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Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial

Katrin Ramsauer, Michael Schwameis*, Christa Firbas, Matthias Müllner, Robert J Putnak, Stephen J Thomas, Philippe Desprès, Erich Tauber, Bernd Jilma, Frederic Tangy*

Summary

Background Chikungunya is an emerging arthropod-borne disease that has spread from tropical endemic areas to more temperate climates of the USA and Europe. However, no specific treatment or preventive measure is yet available. We aimed to investigate the immunogenicity and safety of a live recombinant measles-virus-based chikungunya vaccine.

Methods We did a randomised, double-blind, placebo-controlled, active-comparator, phase 1, dose-escalation study at one centre in Vienna, Austria. Healthy men and women aged 18–45 years with no comorbidities were randomly assigned, by computer-generated block randomisation (block size of 14), to receive either one of three escalating doses of the measles-virus-based candidate vaccine (low dose [$1·5×10^4$ median tissue culture infection doses ($TCID_{50}$) per $0·05$ mL], medium dose [$7·5×10^4$ $TCID_{50}$ per $0·25$ mL], or high dose [$3·0×10^5$ $TCID_{50}$ per $1·0$ mL]), or the active comparator—Priorix. Participants were additionally block-randomised to receive a booster injection on either day 28 or day 90 after the first vaccination. Participants and study investigators were masked to group allocation. The primary endpoint was the presence of neutralising anti-chikungunya antibodies on day 28, as assessed by 50% plaque reduction neutralisation test. Analysis was by intention to treat and per protocol. This trial is registered with EudraCT, number 2013-001084-23.

Findings Between Nov 22, 2013, and Feb 25, 2014, we randomly assigned 42 participants to receive the low dose (n=12), the medium dose (n=12), or the high dose (n=12) of the measles-virus-based candidate vaccine, or Priorix (n=6), of whom 36 participants (86%; n=9, n=12, n=10, n=5, respectively) were included in the per-protocol population. The candidate vaccine raised neutralising antibodies in all dose cohorts after one immunisation, with seroconversion rates of 44% (n=4) in the low-dose group, 92% (n=11) in the medium-dose group, and 90% (n=10) in the high-dose group. The immunogenicity of the candidate vaccine was not affected by pre-existing anti-measles immunity. The second vaccination resulted in a 100% seroconversion for all participants in the candidate vaccine groups. The candidate vaccine had an overall good safety profile, and the rate of adverse events increased with vaccine dose and volume. No vaccination-related serious adverse events were recorded.

Interpretation The live recombinant measles-virus-based chikungunya vaccine had good immunogenicity, even in the presence of anti-vector immunity, was safe, and had a generally acceptable tolerability profile. This vaccine is the first promising measles-virus-based candidate vaccine for use in human beings.

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Introduction

Once restricted to tropical areas with endemic self-limiting outbreaks, chikungunya virus is becoming a global threat with continuous geographical expansion from African and Asian countries to more temperate climates of the industrialised countries.[1–3] Increased travelling and global warming drive the transmission of vector-borne diseases by aiding the establishment and distribution of virus-carrying arthropods. Both *Aedes aegypti* and *Aedes albopictus*—the main vectors of chikungunya virus in urban areas—are already established in the USA and Europe, emphasising the menace of autochthonous chikungunya emergence in these parts of the world.[4,5]

Symptomatic patients with chikungunya virus frequently have high fever and debilitating arthritis, which can persist for years causing substantial long-term morbidity and loss in quality of life.[6–9] Additionally, pathological changes in the joints in a subset (up to 30%) of patients is a major driver for vaccine development. Such symptomatic patients need advanced and prolonged immunotherapy because of joint pain and associated radiographical and virological features of disease, which has a major effect on health-care costs in the affected countries. Although rare, severe complications include respiratory and cardiovascular failure, meningo-encephalitis, hepatitis, renal impairment, and Guillain-

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Barré syndrome. Patients older than 65 years and children younger than 1 year are a high-risk population.[10,11]

During an epidemic, infected people are potentially the amplifying hosts of chikungunya virus through a cycle of human–mosquito transmission.[12] Thus, the size of an epidemic case load is dependent on established human herd immunity,[12] which also protects individuals unsuitable for vaccination, such as pregnant women and immunocompromised patients. Ongoing epidemic outbreaks emphasise the need for an effective vaccine;[13] however, no suitable drugs or preventive measure for chikungunya virus-related disease are yet available. In the past 15 years only two vaccine candidates have been investigated in human trials.[14,15] The first vaccine was assessed in several phase 1 clinical trials, in which it showed an acceptable safety profile with only a mild, very transient, arthralgia in some participants. Later sequence analysis showed two attenuating point mutations.[16] The other candidate has been assessed in a small phase 1 trial[15] to test the effect of high vaccine doses given without an adjuvant.

