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Safety, tolerability, and immunogenicity of two Zika virus DNA vaccine candidates in healthy adults: randomised, open-label, phase 1 clinical trials

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Summary

Background The Zika virus epidemic and associated congenital infections have prompted rapid vaccine development. We assessed two new DNA vaccines expressing premembrane and envelope Zika virus structural proteins.

Methods We did two phase 1, randomised, open-label trials involving healthy adult volunteers. The VRC 319 trial, done in three centres, assessed plasmid VRC5288 (Zika virus and Japanese encephalitis virus chimera), and the VRC 320, done in one centre, assessed plasmid VRC5283 (wild-type Zika virus). Eligible participants were aged 18–35 years in VRC19 and 18–50 years in VRC 320. Participants were randomly assigned 1:1 by a computer-generated randomisation schedule prepared by the study statistician. All participants received intramuscular injection of 4 mg vaccine. In VRC 319 participants were assigned to receive vaccinations via needle and syringe at 0 and 8 weeks, 0 and 12 weeks, 0, 4, and 8 weeks, or 0, 4, and 20 weeks. In VRC 320 participants were assigned to receive vaccinations at 0, 4, and 8 weeks via single-dose needle and syringe injection in one deltoid or split-dose needle and syringe or needle-free injection with the Stratis device (Pharmajet, Golden, CO, USA) in each deltoid. Both trials followed up volunteers for 24 months for the primary endpoint of safety, assessed as local and systemic reactogenicity in the 7 days after each vaccination and all adverse events in the 28 days after each vaccination. The secondary endpoint in both trials was immunogenicity 4 weeks after last vaccination. These trials are registered with ClinicalTrials.gov, numbers NCT02840487 and NCT02996461.

Findings VRC 319 enrolled 80 participants (20 in each group), and VRC 320 enrolled 45 participants (15 in each group). One participant in VRC 319 and two in VRC 320 withdrew after one dose of vaccine, but were included in the safety analyses. Both vaccines were safe and well tolerated. All local and systemic symptoms were mild to moderate. In both studies, pain and tenderness at the injection site was the most frequent local symptoms (37 [46%] of 80 participants in VRC 319 and 36 [80%] of 45 in VRC 320) and malaise and headache were the most frequent systemic symptoms (22 [27%] and 18 [22%], respectively, in VRC 319 and 17 [38%] and 15 [33%], respectively, in VRC 320). For VRC5283, 14 of 14 (100%) participants who received split-dose vaccinations by needle-free injection had detectable positive antibody responses, and the geometric mean titre of 304 was the highest across all groups in both trials.

Interpretation VRC5283 was well tolerated and has advanced to phase 2 efficacy testing.

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Introduction

Zika virus is the latest arboviral infection to cause epidemic disease in the western hemisphere.[1] That this flavivirus can infect human beings has been known for 70 years,[2] but it was not judged to be a public health threat until outbreaks occurred in Yap Island in 2006,[3] French Polynesia in 2013,[4] and the Americas and southeast Asia in 2015.[5] Zika virus infection causes clinical symptoms in about 20% of individuals, of which macular or papular rash, fever, arthritis, arthralgia, non-purulent conjunctivitis, myalgia, headache, retro-orbital pain, oedema, and vomiting are most frequent.[3] *Aedes* spp mosquitoes are the main sources of infection, but sexual[6] and other forms of transmission are possible and can lead to spread to non-endemic regions.[7,8] Over 700 000 cases of autochthonous Zika virus infection have been reported in the Americas since 2015,[9] and have revealed previously unrecognised sequelae. In December, 2015, the Pan American Health Organization and WHO issued an alert linking Zika virus infection with Guillain-Barré syndrome and congenital malformations, including microcephaly. WHO later declared Zika virus to be a Public Health Emergency of International Concern.[10] The causal links between Zika virus infection with these syndromes are supported,[11] but the mechanism of infection-related microcephaly is unknown and its epidemiology is complex.[12] As no effective treatments are

Research in context

Evidence before this study

The Zika virus has been recognised as a pathogenic flavivirus for over 70 years, but was uncharacterised as a pathogen because the threat to public health was limited. Outbreaks in the Pacific region and Brazil led to its recognition as an emerging infectious disease. Previously unknown sequelae of Zika virus infection, most notably congenital abnormalities, served as the impetus for accelerated vaccine development. The Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA, has experience in developing vaccines to protect against emerging infectious diseases, in general, especially against flaviviruses. We searched PubMed with the terms "Zika virus", "Zika vaccine", "flavivirus", and "DNA vaccine." We did not use any other search parameters. We also used news alerts from WHO, Pan American Health Organization, and Centers for Disease Control and Prevention to identify reports. We retrieved 11 445 papers. Two papers provided evidence for immunogenicity and safety of the DNA vaccine platform, given via a needle-free injector system of administration for a different flavivirus (West Nile virus). The DNA vaccine platform was attractive in this context because it allowed for the modification of a previously validated plasmid backbone, which helped facilitate rapid preclinical development, manufacturing, and regulatory approval of a first-in-human vaccine candidate. We also found that using a needle-free injection device could augment immune responses to DNA vaccines compared with administration via needle and syringe.

Added value of this study

Use of previously approved plasmid backbones enabled rapid development and approval of two DNA vaccines for testing in phase 1 studies, and our safety data support this approach. Human immunogenicity data alone, as relates to efficacy, do not currently exist for any Zika virus vaccine. Therefore, human data need to be assessed relative to data derived from animal challenge models to indicate which candidates are likely to succeed in later-stage trials. The results of our two studies and findings from non-human primate studies provided sufficient evidence to support testing of VRC5283 in regions with endemic Zika virus to assess efficacy.

Implications of all the available evidence

VRC5288 and VRC5283 were safe and immunogenic in previous non-human primate studies, eliciting robust neutralising antibody responses and protecting the animals from subsequent viral challenge. In our phase 1 clinical trials, these DNA vaccines were safe and well tolerated in healthy adults and led to the production of detectable cellular responses and neutralising antibody responses against the Zika virus proteins. Administration of the VRC5283 vaccine by needle-free injection resulted in the highest magnitude humoral and cellular responses. While these clinical data indicate that the DNA vaccine candidates are viable options for use against Zika virus infection, additional clinical trials are needed to further the development of VRC5283 and to determine efficacy in at-risk populations. Our previous experience with development of vaccines against emerging diseases shows that rapid development of vaccines requires cooperation between the scientific, medical, regulatory, and political communities toward a common goal.

