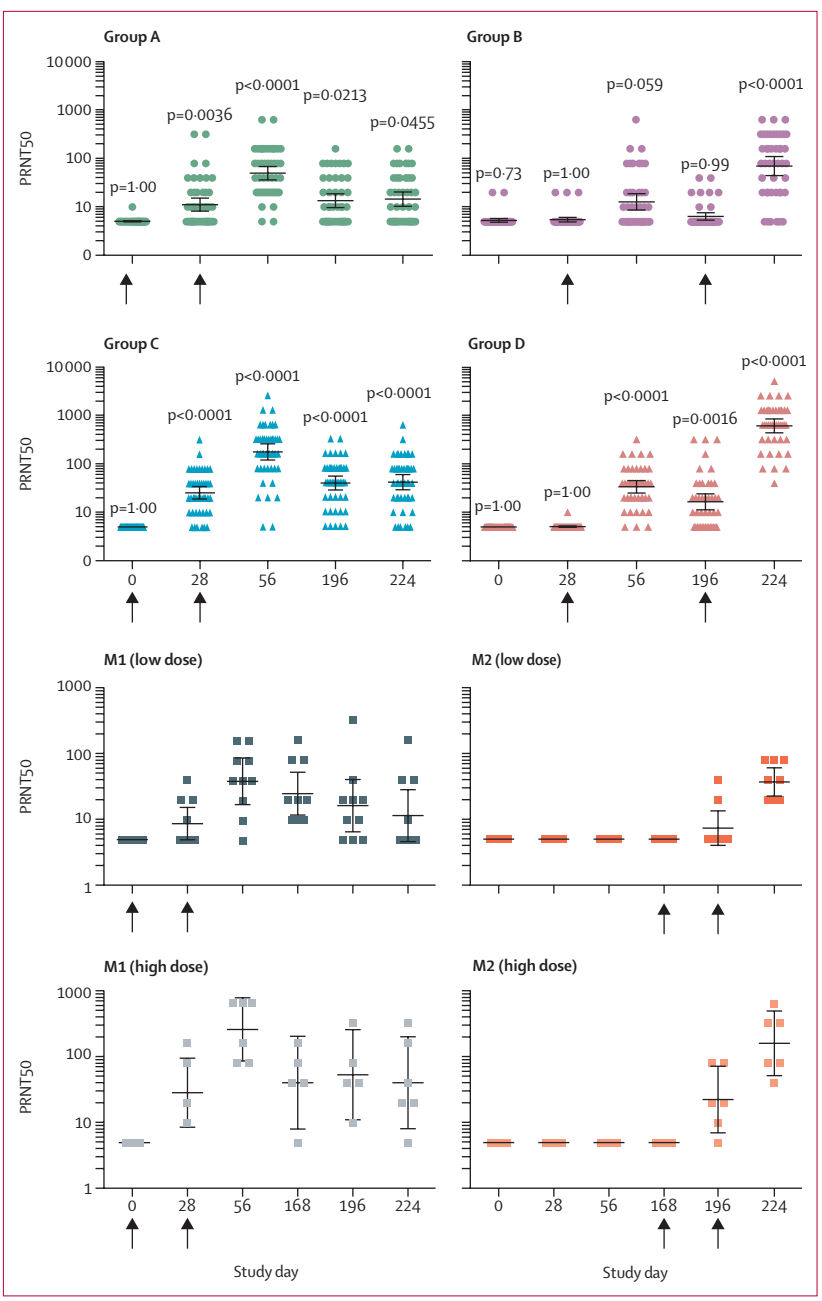

Figure 2: **PRNT50 values over time**

PRNT50 values are shown for each participant. Black horizontal lines indicate geometric mean titres and error bars 95% CIs. Arrows indicate when MV-CHIK was administered. The low dose was 5×10^4 TCID50 and the high dose 5×10^5 TCID50. p values for groups A to D are for comparisons with the control groups (control group 1 for groups A and C and control group 2 for groups B and D) at the same timepoint. PRNT50=serum dilution required to reduce viral plaques by 50% of the control value. M1=measles prime group 1. M2=measles prime group 2. TCID50=tissue culture infectious dose at which 50% of cells are lysed.