The measles-virus-based chikungunya candidate vaccine is a live attenuated recombinant viral vectored vaccine based on the Schwarz strain of measles vaccine, which was originally introduced by the Pasteur Institute in Paris.[17] Recombinant measles-virus vectors provide strong and protective immunity against various arboviruses[18–20] and has been shown to be immunogenic against HIV,[21,22] independent of anti-measles immunity. The immunogenicity and protective efficacy of the measles-virus-based chikungunya vaccine was shown in a measles virus-susceptible mouse model, showing that neutralising antibodies induced by the vaccine confer complete protection against a lethal challenge with chikungunya virus.[23]

We investigated the immunogenicity, safety, and tolerability of the recombinant measles virus-based chikungunya candidate vaccine for prevention of chikungunya virus in the presence of pre-existing anti-vector immunity in healthy adults.

Methods
Study design and participants
We did a randomised, double-blind, placebo-controlled, active-comparator, phase 1, dose-escalation study at the Department of Clinical Pharmacology at the Medical University of Vienna, Austria. Healthy men and women aged between 18 and 45 years with no comorbidities were eligible for inclusion. We excluded participants with a recent infection (within 1 week before their first treatment at visit one) and those vaccinated within 12 weeks before the screening visit. Other exclusion criteria were pregnancy, lactation, history of immunodeficiency or immunosuppressive therapy, known HIV infection, hepatitis B or C infection, and ascertained or presumed hypersensitivity to the active principle or the formulations' ingredients. Any concomitant drug or vaccination was

documented at each visit. The appendix provides a complete list of inclusion and exclusion criteria.

The trial was approved by the Ethics Committee of the Medical University of Vienna (European Research Council number 1655/2013). We designed our study in accordance with the Note For Guidance On Clinical Evaluation Of New Vaccines, and did the study in compliance with the principles of the Declaration of Helsinki for biomedical research involving human beings. Testing, data collection, and analysis at the Walter Reed Army Institute of Research (WRAIR; Silver Springs, MD, USA) was done on de-identified clinical specimens in accordance with WRAIR protocol 2077, with approval from the WRAIR Human Subjects Protection Branch. All participants provided written informed consent.

Randomisation and masking
Participants were randomly assigned, by computer-generated block randomisation (block size of 14), to receive one of three doses of the candidate vaccine, or to receive the active comparator—Priorix. Randomisation was done at the study site, by either the pharmacist or a non-observing investigator, with consecutively numbered randomisation envelopes containing information about the cohort and the volume of vaccine, Priorix, or placebo. The first participant deemed eligible was randomly assigned with the first randomisation envelope, the next with the next envelope, and so forth. Participants were additionally block-randomised to receive a booster injection on either day 28 or day 90 after the first vaccination. Participants and study investigators were masked to group allocation; use of placebo enabled the maintenance of masking.

Procedures
Participants received three escalating doses of the candidate vaccine: low dose ($1 \cdot 5 \times 10^4$ median tissue culture infection doses [$TCID_{50}$] per 0·05 mL), medium dose ($7 \cdot 5 \times 10^4$ $TCID_{50}$ per 0·25 mL), or high dose ($3 \cdot 0 \times 10^5$ $TCID_{50}$ per 1·0 mL). We selected the dosage categories on the basis of preclinical safety studies. The lowest dose was aimed to be at least ten-times higher than the measles vaccine dose in the Priorix group.

We introduced the chikungunya virus (La Reunion strain 06-46) subgenomic open reading frame encoding for structural genes C, E3, E2, 6K, and E1 into the measles virus vector as described elsewhere.[23] The heterologous genes are expressed as the vaccine antigens upon immunisation. The recombinant virus was grown on Vero cells and we determined viral titres on the basis of endpoint dilution assay of these cells. The recombinant virus was presented in a liquid formulation containing ammonium sulfate and HEPES buffer, stored at –80°C (plus or minus 10°C) in a locked freezer. Before vaccination, the ready-to-use vials were placed at room temperature for 30 min in a light-protective cardboard box and had to be given within a further 30 min (ie,

within 60 min after being taken out of the freezer). Expression of the entire open reading frame of the chikungunya virus structural genes led to production of virus-like particles in cells exposed to the vaccine, as characterised by electron microscopy.[23] We validated the presence of chikungunya virus antigens (virus-like particles or proteins) in the final vaccine formulation by immunoblot assay. Such viral compounds were present in the final formulation, but their amounts were not determined. Further quantification will be done according to regulatory requirements.