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yet available and infection is often subclinical, development of a preventive vaccine is urgently needed.

DNA vaccines are safe and immunogenic for many pathogens, including flaviviruses, although none has been licensed for use in human beings.[13–17] A DNA vaccine consists of a plasmid containing coding DNA sequences for virus-specific antigens, a promoter region that enables transcription, and a polyadenylation sequence that facilitates protein translation. Importantly, once a manufacturing process and the safety and immunogenicity of the plasmid have been established, the coding sequences can be changed to those for other known antigens to accelerate identification of novel candidate vaccines, and in some cases reduce the regulatory requirements with minimal preclinical toxicity data. The established manufacturing technologies, previous data on toxicity and safety,[18] inherent DNA stability, and ability to elicit antibody and CD8 T-cell responses make DNA vaccines an attractive option for rapidly responding to emerging infectious diseases.

The Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA, has experience developing DNA vaccines against viruses including HIV, Ebola virus, severe acute respiratory syndrome coronavirus, influenza virus, and West Nile virus.[13,14,16,17,19] The VRC used this experience to develop two DNA vaccine candidates, VRC5288 and VRC5283, against Zika virus. We used West Nile virus vaccines as templates because of the expected biological similarity between members of the Flavivirus genus, and because they have been safe and have induced substantial and durable neutralising activity in clinical trials.[16] Into the plasmid backbone, we inserted sequences from Zika virus *prM* and *E* genes, which encode protein prM (prM) and envelope protein E (E), respectively. When these proteins are expressed in mammalian cells, they assemble into subviral particles that are non-infectious but have structural and antigenic similarities to virion particles and can induce protective immune responses.[20,21] Because preclinical studies were being done in parallel with manufacturing, initial product choice was based on in-vitro data and the final choice was based on animal model data. Therefore, we assessed two DNA vaccine candidates that expressed different prM and E antigen designs because we were uncertain that preclinical

expression and immunogenicity data would predict clinical outcomes.

VRC5288 and VRC5283 are similar, but VRC5288 has an E region that contains both Zika virus and Japanese encephalitis virus sequences, whereas VRC5283 has an E region that contains wild-type Zika virus sequences. The E protein produced by VRC5288 is chimeric, with the extracellular region being of Zika virus origin and the stem and transmembrane regions being made up of 98 aminoacids from the Japanese encephalitis virus. This Japanese encephalitis virus sequence was used because it improved the release of subviral particles in non-human primates, which was thought to have potential for improving immunogenicity, but, without surface exposure on mature particles, were not expected to contribute otherwise to the induction of protective immunity to Japanese encephalitis virus.[22]

In non-human primates, the two Zika virus vaccine candidates induced robust neutralising antibody responses after two doses given 4 weeks apart. 17 of 18 animals who received two 4 mg doses of VRC5288 or two 4 mg or 1 mg doses of VRC5283 were protected from viraemia following Zika virus challenge 8 weeks after vaccination.[22] The animal that broke through received two 4 mg doses of VRC5288. Based on these findings, both vaccines were advanced into phase 1 clinical trials to assess safety, tolerability, and immunogenicity in human beings before selecting one to advance into the next stage of development. Here we report the initial findings of both trials.

Methods

Study design and participants

VRC 319 and VRC 320 are phase 1, randomised, open-label clinical trials of Zika virus DNA vaccine candidates. Eligible participants were healthy adults, aged 18–35 years in VRC 319 and 18–50 years in VRC 320, without abnormal findings in clinical laboratory tests, medical history, or physical examinations. Volunteers for VRC 319 were recruited at the NIH Clinical Center, Bethesda, MD, and the University of Maryland Center for Vaccine Development, Baltimore, MD, USA, and the Hope Clinic of the Emory Vaccine Center, Decatur, GA, USA, and those for VRC 320 were recruited at the NIH Clinical Center.

The NIAID institutional review board reviewed and approved the protocols, and provided oversight for both studies. Reliance agreements with the University of Maryland and Emory Federalwide Assurance were in place for VRC 319. We followed the Department of Health and Human Services guidelines for the protection of human beings in research, and all participants provided written informed consent before enrolment.

Randomisation and masking

In both trials, we used computer-generated randomisation schedules prepared in advance by the study statistician to assign participants to vaccination groups. The schedules were provided to the study site pharmacies and the data management centre. Research nurses enrolled participants. In VRC 319 participants were assigned 1:1 to four different vaccination schedules. In VRC 320 participants were assigned 1:1 to three groups of single-dose or split-dose vaccination.

Vaccines

The vaccines consist of phosphate buffered saline, purified plasmid comprising mammalian expression control elements, coding sequences for Zika virus *prM* and *E* from a French Polynesia isolate (strain H/PF/2013), and standard bacterial origin of replication and selection elements. In the VRC5288 vaccine, the Zika virus coding sequence was modified by substituting Japanese encephalitis virus sequences for the stem and transmembrane regions of the E protein, and in both vaccines, the *prM* signal sequence in the Zika virus coding sequence was exchanged with an analogous Japanese encephalitis virus region to improve secretion of Zika subviral particles from transfected cells. Both vaccines were manufactured by the VRC Pilot Plant, operated under contract by Leidos Biomedical Research (Frederick, MD, USA) according to Good Manufacturing Practices, and supplied in doses of 4 mg/mL.

Study procedures

4 mg vaccine was given in all vaccinations. Volunteers enrolled into VRC 319 received VRC5288 as single intramuscular injections given via needle and syringe. Group 1 received vaccine on weeks 0 and 8, group 2 on weeks 0 and 12, group 3 on weeks 0, 4, and 8, and group 4 on weeks 0, 4, and 20. VRC 319 was originally designed to assess VRC5288 delivered by the needle-free Stratis device (Pharmajet, Golden, CO, USA), but a modification was needed to deliver a DNA vaccine with high viscosity that was not made in time for the trial. The device, therefore, was only used in the VRC 320 trial. Volunteers enrolled into VRC 320 received VRC5283 on weeks 0, 4, and 8. Group 1 received single doses given via needle and syringe into one deltoid; group 2 received split doses (2 mg each), one in each deltoid, given via needle and syringe; and group 3 received split doses (2 mg each), one into each deltoid, given via syringe and needle-free device, in which a spring-powered injector pressurises a narrow stream of vaccine into the tissue without electroporation or other externally applied factors.

