Safety and tolerability of chikungunya virus-like particle vaccine in healthy adults: a phase 1 dose-escalation trial

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Summary

Background **Chikungunya virus—a mosquito-borne alphavirus—is endemic in Africa and south and southeast Asia and has recently emerged in the Caribbean. No drugs or vaccines are available for treatment or prevention. We aimed to assess the safety, tolerability, and immunogenicity of a new candidate vaccine.**

Methods **VRC 311 was a phase 1, dose-escalation, open-label clinical trial of a virus-like particle (VLP) chikungunya virus vaccine, VRC-CHKVLP059-00-VP, in healthy adults aged 18–50 years who were enrolled at the National Institutes of Health Clinical Center (Bethesda, MD, USA). Participants were assigned to sequential dose level groups to receive vaccinations at 10 μg, 20 μg, or 40 μg on weeks 0, 4, and 20, with follow-up for 44 weeks after enrolment. The primary endpoints were safety and tolerability of the vaccine. Secondary endpoints were chikungunya virus-specific immune responses assessed by ELISA and neutralising antibody assays. This trial is registered with ClinicalTrials.gov, NCT01489358.**

Findings **25 participants were enrolled from Dec 12, 2011, to March 22, 2012, into the three dosage groups: 10 μg (n=5), 20 μg (n=10), and 40 μg (n=10). The protocol was completed by all five participants at the 10 μg dose, all ten participants at the 20 μg dose, and eight of ten participants at the 40 μg dose; non-completions were for personal circumstances unrelated to adverse events. 73 vaccinations were administered. All injections were well tolerated, with no serious adverse events reported. Neutralising antibodies were detected in all dose groups after the second vaccination (geometric mean titres of the half maximum inhibitory concentration: 2688 in the 10 μg group, 1775 in the 20 μg group, and 7246 in the 40 μg group), and a significant boost occurred after the third vaccination in all dose groups (10 μg group p=0·0197, 20 μg group p<0·0001, and 40 μg group p<0·0001). 4 weeks after the third vaccination, the geometric mean titres of the half maximum inhibitory concentration were 8745 for the 10 μg group, 4525 for the 20 μg group, and 5390 for the 40 μg group.**

Interpretation **The chikungunya VLP vaccine was immunogenic, safe, and well tolerated. This study represents an important step in vaccine development to combat this rapidly emerging pathogen. Further studies should be done in a larger number of participants and in more diverse populations.**

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Introduction

Chikungunya virus is an arthropod-borne virus of the *Alphavirus* genus of the Togaviridae family and is represented by three clades (west African; east, central, and South African; and Asian), with a high amount of aminoacid homology.[1–3] The virus is transmitted through the bite of an infected *Aedes aegypti* or *Aedes albopictus* mosquito and has been documented in about 40 countries.[3–15] Chikungunya virus is endemic to tropical and subtropical regions of Africa and south and southeast Asia. In 2013, the virus spread to the Americas and is responsible for a rapidly spreading epidemic in the Caribbean. As of June 13, 2014, 19 Caribbean or South American countries or territories have been affected, with an estimated 165 990 suspected chikungunya virus cases.[16–18]

Chikungunya virus causes an acute infection associated with severe morbidity lasting several weeks, although symptoms can persist for months. The incubation period ranges from 2 days to 12 days,[14,19] and the acute symptoms include fever, myalgia, arthralgia, headache, rash, nausea, and fatigue.[20] The hallmark symptom of chikungunya virus infection is severe polyarthralgia, with subacute or chronic arthritis presenting as a long-term sequela in some patients.[3,11,13–15] After presentation with acute-onset arthritis, the virus can be identified on PCR[21] and virus can be detected in the joints of infected patients.[22,23] Therefore, chikungunya-virus-associated arthritis is regarded as a direct consequence of viral infection and the related pro-inflammatory innate immune response, rather than a byproduct of adaptive immune responses.[21,24] Neurological complications (encephalitis and meningoencephalitis) have also been reported in rare cases.[7,15,25–27] Although rarely fatal, deaths have occurred, primarily in the elderly and in those with comorbid disorders.[8,11,13,28]