Priorix (GlaxoSmithKline Pharma GmbH, Vienna, Austria) is a live virus vaccine for immunisation against measles, mumps, and rubella containing the attenuated Schwarz strain of measles virus ($\geq 10^3$ median cell culture infectious dose [$CCID_{50}$] per 0·5 mL), the RIT 4385 strain of mumps virus (derived from the 15 Jeryl Lynn strain; $\geq 10^{3.7}$ $CCID_{50}$ per 0·5 mL), and the Wistar RA 27/3 rubella virus strain ($\geq 10^3$ $CCID_{50}$ per 0·5 mL). The Schwarz strain of measles virus is homologous to the backbone of the candidate vaccine. Each virus strain is separately obtained by propagation in either chick embryo tissue cultures (mumps and measles) or MRC-5 human diploid cells (rubella). Priorix is provided as a white to slightly pink pellet for reconstitution with sterile water containing lactose, neomycin sulfate, and aminoacid, in addition to sorbitol and mannitol as stabilisers.

Placebo injections of sterile saline allowed for assessment of absolute differences in signs and symptoms induced by the candidate vaccine and Priorix. Placebo was given on day 28 or on day 90 depending on the treatment schedule. Participants of each treatment cohort were vaccinated on days 0, 28, and 90; those who were vaccinated on days 0 and 28 received placebo on day 90; and remaining participants were vaccinated on days 0 and 90 and received placebo on day 28. Blood samples for immunogenicity assays and safety analysis were collected at baseline, before each vaccination (days 0, 28 or 90), on day 56, and 30 days after the last vaccination (day 120).

The presence of serum neutralising antibodies was determined with plaque reduction neutralisation tests (PRNT) on Vero-cell monolayers (Vero ATCC CCL-81) in six-well plates, shown by a reduction in the numbers of input virus plaques by at least 50% ($PRNT_{50}$). Appropriate dilutions of sera (serial four-fold dilutions from 1:10 to 1:640) were incubated with 20–100 plaque-forming units of the chikungunya strain 181/clone 25[24] for 30 min at 35°C (plus or minus 2°C). Vero-cell monolayers were inoculated in triplicate wells with the virus–serum mixture, incubated at 35°C to allow for cell attachment, then overlayered with nutrient agarose and incubated for an additional 36–48 h at 35°C (plus or minus 2°C) for plaque development. We determined $PRNT_{50}$ endpoint titres by linear regression (probit) analysis[25] done with the IBM SPSS statistical software package (version 20).

We deemed serum samples with reciprocal $PRNT_{50}$ titres of 10 or greater as positive for chikungunya virus neutralising antibodies. We used four-fold serum dilutions, which can lead to greater titre variations than do two-fold dilutions, for the chikungunya PRNT to increase the dynamic range of the assay, because initially the anti-chikungunya antibody titres were unknown.

We did haemagglutination inhibition assays according to the method of Clarke and Casals[26] with a South African strain of chikungunya virus at a concentration of 4 haemagglutinating units. We tested serum samples at serial two-fold dilutions (1:20 starting dilution). We regarded titres of 20 or greater as positive. To investigate the effect of pre-existing anti-measles immunity on the immunogenicity of the recombinant candidate vaccine, we determined measles titres of all participants assigned to measles-virus-based candidate vaccine with ELISA at all visits on days –14, 0, 14, 28, 56, 90, and 120.

Because this was a first-in-man trial, no more than two participants per day were allowed to receive the first vaccination. Participants were not allowed to receive the vaccination simultaneously, but had to be vaccinated in a 1 h interval. After vaccination, each participant was medically observed for local or systemic side-effects over a period of 1 h. Participants were only discharged home if both the study-related physician and the participant considered a discharge as safe. Participants were meticulously instructed about how to proceed in case of any emergency after discharge. Furthermore, a card-size emergency pass was dispensed including the most relevant information about study participation, the investigational drug, the study site and all names of responsible physicians, and the telephone number of a study-related on-call physician. The principal investigator did a safety analysis after all participants in the low-dose group had been vaccinated once. Medium-dose vaccinations were only initiated pending a written positive safety assessment by the principal investigator and subsequent approval. The same procedure was applied for the transition from the medium-dose to the high-dose group.

Safety analysis
Vital-sign checks, including measurement of blood pressure, pulse rate, and axillary body temperature, in addition to physical examinations, were done at all visits. Additionally, the injection site was inspected and assessed for local side-effects at each visit and before and 6 h after each vaccination. Grading of local pain, tenderness, redness, swelling, itching, and induration was done according to the respective guidance for industry from the US Food and Drug Administration and the guidance of the Brighton Collaboration.[27,28] Accordingly, we graded local side-effects as mild (grade 1), moderate (grade 2), severe (grade 3), and potentially life-threatening (grade 4). All participants were provided with a template that enabled estimation of local reactions

(appendix). Pain, itching, and induration were graded as mild if they did not interfere with daily activity, as moderate if they did compromise activity or necessitated repeated use of non-narcotic pain relief or anti-inflammatory and pain-relieving ointment (itching and induration), and as severe if they prevented daily activity. Grade 4 pain or itching included the need for emergency room visit or hospital admission. We rated swelling and erythema of less than 5 cm in diameter as mild; that exceeding 10 cm in diameter was regarded as severe and necrosis was a sign of grade 4 infection (appendix).