Outcomes

The primary endpoint was vaccine safety, assessed by local and systemic reactogenicity. Safety and tolerability were monitored by clinical and laboratory assessments. Participants used diary cards to record local and systemic reactogenic events occurring in the 7 days after each injection. All adverse events occurring within 28 days after each injection were recorded by clinic staff. Serious adverse events were recorded for the entire duration of the study. These were classified as events or suspected

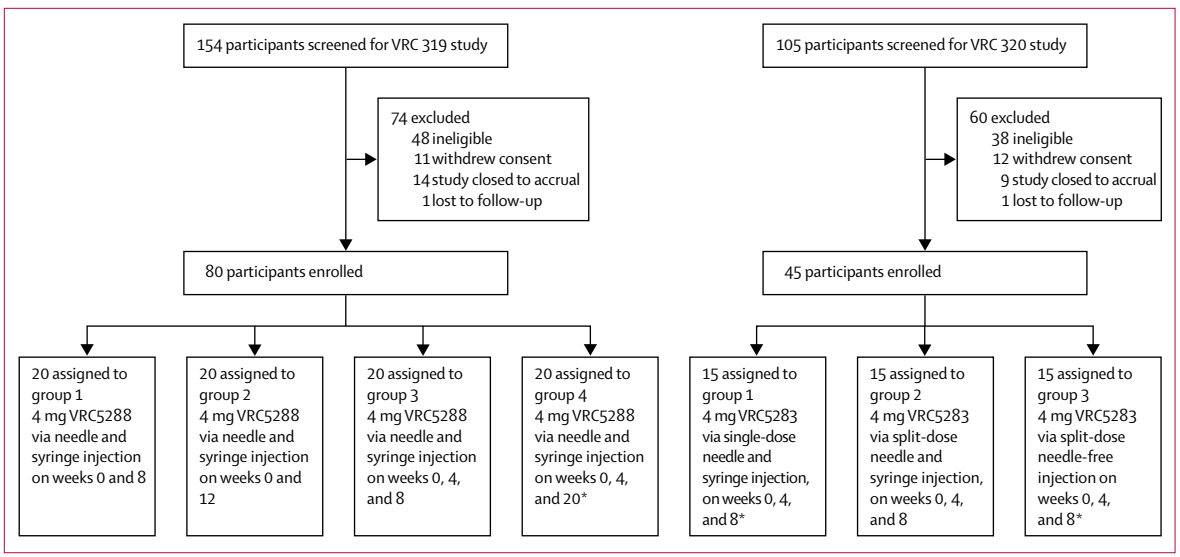

Figure 1: **Trial profiles**
VRC5288=VRC5288 plasmid backbone with Zika virus and Japanese encephalitis chimeric envelope protein E. VRC5283=VRC5283 plasmid backbone with wild-type Zika virus envelope protein E. *A participant withdrew after one dose of vaccine due to time commitments, precluding further trial participation, but was included in the safety analyses.

adverse reactions that, in the view of the investigator or study sponsor, led to death, a life-threatening event, admission to hospital or prolongation of a hospital stay, inability to continue normal life functions, or a congenital anomaly or birth defect, or led to a medical or surgical intervention to prevent one of these outcomes. We used the FDA toxicity grading scale for healthy adults and adolescent volunteers enrolled in preventive vaccine clinical trials. Secondary endpoints were immunogenicity assessed by a reporter virus particle neutralisation assay and antigen-specific T-cell response.

Neutralising antibody responses

Vaccine antibody response was assessed by measuring Zika-virus-specific neutralising antibodies with a previously described reporter virus particle assay.[22] Briefly, Zika virus reporter virus particles were produced in human embryonic kidney 293 T cells by co-transfection with two plasmids, one encoding a green fluorescent protein expression West Nile virus replicon and the other encoding the structural proteins of the Zika virus H/PF/2013 strain. Zika virus reporter virus particles were incubated with serial threefold dilutions of heat-inactivated sera in duplicate technical replicates and added to Raji cells expressing the flavivirus attachment factor DC-SIGNR.[23] Infected cells expressing green fluorescent protein were counted 24 h after infection by flow cytometry. The dilution of sera needed to neutralise half of infection events (EC_{50}) was estimated by non-linear regression with GraphPad Prism version 7. The initial dilution of sera (1:30) was set as the limit of detection of the assay; EC_{50} values of negative samples were reported as half the limit of detection (1:15). Positive antibody response was defined as a EC_{50} greater than or equal to 30.

T-cell response by intracellular cytokine staining

We used intracellular cytokine staining to assess T-cell responses, as previously described.[24] Briefly, cryopreserved peripheral-blood mononuclear cells were stimulated with overlapping peptide pools (length 15 aminoacids, overlapping by 11 aminoacids) for the Zika virus E protein, small envelope protein M, and peptide pr. Peripheral-blood mononuclear cells were collected at baseline, at the time of each vaccination, and 4 weeks after each vaccination. Data were analysed with FlowJo software (version 9.9.6, Treestar, Ashland, OR, USA). The proportions of total CD4 and CD8 T cells producing interleukin 2, interferon γ, tumour necrosis factor α, or a combination of these cytokines, were quantified. Boolean gating was done and all cytokine-positive gates were summed to calculate the total proportion of cytokine-positive cells responding to a peptide pool. For total vaccine responses, the proportions of cytokine-positive T cells responding to pooled peptides were summed. Groups were analysed with background-subtracted data for positive change from baseline.

Statistical analysis

We calculated sample size primarily on ability to identify serious adverse events. For VRC 319 we estimated that 20 participants per group would provide 90% power to detect at least one serious adverse event within a group if the true rate was not less than 0·109. For VRC 320 we estimated that 15 participants per group would provide 90% power to detect at least one serious adverse event within a group if the true rate was not less than 0·142.