collected the immunogenicity data, and, together with the corresponding author, interpreted the data and prepared the manuscript. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Between Aug 17, 2016, and May 31, 2017, 322 healthy volunteers were screened for eligibility, of whom 263 were randomly assigned to receive control vaccine (n=34), MV-CHIK (n=195), or measles prime (n=34; figure 1; appendix). Altogether, 229 participants received at least one injection with MV-CHIK and 34 received control vaccine only; these individuals comprised the safety population. After exclusion of 16 participants because of major protocol deviations, 247 were included in the per-protocol analysis (figure 1). Baseline characteristics for the safety population are shown in table 1.

On day 56 (after one immunisation in groups B and D and two in groups A and C), concentrations of neutralising antibodies against chikungunya virus were detected in all four treatment groups, although the difference compared with the control groups was significant for only groups A, C, and D (figure 2; appendix). Geometric mean titres on day 56 were 50·16 (95% CI 36·39–69·15; p<0·0001 *vs* control group 1) in group A, 12·87 (8·75–18·93; p=0·059 *vs* control group 2) in group B, 174·80 (119·10–256·50; p<0·0001 *vs* control group 1) in group C, and 33·64 (24·96–45·33; p<0·0001 *vs* control group 2) in group D.

Compared with control group 1, geometric mean titres of neutralising antibodies against chikungunya virus were significantly elevated in group A (11·20, 95% CI 8·21–15·27; p=0·0036) and group C (25·70, 19·13–34·53; p<0·0001) 28 days after a single immunisation with MV-CHIK (figure 2). Concentrations of neutralising antibodies were significantly increased in group B (70·53, 44·74–111·20; p<0·0001) and group D (609·80 (438·20–848·60; p<0·0001) compared with control group 2 on day 224 (28 days after the second immunisation; figure 2). Priming with measles vaccine did not affect this outcome: titres of neutralising antibodies were similarly elevated in the measles prime groups 28 days after the second immunisation with MV-CHIK (figure 2). Geometric mean titres on day 56 were 40·00 (95% CI 17·80–89·90; p=0·49 *vs* group A at the same timepoint) for participants in measles prime group 1 who received low-dose MV-CHIK and 254·00 (84·95–759·30; p=0·84 *vs* group C at the same timepoint) for participants in measles prime group 1 who received high-dose MV-CHIK. Geometric mean titres on day 224 were 37·03 (22·59–60·72; p=0·27 *vs* group A value on day 56) for participants in measles prime group 2 who received low-dose MV-CHIK and 160·00 (51·85–493·80; p=0·63 *vs* group C value on day 56) for participants in measles prime group 2 who received high-dose MV-CHIK.

Overall, the 5×10^5 dose (groups C and D) induced significantly higher concentrations of neutralising antibodies than did the 5×10^4 dose (groups A and B) at all timepoints after vaccination (appendix). The rate of seroconversion at 28 days after the second immunisation was 95·9% (47/49) in group A, 86·4% (38/44) in group B, 95·7% (45/47) in group C, and 100% (43/43) in group D (table 2). The results of the ELISA quantification of chikungunya-specific IgG antibodies were similar to those of the PRNT50 assays, with titres significantly increased 28 days after the second immunisation in all treatment groups compared with the appropriate control groups (appendix).

	Group A	Group B	Group C	Group D	M1	M2	Control group 1	Control group 2
Day 0	2·0%	4·5%	0%	0%	0%	0%	0%	0%
Day 28	51·0%	6·8%	87·2%	2·3%	62·5%	0%	0%	0%
Day 56	95·9%	50·0%	95·7%	93·2%	93·8%	0%	0%	0%
Day 168*	..	..	..	..	93·3%	0%	..	..
Day 196	56·5%	18·2%	89·4%	65·1%	80·0%	46·7%	0%	0%
Day 224	56·5%	86·4%	87·2%	100%	56·3%	100%	0%	0%

Percentages indicate proportions of participants with PRNT50 values of 10 or greater. PRNT50=serum dilution required to reduce viral plaques by 50% of the control value. M1=measles prime group 1. M2=measles prime group 2. *Immunogenicity on day 168 was measured only for the measles prime groups.