Participants were asked to keep a study diary to record all local and systemic symptoms and any adverse event taking place in the first 2 weeks after vaccination, starting with the first entry 6 h (plus or minus 1 h) after vaccination. Participants themselves recorded the assessments once daily at the same time every day. A study-related physician verified the completeness and accuracy of self-reported symptoms and side-effects at each visit. Diaries were reviewed together with the particular participant at each visit and participants were interviewed for any adverse events. Diaries had to be signed by both the participant and the reviewing physician at the end of each vaccination period to confirm and ensure completeness and reliability of self-reporting. The diaries were then collected by the investigator at each visit and new ones were dispensed after vaccination.

Solicited and non-solicited adverse events were reported separately. Solicited events included pyrexia, influenza-like ill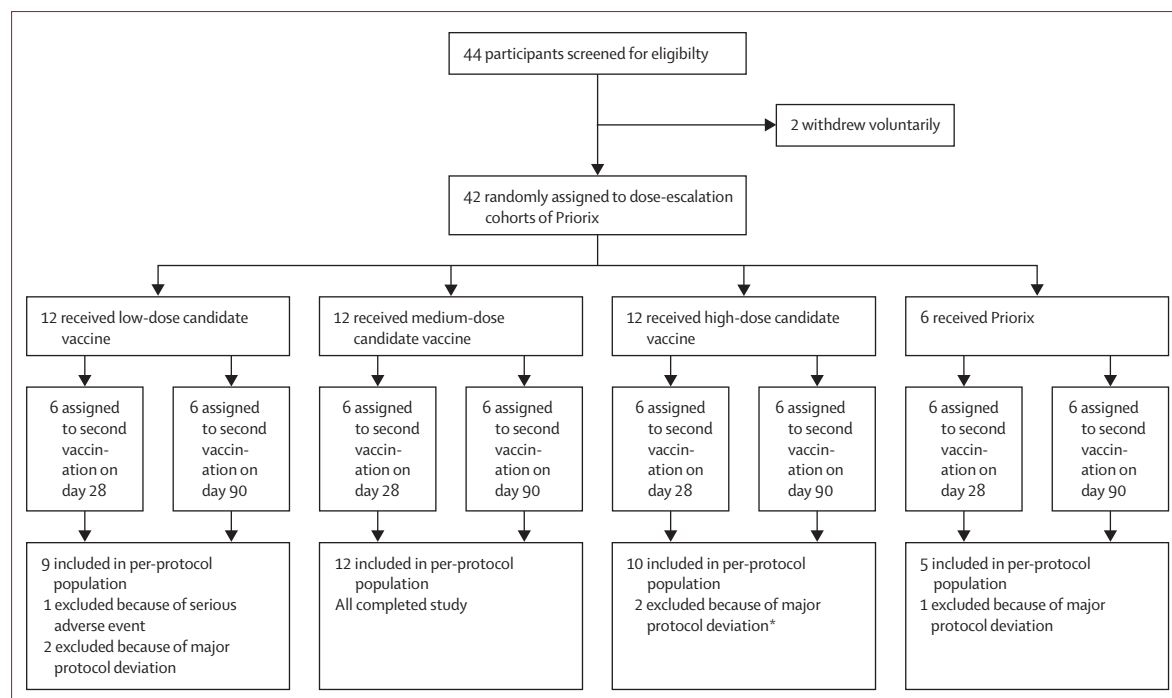ness, headache, and injection-site-related side-effects (erythema, swelling, induration, and pain). We used the term serious adverse event as suggested by the Directive 2001/20/EC of the European Parliament.[29] Safety monitoring was done at the study site by the clinical investigator. Complete blood count, chemistry, and coagulation studies, and urinalyses, were done at visits 0 (day 14), four (day 56), and six (day 120). The safety analysis was based on the safety population, which included all participants who received at least one vaccination.

Outcomes

The primary endpoint was immunogenicity on day 28 in the per-protocol population confirmed by the presence of functional antibodies (geometric mean titres >10) as determined by $PRNT_{50}$. The per-protocol population included only participants who fully adhered to the study protocol instructions and completed the study. Secondary endpoints were immunogenicity at days 0, 14, 28, 56, 90, and 120, assessed by $PRNT_{50}$ and haemagglutination inhibition tests; measurements of anti-measles antibodies on days –14, 0, 14, 28, 56, 90, and 120, assessed by ELISA; the rate of adverse events during the vaccination period until 4 months after the first vaccination; safety laboratory variables (haematology, serum chemistry, urinalysis); and systemic and local tolerability.