Characterisation of the chikungunya virus adaptive immune response shows that IgM antibodies are present as early as 2 days after the onset of symptoms and persist for weeks to months, whereas IgG is generally detected as the virus is cleared and persists for many years.[29,30] The antibody response against chikungunya virus is primarily of the IgG3 isotype.[29] These IgG3 antibodies are neutralising, associated with viral clearance, and associated with a low risk of prolonged arthralgia when they are induced early in the course of infection.[30] Therefore, the adaptive immune response seems to play a part in controlling the arthritis, further implicating the direct role of viral infection as the cause of the joint inflammation.[31] Neutralising antibodies also prevent virus reinfection, which further suggests that antibody-mediated protection occurs.[6,8,15]

Confirmation and diagnosis of chikungunya virus can be made by serology or detection of viral RNA by RT-PCR during acute infection, but access to rapid testing is not widely available. Because of the durable IgG response to chikungunya virus, assays of greatest potential for diagnosis of acute infection are an IgM-based serological test or direct detection of viral nucleic acid.

No vaccine is available for the prevention of chikungunya virus infection and no specific treatment exists. A live attenuated vaccine candidate has been assessed in a phase 2 clinical trial,[32] but did not advance to efficacy testing.[33] Other vaccine strategies under investigation include a formalin-killed vaccine candidate,[34,35] a chimeric alphavirus vaccine candidate,[36] a virus-like particle (VLP)-based vaccine,[37,38] vaccines based on modified vaccinia Ankara and measles vector,[39,40] and DNA candidate vaccines.[41,42]

The Vaccine Research Center (VRC) chikungunya virus candidate vaccine described herein is a VLP that was chosen because VLPs are highly immunogenic, have a proven safety record, and typically elicit high titre neutralising antibodies needed to protect against chikungunya virus.[1] Additionally, there are few containment requirements for manufacturing because live virus production is not needed. The VRC chikungunya VLP candidate vaccine protects non-human primates from infection and illness, and protective immunity is based on the neutralising antibody.[1]

In this phase 1 study of the VRC chikungunya VLP vaccine in healthy adults, we aimed to assess the safety, tolerability, and immunogenicity of this new candidate vaccine.

Methods

Study design and participants

In VRC 311, a phase 1, dose-escalation, open-label clinical trial, we examined the safety, tolerability, and immunogenicity of a VLP chikungunya virus vaccine. Eligible participants were adults aged 18–50 years who were healthy, as defined by 40 inclusion and exclusion criteria related to clinical laboratory tests, medical history, and physical examination (appendix); with no history of chikungunya virus infection; and willing to comply with protocol schedule requirements. This study was open label: both the patients and clinicians knew what dose was being administered for all injections. There were no changes to the design after commencement of the study.

In the sera collection protocol, VRC 200, we obtained convalescent sera from two patients who had recovered from chikungunya virus infection (NCT00067054). The phase 1 study—VRC 311—and VRC 200 were done by the VRC, National Institute of Allergy and Infectious Diseases (NIAID), and National Institutes of Health (NIH) at the NIH Clinical Center (Bethesda, MD, USA).

VRC 311 and VRC 200 were reviewed and approved by the NIAID Institutional Review Board. The US Department of Health and Human Services human experimental guidelines for undertaking clinical research were followed. Informed consent was obtained from participants by study team clinicians before enrolment during their week 0 study visit.

Procedures

The vaccine, VRC-CHKVLP059-00-VP, was manufactured at the VRC, NIAID, Vaccine Pilot Plant operated by Leidos Biomedical Research (Frederick, MD, USA) according to good manufacturing practices and stored in vials at a concentration of 40 μg/mL. The vaccine consists of chikungunya VLPs composed of the E1, E2, and capsid proteins from the chikungunya virus strain 37997. VLPs were produced by transfection of human embryonic kidney VRC293 cells with a DNA plasmid encoding the structural genes of the chikungunya virus. The VRC293 cells are a suspension-adapted serum-free cell line derived from HEK-293 cells. The cell line has been fully characterised for adventitious agents and tumorigenicity according to US Food and Drug Administration (FDA) guidance.[43] The enveloped VLPs self-assemble and are released into the culture medium as particles about 65 nm in diameter. The VLPs were purified through a series of steps including centrifugation, filtration, ultrafiltration and diafiltration, chromatography, and sterile filtration. The VLPs were then formulated at the appropriate dosage and transferred into sterile vials.

The vaccine was administered intramuscularly (deltoid muscle) by needle and syringe at 10 μg (in 0·25 mL), 20 μg (in 0·5 mL), and 40 μg (in 1 mL). Based on preclinical data, the VLP chikungunya virus vaccine was given at weeks 0, 4, and 20.