Table 2: **Seroconversion rate as defined by PRNT50**

	Group A	Group B	Group C	Group D	M1		M2		Control group 1	Control group 2
					Low dose	High dose	Low dose	High dose		
Day –28	..	..	..	..	772·2 (358·3–1665·0)	301·4 (153·8–590·8)	463·5 (212·4–1011·0)	162·2 (89·2–294·9)	..	..
Day 0	456·2 (322·6–645·1)	398·1 (274·3–577·9)	495·0 (353·9–692·4)	401·8 (281·0–574·5)	931·3 (454·5–1908·0)	591·7 (349·9–1001·0)	881·4 (459·9–1689·0)	405·2 (273·6–600·1)	693·9 (370·3–1300·0)	390·4 (192·2–792·9)
Day 28	1509·0 (1203·0–1894·0)	396·9 (279·0–564·7)	2344·0 (1971·0–2787·0)	492·1 (341·5–709·1)	1188·0 (612·9–2301·0)	3398·0 (2222·0–5196·0)	635·3 (429·5–939·5)	465·9 (310·5–698·9)	1201·0 (801·0–1800·0)	447·5 (225·1–889·6)
Day 56	1652·0 (1305·0–2092·0)	1255·0 (990·1–1591·0)	2750·0 (2356·0–3211·0)	2435·0 (1952·0–3038·0)	1236·0 (656·5–2326·0)	3497·0 (2739·0–4464·0)	642·7 (426·8–967·8)	381·1 (234·6–619·0)	1129·0 (764·1–1669·0)	673·8 (446·7–1016·0)

Data in parentheses are 95% CIs. M1=measles prime group 1. M2=measles prime group 2.

Table 3: **Geometric mean titres of anti-measles IgG antibodies, as determined by ELISA**

Concentrations of anti-measles virus antibodies were significantly increased on day 56 compared with baseline in all treatment groups (p<0·0001 for groups A to D *vs* day 0; table 3). High-dose MV-CHIK significantly increased titres of measles-specific antibodies in measles prime group 1 on day 56 (table 3; appendix). By contrast, low-dose MV-CHIK did not boost anti-measles antibody titres in this group between day 0 and day 56 (appendix).

Concentrations of neutralising antibodies against chikungunya virus 28 days after the first immunisation were not affected by serum concentrations of anti-measles antibodies on day 0. Geometric mean titres were 20·00 (95% CI 14·29–27·99) for the lowest quartile compared with 17·46 (11·89–25·66) for the highest quartile (p=0·93; figure 3).

The safety and tolerability profile of MV-CHIK was good, and there were no differences in frequency of solicited adverse events between recipients of MV-CHIK (n=168) and participants in the control groups (n=24; table 4). The most common systemic solicited adverse events were headache and fatigue (table 4). The most frequent local reactions were injection site tenderness, injection site pain, and injection site induration; injection site tenderness and induration were the only adverse events (solicited and unsolicited) to affect a significantly higher proportion of participants in the treatment groups than in the control groups. The majority of solicited adverse events were reported as either mild or moderate; however, ten participants, all in the MV-CHIK treatment groups, reported at least one severe adverse event: one reported injection site pain, one reported injection site induration, four experienced fatigue, two reported headache, two reported flu-like symptoms, and one experienced nausea and vomiting. The frequency of severe adverse events was not significantly different between the MV-CHIK treatment groups and the control groups (p=0·37). Only two participants received remedial medication for severe adverse events, and all severe adverse events were resolved within 1 week.

All unsolicited adverse event data will be made available on EudraCT. The most frequent unsolicited adverse events are listed in table 5. 116 (51%) of 229 recipients of MV-CHIK had unsolicited adverse events compared with 17 (50%) of 34 participants in the control groups (p=1·00). Ten severe unsolicited adverse events occurred in eight participants (one [3%] recipient of control vaccine and seven [3%] recipients of MV-CHIK; p=1·00; appendix).