	VRC 319 trial				VRC 320 trial		
	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	Group 4 (n=20)	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)
Men	6 (30%)	9 (45%)	14 (70%)	8 (40%)	6 (40%)	6 (40%)	8 (53%)
Women	14 (70%)	11 (55%)	6 (30%)	12 (60%)	9 (60%)	9 (60%)	7 (47%)
Age (years)							
Mean (SD)	26·8 (3·0)	28·6 (3·5)	27·9 (4·5)	28·4 (4·0)	30·7 (9·3)	30·5 (8·1)	32·3 (9·8)
Range	20–34	23–35	22–35	23–35	20–47	22–50	21–50
Race							
Asian	4 (20%)	0	2 (10%)	1 (5%)	6 (40%)	1 (7%)	1 (7%)
Black or African American	2 (10%)	6 (30%)	3 (15%)	2 (10%)	1 (7%)	1 (7%)	3 (20%)
White	14 (70%)	14 (70%)	13 (65%)	14 (70%)	8 (53%)	12 (80%)	9 (60%)
Multiracial	0		2 (10%)	3 (15%)	0	1 (7%)	2 (13%)
Ethnicity							
Non-Hispanic Latino	20 (100%)	20 (100%)	20 (100%)	19 (95%)	14 (93%)	11 (73%)	14 (93%)
Hispanic Latino	0	0	0	1 (5%)	1 (7%)	4 (27%)	1 (7%)
Body-mass index (kg/m²)							
Mean (SD)	25·8 (5·4)	27·6 (5·7)	26·4 (4·1)	25·3 (3·9)	26·7 (3·0)	24·5 (4·2)	25·2 (3·5)
Range	18·9–39·6	20·7–39·2	21·3–35·7	19·2–35·1	21·6–32·3	18·7–34·8	19·8–31·8

Table 1: **Baseline characteristics of participants**

We calculated group-wise magnitudes of antibody response as geometric mean titres (GMTs) with 95% CIs. We used a two-sample *t* test to compare group GMTs within and across trials. We compared magnitude of mean T-cell responses before and after vaccination by Wilcoxon's signed-rank test within groups and by Wilcoxon's rank sum test between groups. In accordance with the trial protocols, we made no adjustments for multiple comparisons in the analyses of immunogenicity because the trials were not powered to detect differences. We did all statistical analyses with R version 3.4.1. These trials are registered with ClinicalTrials.gov, numbers NCT02840487 and NCT02996461.

See Online for appendix

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had access to all the data in the studies and final responsibility for the decision to submit for publication.

Results

We enrolled 80 participants of 154 screened in VRC 319, from Aug 2, 2016, to Sept 29, 2016, and 45 of 105 in VRC 320, from Dec 12, 2016, to April 19, 2017 (figure 1). One participant from VRC 319 and two from VRC 320 withdrew after one dose of vaccine due to time commitments, precluding further trial participation, but were included in the safety analyses. Follow-up continues, and is expected to close in August, 2018, for VRC 319 and in February, 2019, for VRC 320. In VRC 319, the groups varied by sex and race, but age, body-mass index, and ethnicity were similar, whereas in VRC 320, only race varied notably (table 1).

Vaccinations were safe and well tolerated in both trials, with local and systemic reactogenic events for VRC5288 and VRC5283 being mild to moderate (appendix). In both studies, pain and tenderness at the injection site was the most frequent local event (37 [46%] of 80 participants in VRC 319 and 36 [80%] of 45 in VRC 320) and malaise and headache were the most frequent systemic events (22 [27%] and 18 [22%], respectively, in VRC 319 and 17 [38%] and 15 [33%], respectively, in VRC 320; table 2) One serious adverse event was reported, which was appendicitis 8 months after vaccination with VRC5288, but was deemed not to be related to vaccination.

The GMTs after vaccination with VRC5288 in VRC 319 were greater after three doses of vaccine than after two doses (table 3, appendix). Positive antibody responses ranged from 60% to 89% 4 weeks after final vaccination (figure 2). The highest GMT and the greatest antibody response and antibody titres were seen in group 4 participants after three doses of vaccine with an extended time between the second and third doses (table 3). After the third dose, the GMT was boosted to greater than the GMTs after the second dose in both three-dose groups ($p=0·0048$ for group 3 and $p<0·0001$ for group 4, figure 3).

The GMT achieved with VRC5283 in VRC 320 was substantially higher with needle-free injection in group 3 than with needle and syringe administration in groups 1 and 2 (table 3). Positive antibody response increased from single-dose needle and syringe administration (77%) to split-dose needle and syringe administration (93%) to split-dose needle-free syringe administration (100%; figure 2). The GMT of 304 in VRC 320 group 3 was the greatest across all groups in both studies ($p<0·0001$ *vs* groups 1–3 and $p=0·0028$ *vs* group 4 in VRC 319; $p=0·0015$