Safety monitoring was done through protocol-specified clinical and laboratory assessments. We measured complete blood count and creatinine and alanine aminotransferase concentrations. Local and systemic reactogenicity parameters were recorded for 7 days after each injection. All adverse events that occurred within 28 days after each vaccination and all serious adverse events and new chronic medical disorders that occurred throughout the study were recorded by study clinicians.

See Online for appendix

We coded adverse events with the Medical Dictionary for Regulatory Activities and severity graded them with a table adapted from a US FDA guidance document.[44]

We assessed vaccine immunogenicity by measuring chikungunya virus-specific humoral immune responses by ELISA and by neutralisation antibody assays throughout the study. Chikungunya virus neutralising antibodies in the sera of volunteers were measured at several timepoints after vaccination (study weeks 0, 4, 8, 20, 22, 24, and 44) by a previously described neutralisation assay,[45] which is reproducible, quantitative, precise, and measures individual infectious events. Briefly, a green fluorescent protein (GFP)-expressing chimeric Semliki Forest virus (SFV)-chikungunya virus encoding the structural proteins of the OPY-1 strain of chikungunya virus was produced in HEK-293T cells by transfection of a molecular clone and harvested 72 h after transfection. The plasmids and methods for virus production are described in detail elsewhere.[45] Stocks of SFV-chikungunya virus used in neutralising antibody assays were passaged once in Vero cells, harvested at 24 h or 48 h after infection, filtered, and stored at –80°C. To measure virus titre, we infected Vero cells with serial twofold dilutions of SFV-chikungunya virus in duplicate. 8 h after infection, cells were trypsinised, fixed with paraformaldehyde, and the number of GFP-positive infected cells was assessed by flow cytometry.

Neutralisation assays were done by incubating serial threefold dilutions of heat-inactivated volunteer serum with the chimeric SFV-chikungunya virus. We created serum–virus complexes (200 µL) in duplicate wells of a 96-well plate, which were incubated for 1 h at room temperature before being added to preplated Vero cells ($2·5×10^4$ cells per well) in a final volume of 300 µL. Infection was done at 37°C in 7% CO_2 for 8 h, after which cells were trypsinised and fixed with paraformaldehyde. We assessed the number of GFP-positive infected cells by flow cytometry. We fitted the resulting serum dose–response data with a sigmoidal dose–response curve with a variable slope to estimate the serum concentration needed to inhibit 50% of infection (IC_{50}; Prism version 6, GraphPad Software, La Jolla, CA, USA). Neutralisation titres were reported only when estimated with confidence by the regression analysis (R^2 value >0·8 and 90% CI within twofold of the estimated neutralisation titre). We assigned samples with low to no neutralisation—for which the above criteria could not be met—a titre of 50, which is the limit of detection in this assay. Tests were repeated on a subset of samples to confirm assay reproducibility; for these samples, we report the mean of both experiments.

Endpoint ELISA titres of antibodies directed against chikungunya VLP antigen (strain 37997), prepared by similar methods to those used for preparation of the vaccine product, were assessed using 96-well Immulon2 (Dynex Technologies, Chantilly, VA, USA) plates coated with optimised concentrations of the VLP that were stored overnight at 4°C, followed by washing and blocking (20% fetal bovine serum and 1% bovine serum albumin buffered solution) for 1 h at 37°C. Serial dilutions of the samples were incubated for 2 h at 37°C. Assay development included biotin-labelled anti-human IgG, IgA, and IgM (1 h at 37°C), followed by streptavidin conjugated with horseradish peroxidase for 30 min at room temperature

	10 µg group (n=5)	20 µg group (n=10)	40 µg group (n=10)	Overall (n=25)
Sex				
Women	2 (40%)	7 (70%)	6 (60%)	15 (60%)
Men	3 (60%)	3 (30%)	4 (40%)	10 (40%)
Age (years)				
Mean (SD)	29 (7)	34 (6)	29 (7)	31 (7)
Range	18–37	28–47	22–42	18–47
Race				
Asian	1 (20%)	1 (10%)	1 (10%)	3 (12%)
Black or African American	1 (20%)	1 (10%)	1 (10%)	3 (12%)
White	3 (60%)	8 (80%)	8 (80%)	19 (76%)
Ethnic origin				
Non-Hispanic or Latino	5 (100%)	10 (100%)	9 (90%)	24 (96%)
Hispanic or Latino	0 (0%)	0 (0%)	1 (10%)	1 (4%)
Body-mass index				
Mean (SD)	23 (2)	25 (3)	26 (5)	25 (4)
Range	22–25	21–31	20–35	20–35
Education				
High school or equivalent	0 (0%)	1 (10%)	0 (0%)	1 (4%)
College graduate	1 (20%)	3 (30%)	6 (60%)	10 (40%)
Advanced degree	4 (80%)	6 (60%)	4 (40%)	14 (56%)

Data are number (%), unless otherwise specified.