Given that chikungunya virus infections are associated with joint pathologies,[7] we considered adverse events related to arthritis as being of special interest. Seven adverse events related to arthritis, all unsolicited, were reported for individuals assigned to MV-CHIK, two of which were deemed to be possibly related to study treatment. One individual in measles prime group 1 reported arthralgia after the prime dose and withdrew from the study. Another participant reported arthritis in both feet 2 months after receiving the first MV-CHIK vaccination and did not receive further vaccination. Overall, the occurrence of adverse events of special interest was not significantly different between the MV-CHIK treatment groups and the control groups (seven [3%] of 229 *vs* none; p=0·60).

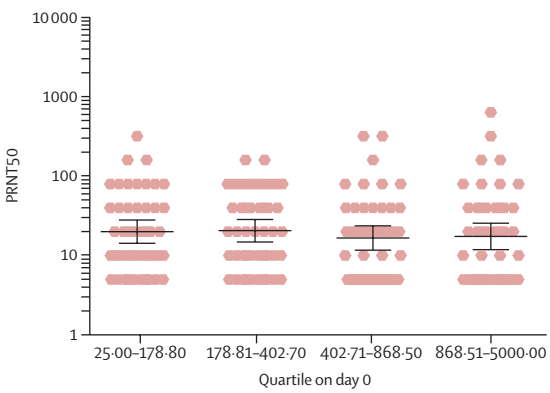

Figure 3: **PRNT50 values by quartile of anti-measles antibody titres at baseline**
PRNT50 values are shown for each participant on day 28 after the first administration of MV-CHIK. Black horizontal lines indicate geometric mean titres and error bars 95% CIs. PRNT50=serum dilution required to reduce viral plaques by 50% of the control value.

	Control (n=34)	MV-CHIK (n=229)	p value
Any solicited adverse event	24 (71%)	168 (73%)	0·84
Fatigue	8 (24%)	52 (23%)	1·00
Mild	4 (12%)	32 (14%)	..
Moderate	4 (12%)	16 (7%)	..
Severe	0	4 (2%)	..
Rash	2 (6%)	3 (1%)	0·13
Mild	1 (3%)	2 (1%)	..
Moderate	1 (3%)	1 (<1%)	..
Vomiting	1 (3%)	2 (1%)	0·34
Mild	0	1 (<1%)	..
Moderate	1 (3%)	0	..
Severe	0	1 (<1%)	..
Nausea	6 (18%)	19 (8%)	0·11
Mild	4 (12%)	15 (7%)	..
Moderate	2 (6%)	3 (1%)	..
Severe	0	1 (<1%)	..
Flu-like symptoms	4 (12%)	41 (18%)	0·47
Mild	4 (12%)	27 (12%)	..
Moderate	0	12 (5%)	..
Severe	0	2 (1%)	..
Arthralgia	5 (15%)	23 (10%)	0·38
Mild	2 (6%)	16 (7%)	..
Moderate	3 (9%)	7 (3%)	..
Limb discomfort	5 (15%)	27 (12%)	0·58
Mild	5 (15%)	21 (9%)	..
Moderate	0	6 (3%)	..

(Table 4 continues on next page)

	Control (n=34)	MV-CHIK (n=229)	p value
(Continued from previous page)			
Myalgia	6 (18%)	34 (15%)	0·62
Mild	4 (12%)	29 (13%)	..
Moderate	2 (6%)	5 (2%)	..
Headache	16 (47%)	76 (33%)	0·13
Mild	9 (26%)	42 (18%)	..
Moderate	7 (21%)	32 (14%)	..
Severe	0	2 (1%)	..
Fever	1 (3%)	4 (2%)	0·50
Mild	0	2 (1%)	..
Moderate	1 (3%)	2 (1%)	..
Injection site induration	0	37 (16%)	0·0067
Mild	0	31 (14%)	..
Moderate	0	5 (2%)	..
Severe	0	1 (<1%)	..
Injection site oedema	0	15 (7%)	0·23
Mild	0	13 (6%)	..
Moderate	0	2 (1%)	..
Injection site erythema	4 (12%)	34 (15%)	0·80
Mild	4 (12%)	33 (14%)	..
Moderate	0	1 (<1%)	..
Injection site pruritus	0	8 (3%)	0·60
Mild	0	7 (3%)	..
Moderate	0	1 (<1%)	..
Injection site tenderness	7 (21%)	120 (52%)	0·0007
Mild	7 (21%)	98 (43%)	..
Moderate	0	19 (8%)	..
Severe	0	3 (1%)	..
Injection site pain	7 (21%)	75 (33%)	0·17
Mild	7 (21%)	61 (27%)	..
Moderate	0	13 (6%)	..
Severe	0	1 (<1%)	..