	VRC5288					VRC5283			
	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	Group 4 (n=20)	Overall (n=80)	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	Overall (n=45)
Local symptoms									
Pain/tenderness									
None	9 (45%)	11 (55%)	10 (50%)	13 (65%)	43 (54%)	4 (27%)	4 (27%)	1 (7%)	9 (20%)
Mild	11 (55%)	9 (45%)	10 (50%)	7 (35%)	37 (46%)	11 (73%)	10 (67%)	12 (80%)	33 (73%)
Moderate	0	0	0	0	0	0	1 (7%)	2 (13%)	3 (7%)
Swelling									
None	19 (95%)	20 (100%)	20 (100%)	20 (100%)	79 (99%)	15 (100%)	15 (100%)	12 (80%)	42 (93%)
Mild	1 (5%)	0	0	0	1 (1%)	0	0	3 (20%)	3 (7%)
Redness									
None	19 (95%)	20 (100%)	20 (100%)	16 (80%)	75 (94%)	15 (100%)	15 (100%)	14 (93%)	44 (98%)
Mild	1 (5%)	0	0	4 (20%)	5 (6%)	0	0	1 (7%)	1 (2%)
Any									
None	9 (45%)	11 (55%)	10 (50%)	11 (55%)	41 (51%)	4 (27%)	11 (73%)	1 (7%)	9 (20%)
Mild	11 (55%)	9 (45%)	10 (50%)	9 (45%)	3 (49%)	11 (73%)	3 (20%)	12 (80%)	33 (73%)
Moderate	0	0	0	0	0	0	1 (7%)	2 (13%)	3 (7%)
Systemic symptoms									
Malaise									
None	15 (75%)	14 (70%)	15 (75%)	14 (70%)	58 (73%)	10 (67%)	10 (67%)	8 (53%)	28 (62%
Mild	4 (20%)	5 (25%)	5 (25%)	6 (30%)	20 (25%)	4 (27%)	5 (33%)	6 (40%)	15 (33%)
Moderate	1 (5%)	1 (5%)	0	0	2 (3%)	1 (7%)	0	1 (7%)	2 (4%)
Myalgia									
None	17 (85%)	16 (80%)	16 (80%)	14 (70%)	63 (79%)	9 (60%)	14 (93%)	13 (87%)	36 (80%)
Mild	2 (10%)	3 (15%)	4 (20%)	5 (25%)	14 (18%)	4 (27%)	1 (7%)	1 (7%)	6 (13%)
Moderate	1 (5%)	1 (5%)	0	1 (5%)	3 (4%)	2 (13%)	0	1 (7%)	3 (7%)
Headache									
None	16 (80%)	16 (80%)	16 (80%)	14 (70%)	62 (78%)	10 (67%)	15 (100%)	10 (67%)	30 (67%)
Mild	4 (20%)	2 (10%)	4 (20%)	5 (25%)	15 (19%)	4 (27%)	0	5 (33%)	13 (29%)
Moderate	0	2 (10%)	0	1 (5%)	3 (4%)	1 (7%)	0	0	2 (4%)
Chills									
None	20 (100%)	18 (90%)	19 (95%)	17 (85%)	74 (93%)	15 (100%)	15 (100%)	13 (87%)	43 (96%)
Mild	0	1 (5%)	1 (5%)	3 (15%)	5 (6%)	0	0	1 (7%)	1 (2%)
Moderate	0	1 (5%)	0	0	1 (1%)	0	0	1 (7%)	1 (2%)
Nausea									
None	19 (95%)	18 (90%)	20 (100%)	16 (80%)	73 (91%)	14 (93%)	14 (93%)	15 (100%)	43 (96%)
Mild	1 (5%)	1 (5%)	0	4 (20%)	6 (8%)	1 (7%)	1 (7%)	0	2 (4%)
Moderate	0	1 (5%)	0	0 (%)	1 (1%)	0	0	0	0
Joint pain									
None	19 (95%)	20 (100%)	20 (100%)	17 (85%)	76 (95%)	12 (80%)	14 (93%)	12 (80%)	37 (82%)
Mild	1 (5%)	0	0	3 (15%)	4 (5%)	2 (13%)	1 (7%)	3 (20%)	7 (16%)
Moderate	0	0	0	0	0	1 (7%)	0	0	1 (2%)
Temperature									
None	20 (100%)	20 (100%)	20 (100%)	20 (100%)	80 (100%)	15 (100%)	15 (100%)	15 (100%)	45 (100%)
Any									
None	13 (65%)	13 (65%)	10 (50%)	12 (60%)	48 (60%)	6 (40%)	9 (60%)	6 (40%)	21 (47%)
Mild	6 (30%)	5 (25%)	10 (50%)	7 (35%)	28 (35%)	7 (47%)	5 (33%)	8 (53%)	20 (44%)
Moderate	1 (5%)	2 (10%)	0	1 (5%)	4 (5%)	2 (13%)	1 (7%)	1 (7%)	4 (9%)

VRC5288=VRC5288 plasmid backbone with Zika virus and Japanese encephalitis virus envelope protein E. VRC5283=VRC5283 plasmid backbone with wild-type Zika virus envelope protein E.

Table 2: **Local and systemic reactogenicity**

	GMT (95% CI)	Mean change in CD4 response from baseline (95% CI)				Mean change in CD8 response from baseline (95% CI)			
		E peptides	M peptides	pr peptides	Pooled peptides	E peptides	M peptides	pr peptides	Pooled peptides
VRC 319 trial plasmid VRC5288 (Zika virus/JEV chimera)									
Group 1	67 (40 to 114)	0·031 (0 to 0·061); p=0·0362	0·01 (–0·011 to 0·03)	0·012 (–0·006 to 0·029)	0·051 (–0·012 to 0·114)	0·013 (–0·005 to 0·032)	0·017 (–0·003 to 0·037)	0·023 (–0·004 to 0·051); p=0·0318	0·054 (–0·007 to 0·115)
Group 2	55 (39 to 78)	0·037 (0·005 to 0·069); p=0·0296	0·025 (–0·004 to 0·054)	0·022 (–0·012 to 0·055)	0·082 (–0·009 to 0·173)	0·023 (–0·016 to 0·061)	0·017 (–0·014 to 0·048)	0·017 (–0·018 to 0·051)	0·056 (–0·04 to 0·152)
Group 3	81 (51 to 127)	0·019 (0·002 to 0·037)	0·006 (–0·009 to 0·02)	0·009 (–0·005 to 0·024)	0·035 (0·001 to 0·069)	0·015 (–0·01 to 0·039)	0·01 (–0·011 to 0·031)	0·026 (0·014 to 0·038); p=0·0002	0·05 (0·005 to 0·096); p=0·0304
Group 4	120 (73 to 197)	0·041 (0·018 to 0·063); p=0·0012	0·005 (–0·009 to 0·018)	0·019 (–0·01 to 0·049)	0·064 (0·017 to 0·110); p=0·0108	0·055, p=0·0046 (0·024 to 0·087)	0·007 (–0·007 to 0·021)	0·024 (–0·001 to 0·049); p=0·0494	0·086 (0·039 to 0·134); p=0·0039
VRC 320 trial plasmid VRC5283 (wild-type Zika virus)									
Group 1	48 (28 to 83)	0·014 (–0·014 to 0·042)	–0·003 (–0·012 to 0·007)	–0·011 (–0·04 to 0·018)	0·002 (–0·049 to 0·052)	0·016 (–0·005 to 0·037)	0·002 (–0·01 to 0·013)	0·009 (–0·004 to 0·023)	0·027 (–0·007 to 0·061)
Group 2	150 (99 to 226)	0·036 (–0·007 to 0·08)	0·006 (–0·008 to 0·021)	0·007 (–0·012 to 0·025)	0·05 (–0·007 to 0·106); p=0·0353	0·008 (–0·043 to 0·06)	–0·009 (–0·032 to 0·015)	0·018 (–0·007 to 0·043)	0·016 (–0·053 to 0·085)
Group 3	304 (215 to 430)	0·083 (0·029 to 0·136); p=0·0001	0·014 (–0·006 to 0·035)	0·014 (–0·003 to 0·032)	0·111 (0·05 to 0·172); p=0·0004	0·091 (0·014 to 0·168); p=0·0004	–0·007 (–0·03 to 0·017)	0·029 (–0·037 to 0·094)	0·113 (0·016 to 0·21); p=0·0166

Values are displayed as group means; p values are given only for significant differences from baseline. GMT=geometric mean titre. E=envelope protein E. M=small envelope protein M. pr=peptide pr. JEV=Japanese encephalitis virus.