Table 1: **Baseline demographics of participants**

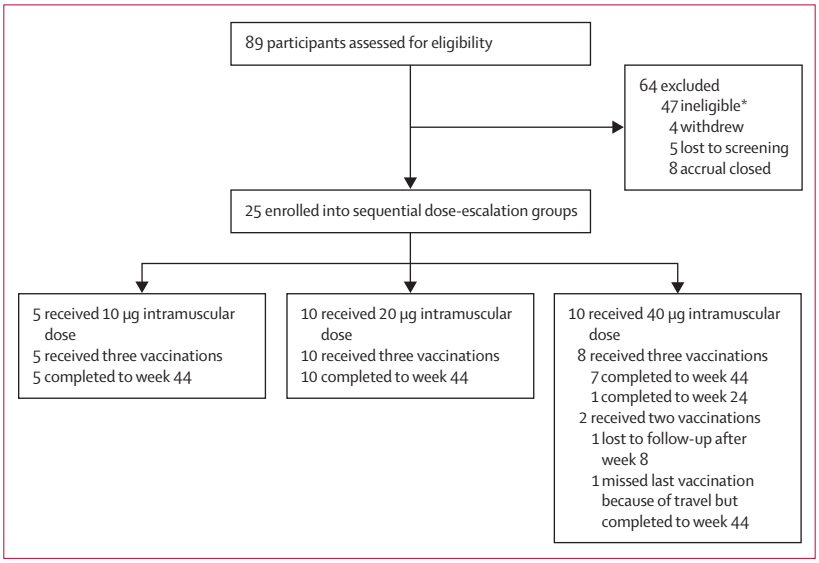

Figure: **Trial profile**

*Seven of whom did not meet several criteria. Ineligibility reasons were laboratory test (n=13), medical history (n=22), physical findings (n=10), and inability to comply with protocol requirements (n=10).

	10 μg group (n=5)	20 μg group (n=10)	40 μg group (n=10)	Overall (n=25)
Local symptoms				
Pain or tenderness				
None	4 (80%)	6 (60%)	6 (60%)	16 (64%)
Mild	1 (20%)	4 (40%)	4 (40%)	9 (36%)
Swelling				
None	5 (100%)	10 (100%)	10 (100%)	25 (100%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Redness				
None	5 (100%)	10 (100%)	10 (100%)	25 (100%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Any local symptom				
None	4 (80%)	6 (60%)	6 (60%)	16 (64%)
Mild	1 (20%)	4 (40%)	4 (40%)	9 (36%)
Systemic symptoms				
Malaise				
None	4 (80%)	7 (70%)	8 (80%)	19 (76%)
Mild	1 (20%)	3 (30%)	2 (20%)	6 (24%)
Myalgia				
None	5 (100%)	8 (80%)	9 (90%)	22 (88%)
Mild	0 (0%)	2 (20%)	1 (10%)	3 (12%)
Headache				
None	4 (80%)	10 (100%)	7 (70%)	21 (84%)
Mild	1 (20%)	0 (0%)	3 (30%)	4 (16%)
Chills				
None	5 (100%)	10 (100%)	10 (100%)	25 (100%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nausea				
None	4 (80%)	10 (100%)	7 (70%)	21 (84%)
Mild	1 (20%)	0 (0%)	3 (30%)	4 (16%)
Raised temperature				
None	5 (100%)	10 (100%)	10 (100%)	25 (100%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Joint pain				
None	5 (100%)	10 (100%)	10 (100%)	25 (100%)
Mild	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Any systemic symptom				
None	3 (60%)	7 (70%)	5 (50%)	15 (60%)
Mild	2 (40%)	3 (30%)	5 (50%)	10 (40%)

Data are number (%). Each vaccine recipient is counted once at worst severity for any local and systemic parameter. There was no moderate or severe reactogenicity.