Table 4: **Solicited adverse events**

	Control (n=34)	MV-CHIK (n=229)	p value
Nasopharyngitis	0	22 (10%)	0·76
Mild	0	10 (4%)	..
Moderate	0	12 (5%)	..
Headache	3 (9%)	17 (7%)	0·73
Mild	1 (3%)	9 (4%)	..
Moderate	2 (6%)	8 (3%)	..
Dysmenorrhoea	0	8 (3%)	0·60
Mild	0	1 (<1%)	..
Moderate	0	7 (3%)	..
Oropharyngeal pain	2 (6%)	10 (4%)	0·66
Mild	2 (6%)	7 (3%)	..
Moderate	0	3 (1%)	..
Rhinitis*	1 (3%)	11 (5%)	1·00

Data are n (%). *All cases of rhinitis were mild.

Table 5: **Five most frequent unsolicited adverse events**

Six participants (four recipients of MV-CHIK and two recipients of control vaccine) had serious adverse events, of which all were deemed to be unrelated to study medication. In the MV-CHIK groups, one participant had an umbilical hernia, one ruptured the cruciate ligament of the left knee, and one was diagnosed with papillary thyroid cancer. Additionally, a woman aged 29 years had a miscarriage; she became pregnant despite use of oral contraception and was removed from the study after receiving a single vaccination. Upon unmasking of the data, we found that she had received placebo, indicating that the miscarriage was unrelated to the vaccine. In the control groups, one participant was diagnosed with laryngeal cancer and one was diagnosed with type 2 diabetes.

Shedding of MV-CHIK was assessed in 33 participants, including seven in group A, four in group B, 11 in group C, four in group D, three in control group 1, and two in control group 2. MV-CHIK RNA was not detected in any of the samples analysed (data not shown).

Discussion

Chikungunya virus presents a substantial threat to public health, with no specific treatment or preventive measures available. We found that the MV-CHIK vaccine had a safety and tolerability profile that was similar to the measles control vaccine. Although the proportions of participants who had injection site tenderness or induration were higher in MV-CHIK recipients than in the control groups, the comparison of overall adverse events did not reveal significant differences. We also showed that a single MV-CHIK immunisation induced neutralising antibodies in 50–93% of participants and that a second vaccination induced high titres, with seroconversion rates of 86–100% in all MV-CHIK treatment groups after a vaccine boost at 1 month or 6 months. The higher dose of MV-CHIK significantly increased concentrations of neutralising antibodies against chikungunya virus compared with the lower dose. Moreover, the vaccine boost at 6 months appeared to increase neutralising antibody titres to a greater extent than did the boost at 1 month. These results suggest a that a prime–boost immunisation approach should be considered for further development.

MV-CHIK was previously found to be immunogenic and able to protect against lethal challenge in both mice[17] and non-human primates (Themis, unpublished). Moreover, MV-CHIK was safe and well tolerated and led to 100% seroconversion after two immunisations in a phase 1 trial in 36 healthy adults.[18] By 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.[19] In this study, 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. The

MV-CHIK vaccine is based on a measles backbone vector that lacks chikungunya virus non-structural genes, reducing the likelihood of side-effects related to the virus.