Table 3: **Neutralising antibody titres and T-cell responses 4 weeks after final vaccination**

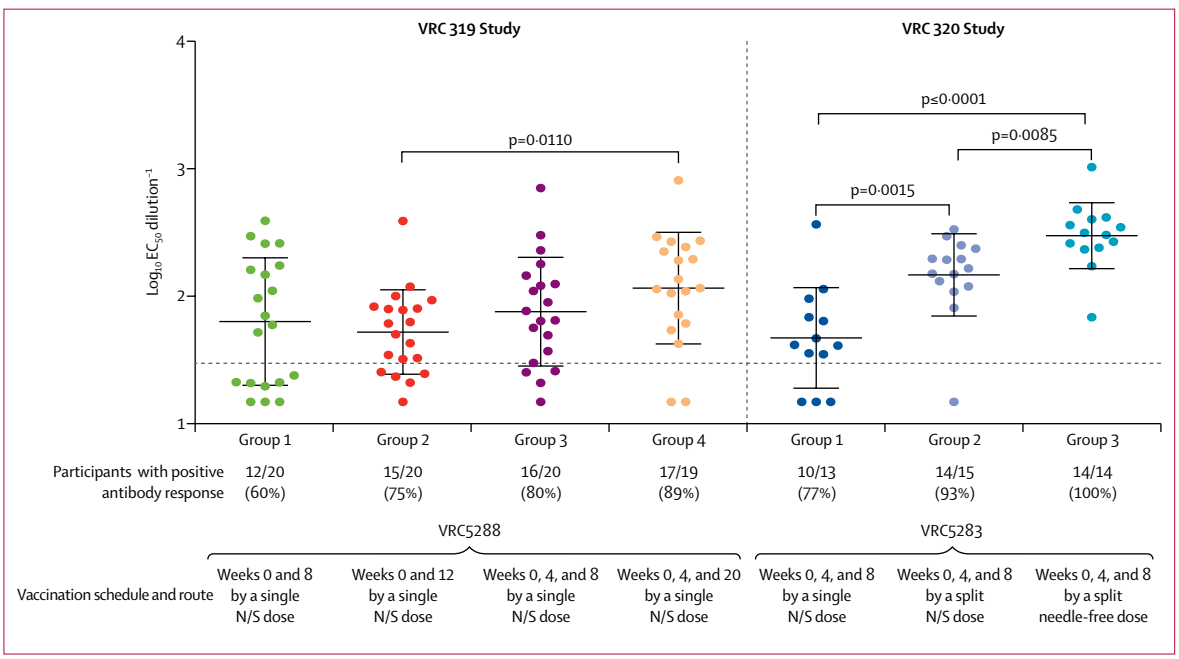

Figure 2: **Neutralising activity 4 weeks after last vaccination, measured by reporter virus particle assay**
In the VRC 319 study, samples were collected in week 12 for groups 1 and 3, week 16 for group 2, and week 24 for group 4. In the VRC 320 study, all samples were collected at week 12. Data are geometric mean titres and SDs derived from two to four independent assays per sample. The dotted line represents the limit of detection of the assay (dilution 1:30). Negative samples were reported as half the limit of detection (dilution 1:15). EC_{50}=dilution of sera required to neutralise half of infection events. N/S=needle and syringe.

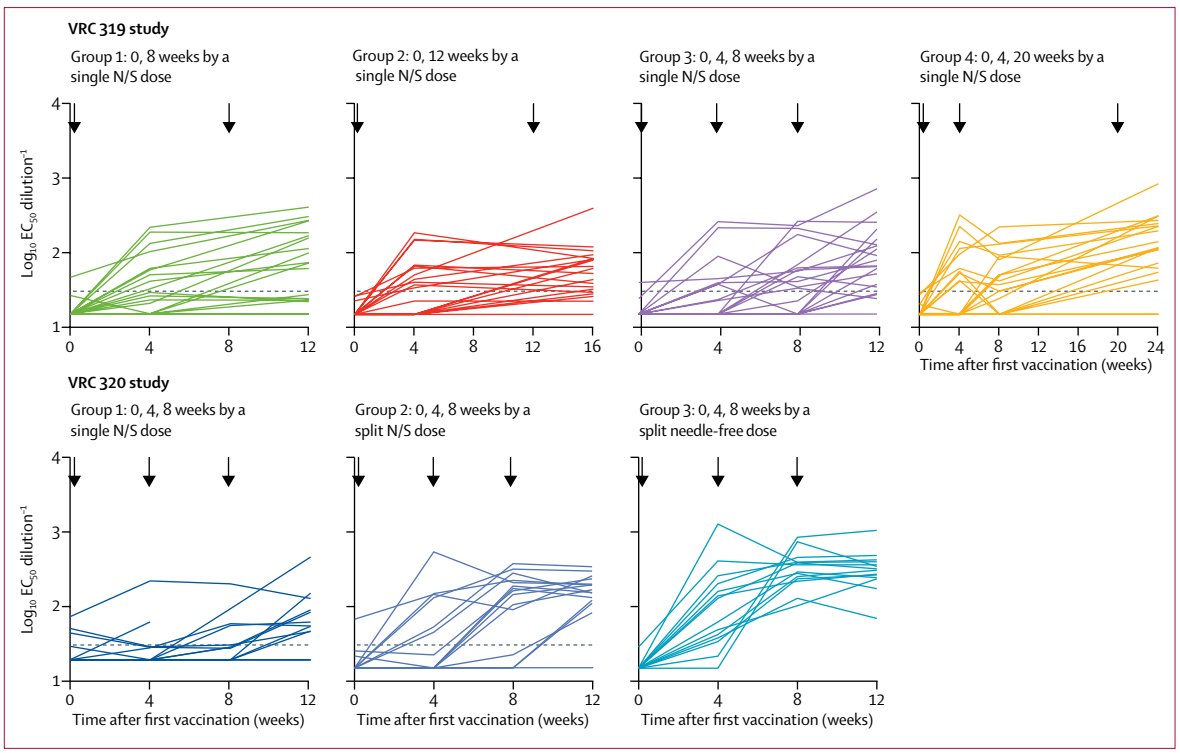

Figure 3: **Neutralising activity 4 weeks after each vaccination, measured by reporter virus particle assay**
Each line represents the EC_{50} of an individual participant over time. Arrows indicate the timings of vaccinations. Values shown are means of two to four independent assays per sample. The dotted line represents the limit of detection of the assay (dilution 1:30). Negative samples were reported as half the limit of detection (dilution 1:15). EC_{50}=dilution of sera required to neutralise half of infection events. N/S=needle and syringe.