Table 2: **Maximum local and systemic reactogenicity**

and 3,5′,5,5′-tetra-methylbenzidine substrate for 30 min at room temperature. Plates were read on a Spectramax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Samples collected after vaccination were corrected using the matched volunteer sample taken before vaccination to eliminate inherent volunteer non-specific reactivity. We calculated endpoint titres as the most dilute serum concentration that gave an optical density reading of greater than 0·2 above background. Run-to-run consistency was monitored by a positive control of polyclonal sera generated in non-human primates.

Outcomes

The primary endpoints were safety and tolerability of the vaccine. Secondary endpoints were chikungunya virus-specific immune responses assessed by ELISA and neutralising antibody assays.

Statistical analysis

The sample size was prespecified as five or ten participants per dose group because this was the first human phase 1 clinical trial of a novel vaccine. For the antibody response measured by ELISA and neutralising antibody titre, we computed the geometric mean titre along with the 95% CIs. We used the two-sample *t* test for between-group comparisons and the paired *t* test for within-group comparisons for both ELISA and neutralising antibody titre in log scales.

This trial is registered with ClinicalTrials.gov, NCT01489358.

Role of the funding source

The study was fully funded by the NIH intramural research programme. The vaccine was manufactured by the VRC (an intramural centre within the NIAID, NIH), and the VRC funded and undertook the study. The VRC 311 Study Team was responsible for the study design, study management, regulatory submissions to the NIAID Institutional Review Board and US FDA, data collection, data analysis, data interpretation, and writing of the report. The principal investigator and associate investigators had full access to all the data in the study and JEL had final responsibility for the decision to submit for publication.

Results

25 participants were enrolled from Dec 12, 2011, to March 22, 2012, into the three dosage groups: 10 μg (n=5), 20 μg (n=10), and 40 μg (n=10). 73 vaccinations were administered. The final study vaccination was on Aug 14, 2012, and the final study follow-up visit was on March 4, 2013. The study population consisted of 15 (60%) women and ten (40%) men with a mean age of 31 years (range 18–47; table 1). Of the 25 participants, 23 received all three vaccinations (figure).

Vaccinations were well tolerated, with no serious adverse events reported. Overall, for the solicited reactogenicity parameters, nine (36%) of 25 participants reported mild local reactogenicity, whereas ten (40%) reported mild systemic reactogenicity at least once after a vaccination (table 2). There were no reports of arthralgia after vaccination.

Seven mild-to-moderate adverse events (occurring in four participants) were deemed to be related to study vaccine because they occured within 2–4 weeks after vaccination: four mild transient alanine aminotransferase

increases and two mild and one moderate transient neutropenia. All resolved without clinical sequelae.

Antibodies were detected by ELISA in most participants after the first vaccination (100% in the 10 µg and 40 µg groups and 80% in the 20 µg group), were substantially boosted by 4 weeks after the second vaccination (10 µg group p=0·07, 20 µg group p=0·0002, and 40 µg group p<0·0001), and were boosted to peak titres at 4 weeks after the third vaccination (table 3). The geometric mean titres were not significantly different between the three dose groups, except for at week 24 (table 3). Furthermore, there was no significant difference between the group geometric mean titres 4 weeks after the second vaccination compared with 4 weeks after the third vaccination (group 1 p=0·10, group 2 p=0·20, group 3 p=0·77, by paired *t* test; appendix).

Neutralising antibodies against an outbreak strain, OPY1 (east, central, and south African clade), were found in all participants 4 weeks after the second vaccination (week 8, appendix). A higher mean neutralising antibody titre was noted in the 40 µg dose group compared with the 20 µg dose group at both 4 weeks after the second vaccination (week 8, p=0·0001, unpaired *t* test) and at the time of the third vaccination (week 20, p=0·0082, unpaired *t* test). After the third vaccination, there were no significant differences between the group mean titres (table 3; appendix). 6 months after the third vaccination (week 44), neutralising antibodies remained detectable in participants in all three groups (table 3). In the one participant who received only the first two 40 µg vaccinations but who was followed up to week 44, neutralising antibodies were still present 10 months after the second vaccination. A significant boost of neutralising activity against the chikungunya virus OPY1 strain was noted after the third vaccination in all dose groups (10 µg group p=0·0256, 20 µg group p<0·0001, and 40 µg group p=0·0082, paired *t* test). Tests were repeated for 36 samples and repeated values were within 2·5-fold of the initial titre.