Statistical analysis

In the primary immunogenicity analysis, we compared $PRNT_{50}$ antibody geometric mean titres, haemag-

Figure 1: Trial profile
*Major protocol deviations were time-window deviations (n=3) and two voluntary withdrawals (for any reason) from further vaccinations.

glutination inhibition test antibody titres, and anti-measles antibody titers on day 28 between the treatment groups. We used vaccination doses (Priorix, and the low, medium, and high doses of the candidate vaccine) and vaccination schedules (boost on day 28 vs day 90) as fixed factors. Immunogenicity results are given for the per-protocol population; the appendix provides results of the intention-to-treat analysis.

We estimated geometric mean titres and ratios of these titres by applying ANOVA, with Tukey's range test for post-hoc multiple comparisons. For this method we used $\log_{10}$ transformed data and took the anti-log of the resulting point estimates for the least-squares means, differences in least-squares means, and the corresponding 95% CIs. Tukey's range test is a method for handling the issue of multiple statistical tests and applies the necessary adjustment to p values and confidence limits when differences in least-squares means are calculated between several treatment groups.

We report solicited and unsolicited adverse events as total numbers and percentages. Continuous variables are given as median (95% CI) or mean (SD). Sample sizes used in this study have previously proved sufficient for assessment of the immunogenicity of vaccines in early phase 1 trials[30] and provide a fairly good estimate of the variability;[31] we did no formal sample-size calculation for our study. All statistical tests were two-tailed. We did statistical analyses with SAS (version 9.3) and used STATA statistical software (release 12 and origin pro version 9.0) to construct graphs. This trial is registered with EudraCT, number 2013-001084-23.

Role of the funding source

The sponsor of the study sponsored the data management, statistical analysis, and made financial contributions to the study site to undertake the trial. Together with the corresponding author, the sponsor designed the study, collected the immunogenicity data, interpreted the data, and had a role in manuscript preparation. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.
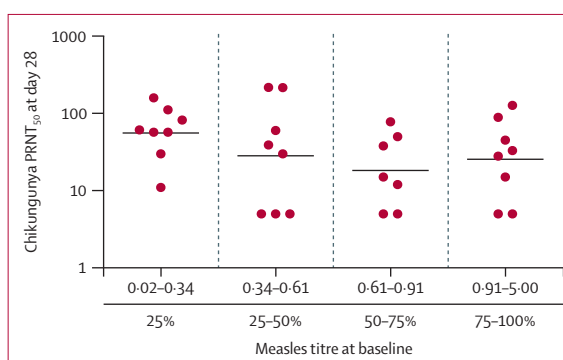
Results

Figure 1 shows the trial profile. Between Nov 22, 2013, and Feb 25, 2014, we randomly assigned 42 participants to receive the low, medium, or high dose of the candidate vaccine, or priorix, of whom 36 participants (86%) were included in the per-protocol population. The overall study duration per participant was 4 months. Baseline characteristics were similar between groups (table 1).

The candidate vaccine induced chikungunya virus neutralising antibodies in all dose cohorts after one immunisation. Immunogenicity of the candidate vaccine was not affected by pre-existing anti-measles immunity (figure 2). On day 28 after the first immunisation, the geometric mean titres induced in the medium-dose

	Low-dose group (n=12)	Medium-dose group (n=12)	High-dose group (n=12)	Priorix group (n=6)
Sex				
Male	4 (33%)	6 (50%)	6 (50%)	3 (50%)
Female	8 (67%)	6 (50%)	6 (50%)	3 (50%)
Ethnic origin				
White	12 (100%)	12 (100%)	12 (100%)	5 (83%)
Black	0	0	0	1 (17%)
Other	0	0	0	0
Age, years	34 (7·1)	30 (6·0)	28 (7·6)	30 (8·8)

Data are n (%) or mean (SD).

Table 1: **Baseline characteristics**

Figure 2: **Pre-existing anti-measles immunity**
Figure shows basal measles titre quartiles obtained at visit one from all participants in all dose groups against chikungunya neutralising titres on day 28 after the first vaccination. $PRNT_{50}$=50% plaque reduction neutralisation test.

(48, 95% CI 24–95) and high-dose (46, 22–99) groups were higher than those induced in the low-dose group (10, 5–19) or the priorix group (7, 4–14). The chikungunya geometric mean titre in the low-dose and medium-dose groups decreased over a period of 3 months; however, the titre in the high-dose group persisted over this period (figure 3). Geometric mean titres were significantly lower in participants in the low-dose group than in those in the medium-dose and high-dose groups (table 2). Importantly, however, the second immunisation yielded a seroconversion rate of 100% in all dose cohorts.