vs group 1 and p=0·0085 *vs* group 2 in VRC 320; appendix). Split-dose administration of vaccine with needle and syringe also improved GMT compared with single-dose administration via the same method (appendix). In the two groups receiving VRC5283 by needle and syringe, the antibody levels were higher after splitting the dose (p=0·0015 for group 2). Boosting with the third dose only significantly increased the GMT to greater than that after the second dose in group 1 (p=0·0016). EC_{80} results are shown in the appendix.

4 weeks after last vaccination with VRC5288 in VRC 319, in group 4, T-cell responses to pooled peptides were significantly increased (CD4 p=0·0108 and CD8 cells p=0·0039) compared with baseline (table 3, figure 4). Group 3 showed increased CD8 (p=0·0304) responses to pooled peptides. The greatest T-cell responses overall were seen 4 weeks after needle-free administration of VRC5283 in VRC 320 (table 3, figure 4). CD8 cell counts in participants who received VRC5283 via needle-free injection had increased total cytokine responses compared with baseline for pooled peptides (p=0·0166) and specifically for E-protein peptides (p=0·0004, appendix). CD4 cell counts from this group were also increased with pooled peptides (p=0·0004), again specifically for E-protein peptides (p=0·0001, appendix). VRC5283 given in split doses via needle and syringe also produced a significant CD4 response to pooled peptides (p=0·0353), but not a significant CD8 response. There were no significant responses to small envelope protein M or pr peptide.

Discussion

The emergence of Zika virus challenged the scientific community to address a relatively uncharacterised pathogen posing a substantial threat to international public health. Although symptoms of Zika virus infection are typically mild, infection during pregnancy is associated with a high teratogenic risk. Moreover, sexual transmission by travellers to endemic regions might extend the epidemic to non-endemic regions without requiring a mosquito vector. Rapid development of vaccines has, therefore, been started. The two DNA vaccines we assessed were safe and well tolerated, with most adverse events being mild. Both vaccines were immunogenic, but the greatest effects were seen with VRC5283 given in split doses via needle-free injection in the VRC 320 trial.

Despite the novelty of Zika virus, previous knowledge of flavivirus biology and immunity has pointed towards likely immunogenicity correlates and facilitated development of Zika virus vaccines. For example, neutralising activity is an established correlate of protection for most licensed flavivirus vaccines.[25] In studies of VRC5288 and VRC5283 in non-human primates, neutralising activity correlated with protection against viraemia following

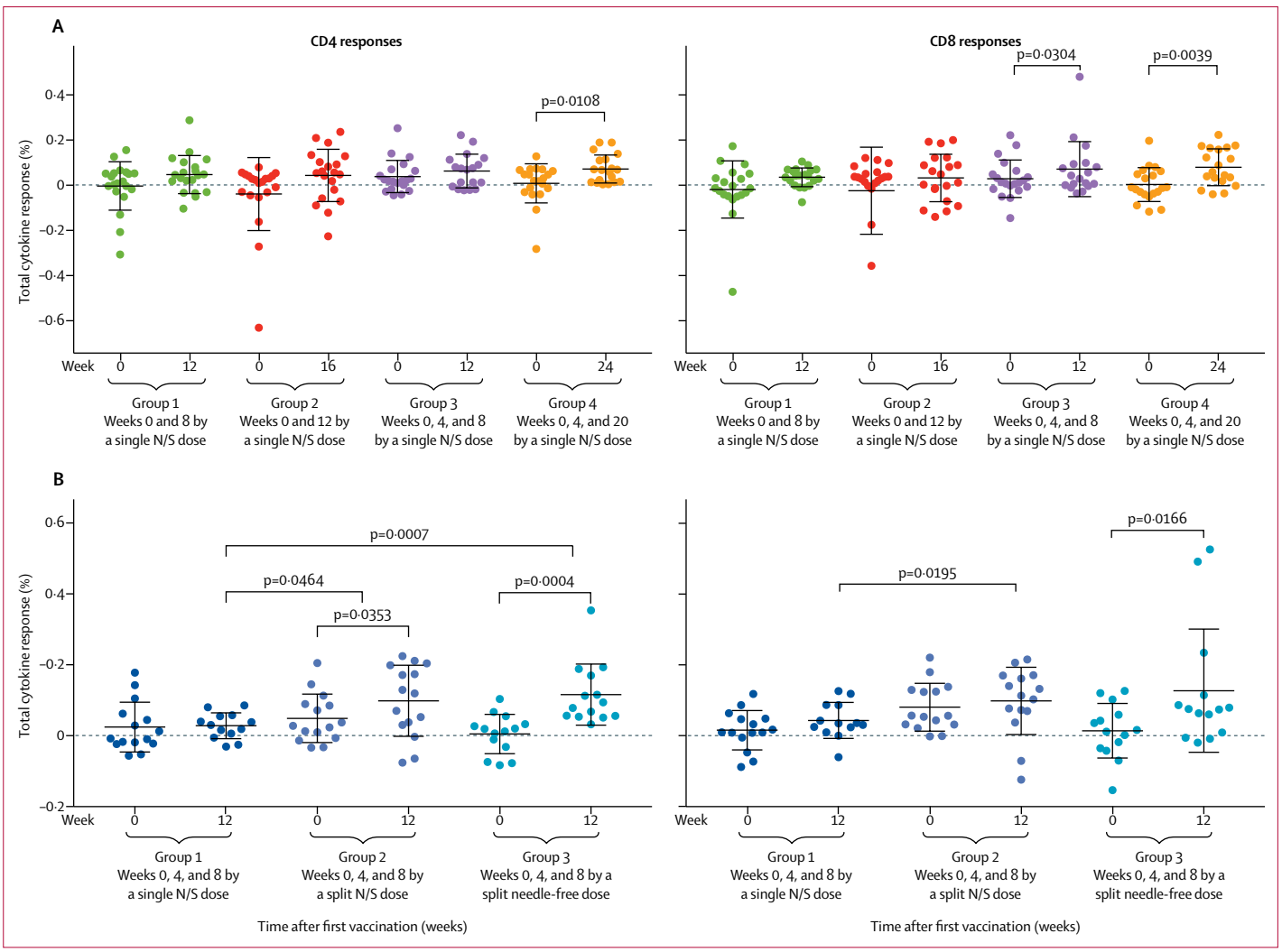

Figure 4: **Immunogenicity at baseline and 4 weeks after last vaccination, measured by intracellular cytokine staining showing T-cell responses**
(A) VRC 319 study. (B) VRC 320 study. Data are group arithmetic mean proportions and SDs of total T cells producing interleukin 2, interferon γ, tumour necrosis factor α, or a combination of these cytokines, against pooled envelope protein E, small envelope protein M, and peptide pr. N/S=needle and syringe.