Convalescent sera from two patients who had chikungunya virus infection while in the Philippines in 2014 were assessed 3 months after onset of infection. The convalescent IC_{50} titres were 7057 and 4227, which are comparable with the titres of the vaccine recipients 4 weeks after completion of the vaccine regimen (geometric mean titre of the IC_{50}: 10 µg group 8745, 20 µg group 4525, and 40 µg group 5390).

	ELISA titre (strain 37997)			Neutralisation IC_{50} titre (strain OPY1)		
	10 µg group (n=5)	20 µg group (n=10)	40 µg group (n=10)	10 µg group (n=5)	20 µg group (n=10)	40 µg group (n=10)
0*†	..	..	..	50 (50–50)	51 (49–52)	52 (50–54)
4*	160 (19–1317)	278 (98–788)	424 (134–1338)	188 (30–1179)	236 (90–614)	346 (120–999)
8	3378 (358–31856)	5881 (2026–17077)	20480 (12144–34539)	2688 (885–8166)	1775 (1129–2791)	7246 (4512–11637)
20*	2560 (758–8645)	1114 (557–2229)	4740 (1852–12133)	650 (251–1680)	510 (288–901)	1485 (831–2655)
22	31042 (14378–67021)	13512 (4852–37626)	14482 (4362–48073)	NA	NA	NA
24	40960 (40960–40960)	15521 (6058–39763)	34443 (22862–51890)	8745 (1514–50516)	4525 (2252–9093)	5390 (1865–15573)
44	4457 (442–44860)	5881 (2026–17077)	8611 (2730–27161)	940 (141–6254)	717 (267–1927)	1385 (605–3171)

Data are the geometric mean titre (95% CI). All available samples were used for each reported result. IC_{50}=half maximum inhibitory concentration. NA=not assessed. *Visit at which vaccine was administered. †For ELISA, week 0 values were used to background correct titres for subsequent weeks.

Table 3: **Antibody titres**

Discussion

In this phase 1 study, the chikungunya VLP vaccine VRC-CHKVLP059-00-VP was safe, well tolerated, highly immunogenic, and was given without adjuvant (panel). The first and second vaccinations were highly immunogenic, and after the second vaccination all participants in all dose groups had developed robust titres of chikungunya virus neutralising antibodies. Immune responses after the initial dose of vaccine and the boost in neutralising antibody titre noted after the second and third doses of vaccine is consistent with other efficacious VLP vaccine regimens.[46] Vaccine-induced antibodies were durable because they were detected in all participants 6 months after their last vaccination, including in those who received the lowest dose of vaccine.

There are three genotypes of chikungunya virus, which share high aminoacid sequence identities (95·2–99·8%).[2] Our VLP vaccine was based on the west African strain 37997. The west African strain and the east, central, and south African outbreak strain (OPY1) have the greatest genotypic differences, whereas the Asian strain is similar to the east, central, and south African strain.[2] Therefore, the amount of cross-reactive neutralising activity against the east, central, and south African outbreak strain (OPY1) induced by the chikungunya VLP vaccine suggests cross-protection could be achieved for several strains.

Several lines of evidence support a crucial role for neutralising antibodies in protection against chikungunya virus infection.[1,30,47] In this study, we used a GFP-expressing chimeric chikungunya virus in a flow-cytometry-based assay to measure neutralisation titres. This assay offers many advantages when assessing the results of a clinical trial. The use of a flow cytometer provides a high-throughput and less operator-dependent approach compared with traditional plaque reduction neutralisation test (PRNT)-type assays, while maintaining a reproducible and precise measurement of individual infectious events. IC_{50} values calculated for chikungunya virus monoclonal antibodies using this method are similar to IC_{50} values calculated from focus reduction neutralisation tests;[45] thus, the flow-cytometry-based neutralisation assay we used is comparable with more traditional live virus neutralisation