In general, the candidate vaccine had an acceptable tolerability profile. Table 3 shows solicited and unsolicited adverse events by treatment cohort. The most frequently recorded unsolicited adverse events were nasopharyngitis in 11 participants (26%) and oropharyngeal pain in five participants (12%). Solicited adverse events were headache in 24 participants (57%), injection-site pain in 21 participants (50%), and influenza-like illness in 19 participants (45%). Overall, transient musculoskeletal pain was reported in five (12%) of 42 participants in the safety population group. One participant (8%) each in the low-dose and the medium-dose group had mild limb pain associated with flu-like symptoms, as did three (25%)

	Boost on day 28				Boost on day 90			
	Low-dose group (n=4)	Medium-dose group (n=6)	High-dose group (n=5)	Priorix group (n=2)	Low-dose group (n=5)	Medium-dose group (n=6)	High-dose group (n=5)	Priorix group (n=3)
Day 0	5 (5–5)	5 (5–5)	5 (5–5)	8 (0–2017)	5 (5–5)	5 (5–5)	5 (5–5)	5 (5–5)
Day 14	16 (4–57)	31 (8–131)	34 (7–174)	10 (0–93 360)	6 (4–8)	32 (17–62)	31 (6–147)	5 (5–5)
Day 28	14 (2–86)	36 (10–136)	73 (31–172)	9 (0–21 940)	7 (4–14)	63 (25–158)	29 (6–140)	5 (5–5)
Day 56	73 (21–257)	150 (41–552)	433 (262–713)	23 (0–5)	15 (4–61)	21 (8–54)	23 (6–95)	14 (0–1105)
Day 90	27 (13–57)	91 (17–478)	123 (38–397)	10 (0–66 827)	5 (5–5)	8 (3–22)	15 (4–49)	5 (5–5)
Day 120	16 (4–75)	28 (9–90)	66 (22–203)	8 (0–2017)	63 (19–208)	130 (53–316)	416 (145–1192)	5 (5–5)

Data are geometric mean titre (95% CI). Assessed by 50% plaque reduction neutralisation test.

Table 2: Geometric mean antibody titres by treatment group in the per-protocol population

	Low-dose group (n=12)	Medium-dose group (n=12)	High-dose group (n=12)	Priorix group (n=6)
Solicited adverse events				
Mild to moderate	9 (75%)	12 (100%)	12 (100%)	4 (67%)
Severe	0	1 (8%)	3 (25%)	0
Serious	0	0	0	0
Related	6 (50%)	11 (92%)	12 (100%)	4 (67%)
Action taken*	3 (25%)	3 (25%)	10 (83%)	1 (17%)
Unsolicited adverse events				
Mild to moderate	10 (83%)	8 (67%)	8 (67%)	2 (33%)
Severe	1 (8%)	1 (8%)	0	0
Serious	1 (8%)	1 (8%)	0	0
Related	0	2 (17%)	3 (25%)	0
Action taken*	6 (50%)	5 (42%)	7 (58%)	1 (17%)

*Such as concomitant drug or hospital admission (in that case rated as a serious adverse event).

Table 3: Adverse events in the safety population (n=42)

participants in the high-dose group. Pain in the limbs associated with immunisation with the candidate vaccine was largely graded as mild (five [83%] of six participants) and did not engender any withdrawals from the study. The rate of musculoskeletal pain decreased from 12% (n=5) at the first visit to 2% (n=1) at visit five. No signs of inflammation were recorded.

Overall, we recorded seven severe adverse events in six participants, of which five events were solicited and related to vaccination, including headache, local erythema, local induration, local pain and pyrexia (table 3). Two serious adverse events, which were unsolicited and clearly unrelated to study treatment, were reported (table 3): one participant attempted suicide and was therefore removed early from the study, the
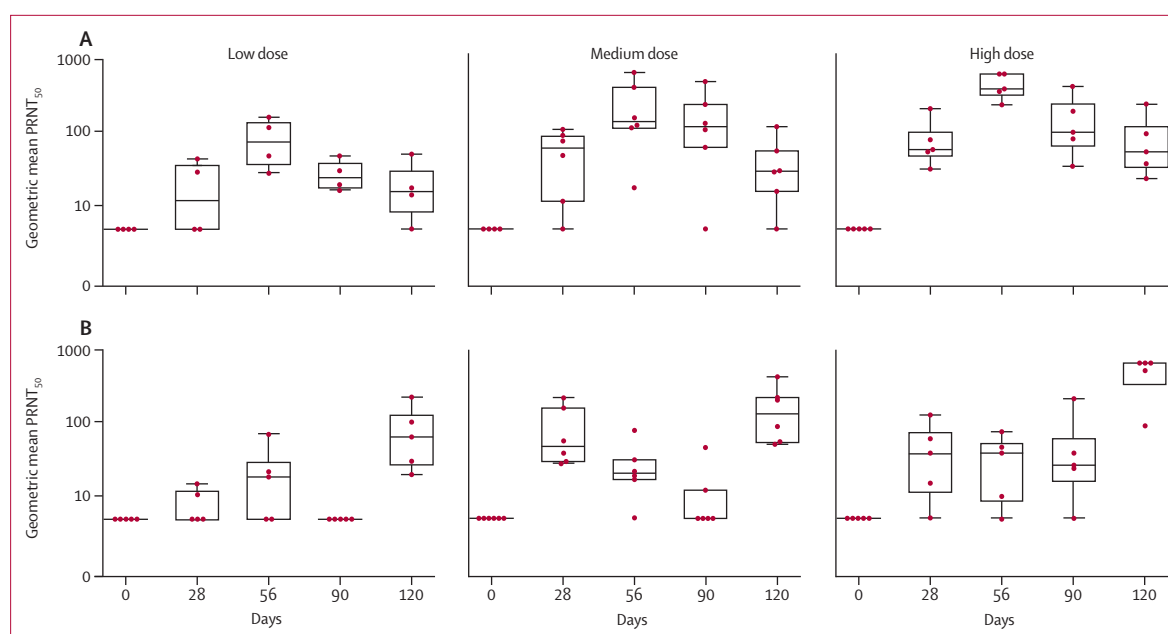
other participant had a meniscus injury. The number of overall and related adverse events increased with the dose and volume of the candidate vaccine (data not shown). We also noted this association for non-local, solicited, and unsolicited adverse events. Local reactions to immunisation with the candidate vaccine increased dose-dependently and were regarded as related to the high inoculation volume (1 mL) together with the formulation's salt buffer content, rather than the active ingredient. No clinically relevant abnormalities in safety laboratory measures or urinalysis results were noted.