Protection against chikungunya virus has been associated with the induction of neutralising antibodies, primarily directed against structural proteins.[16,20] This observation was substantiated by findings in mice through passive transfer of pre-immune sera.[17,21] Epidemiological studies in the Philippines[22] and Cambodia[23,24] found that positivity for neutralising antibodies against chikungunya virus at baseline, as established with plaque reduction neutralisation tests, was associated with 100% (95% CI 46–100) protection against symptomatic infection. Additionally, those studies found that broad cross-neutralisation among lineages of chikungunya virus exists, and that it is highly probable that the induction of a neutralising antibody response will provide long-lasting (if not lifelong) immunity across all genotypes of the virus. Future studies will need to establish an immune correlate of protection and a protective threshold to assess the feasibility of using single-dose MV-CHIK to provide short-term protection for travellers or for outbreak intervention. A flexible booster dose (between 1 month and 12 months) could then potentially provide a durable immune response. Although the data from this study are encouraging, development of target-specific product profiles that address the needs of different at-risk populations (ie, travellers, children, people in endemic areas) is needed.

A role for cell-mediated responses in protection against chikungunya virus infection seems less likely than a role for humoral immunity. Naturally occurring infections induce T-cell responses, but genetic experiments in mice suggest that CD8 T cells might not have a part in the defence against chikungunya virus, and CD4 T-cell responses might even promote the development of joint pathologies.[25] PBMCs were collected from a subset of vaccinated participants in this study, but these analyses are ongoing and the results will be presented in a future manuscript.

A large part of the population in Europe is vaccinated against measles.[26] A 2018 report from the Austrian Federal Ministry of Labour, Social Affairs, Health and Consumer Protection states that more than 95% of children aged 6 years have received at least one dose of a measles vaccine,[27] and more than 85% of children in all monitored countries receive at least one vaccination against measles.[28] Therefore, it is important to assess the effect of pre-existing immunity for measles-vectored vaccines on the immunogenicity of a new vaccine. In this study, we addressed this matter in two ways. First, we quantified antibodies against measles virus on day 0 of the study, allowing the concentrations of neutralising antibodies to MV-CHIK 28 days after the first vaccination in the quartile with the lowest baseline concentrations of measles-specific antibodies to be compared with those in the quartile with the highest baseline concentrations. Second, we assessed the immunogenicity of MV-CHIK in participants who received a measles prime shortly before administration of the candidate vaccine. Both experimental approaches suggested that pre-existing immunity against measles does not affect the immunogenicity of MV-CHIK. These results are in line with data gathered during the phase 1 trial MV-CHIK-101.[18]

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.

This study has some limitations. Although we showed that responses to the vaccine were durable up to 6 months after one or two immunisations, future studies will need to assess durability in the longer term. Furthermore, we did not assess the ability of MV-CHIK to induce neutralising antibodies against all genetic lineages of chikungunya virus, although this will be analysed in upcoming serological studies. We also did not investigate potential cross-reactivity against related alphaviruses, which will need to be investigated in future studies. Finally, the vaccine safety and immunogenicity was shown in a homogeneous European population. Studies in endemic areas and in the USA are ongoing (NCT03028441 and NCT03101111).

In conclusion, we showed that the MV-CHIK candidate vaccine is safe, well tolerated, and highly immunogenic in healthy volunteers aged 18–55 years. On the basis of these data, we are preparing a phase 3 study of MV-CHIK. Our findings suggest that the 5×10^5 TCID50 dose induces protective titres of functional antibodies in the majority of recipients after a single immunisation, and that a prime–boost vaccination schedule is even more effective.

Contributors

ECR, AP, MM, ET, and KR contributed to the design of the trial. ECR, EB, UW, CF, and ML collected data. ECR, RT, and KR performed the literature search and wrote the manuscript. RT constructed the figures and tables. All authors contributed to data interpretation, critically reviewed the manuscript, and approved the final version of the manuscript for publication.

Declaration of interests

AP, MM, RT, ET, and KR are employees of Themis. ET is managing director and shareholder of Themis. ECR, UW, and CF were principal investigators at the trial sites and their respective organisations received trial fees. The institutions of EB and ML received site fees from Themis for their contributions to this study. ET and KR have patents pending related to this work.

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