Panel: Research in context

Systematic review

Research and vaccine development for emerging infectious diseases is part of the mission of the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Based on evidence of the increasing spread of chikungunya virus infections, we started to develop a vaccine. We searched PubMed using the keywords "chikungunya", "chikungunya virus", "chikungunya virus vaccine", "alphavirus", "alphavirus vaccine", and "virus-like particle vaccine". We also used the following search terms: general reviews, vaccine, immunisation, genomics, virology, pathology, diagnostics, transmission, epidemiology, immunology, immunity, basic and clinical research, and region-specific outbreaks. We also searched reports, white papers, and news alerts from ProMED-mail, WHO, the Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control, the Australia Department of Health and Aging, the UK Health Protection Agency, and the Indian National Vector Borne Disease Control Programme. Relevant to our development of the product and methods for this trial, we assessed the published work on previous and ongoing chikungunya virus vaccine development strategies, including a live attenuated vaccine candidate, a formalin-killed vaccine candidate, a chimeric alphavirus vaccine candidate, a virus-like particle-based vaccine, vaccines based on modified vaccinia Ankara and measles vector, and DNA candidate vaccines. From our review of the publicly available information, we concluded that although many vaccine platforms showed immunogenicity in preclinical testing, none, other than the live attenuated candidate, had advanced through clinical assessment.[1,32–36,42] We found evidence that development of a safe vaccine that could be manufactured without live virus and thus in the absence of biocontainment could have a public health benefit and contribute to a better understanding of protection from alphavirus infections in general.

Interpretation

In this phase 1, dose-escalation, open-label clinical trial of the safety and immunogenicity of a virus-like particle chikungunya virus vaccine that was given without adjuvant in 25 healthy adults, the product was safe, well tolerated, highly immunogenic, and easy to produce. The first and second vaccinations were highly immunogenic and after the second vaccination all participants in all dose groups had developed robust titres of chikungunya virus neutralising antibodies. These clinical data represent an important step in vaccine development to combat this rapidly emerging pathogen. To expand on the safety and immunogenicity data gained in this trial and to further the clinical development of this vaccine, additional clinical trials need to be done in additional populations, including at-risk populations.

assays. An additional benefit of this assay is that negligible virus input is needed, eliminating potentially confounding effects of antigen excess when measuring antibody neutralisation.[45] Also, the chikungunya VLP vaccine used herein has been tested in non-human primates,[1] in which it elicited similar amounts of neutralising antibodies and provided complete protection against viraemia and clinical measures of illness. Additionally, IgG from non-human primates vaccinated with chikungunya VLP provided passive protection in an otherwise lethal mouse model of chikungunya virus infection,[1] further showing the antibody-mediated mechanism of protection from chikungunya virus infection. No studies have identified the specific neutralisation titres needed for protection in human beings. However, in a mouse challenge model, convalescent human sera with low in-vivo neutralisation potency ($PRNT_{80}$=40) protected mice from chikungunya virus challenge.[47] The neutralisation titres of recipients of the VRC chikungunya VLP vaccine represent a potentially durable protective response: at 11 months after vaccination, titres (geometric mean titre of the IC_{50} 1014) were comparable to those reported after natural chikungunya virus infection, which have been inferred to be protective.[29,30,47] Additionally, we assessed convalescent sera for neutralising antibody from two patients with recent chikungunya virus infection. Convalescent titres were comparable with the titres reported in our participants 4 weeks after completion of the vaccine regimen. By comparison, in a phase 2 trial[32] of a live attenuated chikungunya virus vaccine candidate, mean $PRNT_{50}$ was less than 200 at 11 months after vaccination. 8% of participants in that trial displayed arthralgia as a side-effect, suggesting possible unexpected virulence of the attenuated candidate.

The process used to make VLPs is a platform technology that can be applied to the production of other alphavirus vaccine candidates. During advanced development, the possibility of dose sparing by formulating the vaccine with an adjuvant should be considered.

The results reported here are limited by the size and geographic restrictions of a small, single-site phase 1 clinical trial. The initial human data with this candidate chikungunya virus vaccine, which showed it is a candidate vaccine that induces immune responses consistent with present knowledge of what is hypothesised to be protective immunity, needs to be confirmed by larger studies in diverse populations, including those at risk of chikungunya virus infection.

Contributors

L-JC was the lead associate investigator and JEL was the principal investigator. SHP, UNS, and BSG were study investigators. RMS, WA, and GJN developed and produced the vaccine. ZH did the statistical analysis. L-JC, KAD, FHM, JGS, SS, SHP, GY, UNS, MEE, RTB, RAK, and JEL collected data. L-JC, KAD, GY, ZH, MEE, RTB, RAK, WA, JRM, TCP, BSG, and JEL analysed and interpreted data. L-JC, FHM, JGS, SS, SHP, GY, ZH, MEE, RMS, WA, GJN, JRM, BSG, and JEL designed the study. All authors contributed to the writing of the report and approved the final version.

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Declaration of interests
We declare no competing interests.

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