Discussion

Our findings show, for the first time, the ability of a measles-vectored vaccine to induce functional, neutralising anti-chikungunya virus antibodies in healthy adults, even in the presence of pre-existing anti-vector immunity. This asset is fundamental for this vaccine platform, which is based on a replicating vector derived from one of the safest and most efficient human vaccines.

Although no specific therapy or preventive treatment for chikungunya disease is available, only two candidate vaccines have been tested in human beings.[14,15] The first vaccine (strain 181/clone 25), a live-attenuated derivative of southeast Asian human isolate strain AF15561, entered phase 2 in 2000. The candidate had an acceptable safety profile with only a few participants reporting arthralgia. Additional development was not completed because of the low incidence of disease at the time, and the existence of other higher priority development efforts. The recombinant measles-virus-based chikungunya vaccine candidate has several advantages compared with previous efforts to develop a chikungunya vaccine. First, the construct is based on a recombinant measles virus with no live attenuated and replicating chikungunya virus. Thus, we did not expect the recombinant candidate vaccine to induce chikungunya-like symptoms including chronic arthritis. Accordingly, no clinical or laboratory signs suggesting inflammatory arthralgia were recorded. Second, use of a live attenuated vaccine does not require use of adjuvants. Comparably low doses can be effective, which will aid manufacturing and reduce the costs of a final vaccine product.

A second candidate vaccine for prevention of human chikungunya fever consists of non-replicating chikungunya virus-like particles and was investigated by Chang and colleagues in a phase 1 dose-escalation, open label, clinical trial in 25 adult volunteers.[15] Similar to our findings, primary vaccination was immunogenic, with a booster vaccination leading to a significant increase in antibody titers and a 100% seroconversion rate. However, because of interassay differences, no direct comparisons of geometric mean titres between the two studies can be made. Furthermore, no data exist showing a geometric mean titre antibody threshold associated with protection against chikungunya-virus-related disease in human beings.

Figure 3: Dose-dependent increases in geometric mean titers (95%CI) from days 0 to 120 in the per-protocol population
The second immunisation was given on day 28 (A) or after day 90 (B). $PRNT_{50}$=50% plaque reduction neutralisation test.

Our findings show the functionality of a measles virus vectored vaccine in preimmune participants. Descriptive analyses did not show a uniform reduction or increase of functional antibodies (anti-chikungunya virus $PRNT_{50}$ geometric mean titre) by measles immunoglobulin G baseline titre, and the applied ANOVA model was non-significant. This finding needs to be confirmed in a phase 2 study with larger cohort sizes, which will then have the statistical power to make a final conclusion about the role of anti-vector immunity.

The present candidate vaccine is designed on the basis of the genomic RNA sequence from the strain 06-49, which was isolated from a patient with viraemia during the major epidemic in 2006 at La Réunion island. This chikungunya strain is a member of the East, Central, South African (ECSA) lineage, which accounts for most epidemics worldwide.[12] Virus neutralisation assays in the present study were done with the attenuated 181/25 vaccine strain,[24] which is derived from an Asian lineage virus isolate.[32] Thus, the candidate vaccine elicits a cross neutralising immune response.

In our study, the medium vaccine dose might have yielded the best ratio of immunogenicity to tolerability, combining robust immunogenicity with the lowest solicited and unsolicited adverse events necessitating treatment. This finding will be addressed in detail in future clinical trials with larger group sizes. Throughout the development process of this candidate vaccine towards licensure, the vaccine formulation will be optimised to allow for improved tolerability of high vaccine doses.

By contrast with previous studies, we used a block-randomised design with two vaccination sequences, which allowed us to assess vaccine immunogenicity after both primary and booster vaccination at days 28 and 90. The 28 day vaccination scheme enabled testing of the immunogenicity of two closely spaced doses of the candidate vaccine, a scheme that is most suitable for tourists and health-care workers travelling to chikungunya-endemic regions. The other injection sequence assessed (ie, days 0 and 90) provided valuable information about the persistence of neutralising antibody titres after one dose, and about the size of the immune response when the booster was given after a longer time interval than 28 days, which might allow for better immune maturation. Indeed, long-term memory and the ability to be boosted is a hallmark of live viral vaccines and particularly of measles vaccine. We showed that the antibody response was significantly boosted after a second vaccination, irrespective of the spacing between the two immunisations. After one vaccination the antibody titres declined in the lower dose groups, whereas the titre persisted in the high-dose group over a period of 3 months. Thus, for a one-shot vaccine approach, we anticipate a high-dose immunisation ($>1 \times 10^5$ $TCID_{50}$). Monitoring of antibody persistence is key for the clinical development of the vaccine candidate and this question will be addressed in a larger phase 2 trial.

Residents of and travellers to endemic areas constitute the major at-risk populations that would benefit from a chikungunya vaccine. Aside from the risk of direct and long-lasting health damage,[33] viral epidemics can have a substantial long-term effect on a country's economy and social health because of increased health-care costs, loss of

working days, and a declining tourist trade.[34–37] Tourism is a crucial source of earnings for several tropical countries and constitutes an important driver of economic prosperity. An outbreak of chikungunya fever on the French island of La Réunion in 2006, with more than 260 000 islanders infected,[38] diminished tourism by 60%. The economic costs related to this epidemic were estimated at €43·9 × 10⁶, including 60% due to direct medical costs and 40% due to sick leave.[34] The epidemic spread was aided by a virus-naive population with no previous herd immunity, again emphasising the need for a sufficient rate of immunisation coverage to avoid future outbreaks.

Our trial was limited by a volunteer pool in which most participants were white. Thus, our results warrant further clinical trials of the present measles virus-based chikungunya vaccine in populations of differing ethnic origins and ages, especially those at high risk for chikungunya infection (panel). This vaccine is the first promising measles virus-based candidate vaccine for the prevention of chikungunya fever in human beings.

Panel: Research in context

Systematic review
We searched Pubmed with the keywords "chikungunya", "chikungunya virus", chikungunya vaccine", "measles-vector", and "pre-existing vector immunity". We also searched for news alerts from WHO, the US Centres for Disease Control and Prevention (CDC), and the European CDC. Previous chikungunya virus vaccine projects were carefully reviewed. In the past few years great efforts have been made towards the development of vaccines against re-emerging chikungunya fever. Heterogeneous approaches using virus-like particles, live attenuated chikungunya viruses, DNA-based, poxvirus-based, and adenovirus-based vaccines have been tested with variable results.[39–44] In 2003, Frederic Tangy and colleagues introduced the live attenuated measles-virus vaccine (Schwarz strain) as a vector to express heterologous viral antigens.[17] This vector has since been used to generate various recombinant measles clones eliciting strong and protective immunity against various arthropod-borne viruses such as West Nile virus and dengue virus. Mice and monkeys immunised with measles-virus vectors expressing full-length West Nile virus envelope protein E are fully protected against wild-type West Nile viral challenges,[20] and measles virus vaccines containing dengue virus antigens induce strong functional antibody responses against all four dengue virus serotypes.[18,19] Even in the presence of measles immunity, measles-virus-based vaccinations yield robust humoral and cellular immune responses.[21,22] Accordingly, excellent immunogenicity has also been reported for the present measles virus-based chikungunya vaccine—the first measles-virus-based candidate vaccine against chikungunya virus.[22] However, proof of the immunogenic efficacy of this vaccine in human beings was still outstanding.

Interpretation
Our findings show the immunogenicity of the live, recombinant, measles virus-based chikungunya vaccine in healthy human beings, warranting the advancement of this vaccine into further trials, including in populations at risk for chikungunya infection. In view of an ongoing major epidemic of chikungunya in the Americas, with more than 1·1 million currently suspected cases,[45] an effective vaccine is urgently needed. Although further work will be needed to show safety, tolerability, and ability of the vaccine to protect against chikungunya virus, our trial data suggest that this novel vaccine could be a promising candidate. The threat of chikungunya virus has been a major burden for large parts of the world before. The more recent outbreaks in La Réunion and in the Caribbean have emphasised the importance of chikungunya virus worldwide, and will hopefully increase awareness of this disease, and lead to the development of techniques for its prevention and treatment.

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