vaccination and Zika virus challenge.[22] Additionally, the role of antibody-mediated protection against Zika virus infection is supported by protection against infection after adoptive transfer of purified IgG from mice vaccinated with a DNA vaccine against Zika virus into CD4 and CD8 T-cell-depleted mice.[26] In addition to humoral immunity, cellular immunity might be relevant, particularly because CD8 T-cell responses have been detected in human beings after flavivirus infections.[27–29] With this in mind, the DNA vaccine platform has an advantage over protein-based vaccines because it induces antibody and substantial T-cell responses. CD4 T-cell responses are necessary for optimum memory B-cell response, and CD8 T cells are thought to facilitate viral clearance important to fetal protection.[30]

Based on our findings, VRC5283 was more promising than VRC5288 to advance into later stage development because 100% (14 of 14) of participants who received the vaccine by needle-free injection in split doses had detectable antibody responses and had neutralising antibody and T-cell responses of the greatest magnitude. Additionally, CD4 and CD8 T-cell responses were greater with VRC5283 than VRC5288. Since VRC5288 encodes the Japanese encephalitis virus E transmembrane sequence, some cellular immune responses induced by vaccination might have been specific to this virus and not captured by our intracellular cytokine staining assay or might not have been able to respond to Zika virus. The wild-type Zika virus E transmembrane protein in the VRC5283 plasmid might, therefore, elicit cellular immune responses to this region, giving this vaccine a further advantage.

Immune responses differed by vaccine delivery method. Needle-free injectors are known to augment

DNA vaccine response, possibly by increased dispersion of injectate or through tissue damage that increases immunogenicity.[31] The GMT after split-dose needle-free delivery of VRC5283 was six times higher than that after single-dose delivery via needle and syringe. The number of injections was also important, as the GMT achieved after VRC5283 was given in split doses via needle and syringe was two to three times greater than those seen with VRC5288 or VRC5283 given in single doses by the same vaccination schedule. The VRC 319 trial used only needle and syringe delivery and, therefore, we could not compare specific variables responsible for different outcomes between the two trials. In non-human primate studies, however, VRC5288 and VRC5283 were both administered by needle-free injection, and VRC5283 prevented viraemia more effectively than VRC5288.[22]

The antibodies induced by each vaccine are being investigated for qualitative differences that might contribute to their differing protective capacity. Additionally, dose-reduction studies for VRC5283 in non-human primates are being done to define the serological correlate of protection from viraemia. Based on all the available information and the need for a shortened assessment schedule, VRC5283 has been advanced into an international placebo-controlled phase 2 efficacy trial of vaccination at 0, 4, and 8 weeks via needle-free delivery with the Stratis device (NCT03110770). This study aims to assess safety, immunogenicity, and efficacy in populations in regions in South and Central America, the Caribbean, and the USA endemic for Zika virus.

The basis for comparing the serological correlate of protection from viraemia in non-human primates and vaccine immunogenicity in human volunteers is serum neutralising activity measured by the reporter virus particle assay, which yields highly reproducible and sensitive results. This assay measures inhibition of viral entry across a large dynamic range. Moreover, it has been used extensively to define mechanisms of flavivirus neutralisation and has been a reliable endpoint for determining immune correlates in studies of non-human primates[22] and human beings.[16] The assay correlates with traditional measurements of neutralisation, such as the plaque reduction neutralisation test, that have been used in assessments of previously licensed flavivirus vaccines. The plaque reduction neutralisation test and the microneutralisation assay, however, assess entry and spread of replicating virus in cell culture, and might be unable to detect replication-competent virus that is not neutralised at any concentration. In a phase 1 trial of another Zika virus DNA vaccine, neither the plaque reduction neutralisation test nor the microneutralisation assay predicted whether passively transferred human sera would protect mice against Zika virus challenge.[32] Ultimately, efficacy data from field trials will be needed to establish reliable correlates of protection that can be used to refine sufficient doses and schedules for effective immunity.

Much still needs to be done before any vaccine against Zika virus can be deployed. Human trials so far show data from healthy populations suitable for open-label phase 1 trials. Randomsied placebo-controlled efficacy trials in volunteers from regions where Zika virus and other flaviviruses are endemic are still needed. An important limitation in the development of any Zika virus vaccine is the lack of established correlates of protection from fetal malformations. Whether established correlates of protection from clinical disease in non-teratogenic flaviviruses will be predictive of prevention of fetal disease with Zika virus is unknown. Whether completely preventing viraemia, reducing peak viraemia, or preventing persistent viraemia in pregnant mothers will provide sufficient fetal protection is also unknown. Similar to the situation for congenital rubella syndrome in the 1960s, sustained epidemiological surveillance of large populations of vaccinated individuals will be needed to confirm effects on congenital infection.

A limitation of this study is the small number of participants. This is, however, typical of phase 1 studies.

Our studies of two ZIKV DNA vaccines, VRC5288 and VRC5283, advance the effort to quickly curb the effects of the Zika epidemic. VRC5283 showed the most robust neutralising antibody and T-cell responses, and has been advanced into an international phase 2 efficacy trial. Several other vaccine approaches are also being pursued. Findings from a human study of another DNA vaccine have been reported,[32] and an inactivated vaccine is being assessed in human beings (NCT02963909). Differing vaccines might be designed for distinct target populations, provide various immune response patterns, and varying durability. As the joint efforts continue and knowledge of the immune response to Zika virus deepens, guidance on developing a definitive solution to the epidemic will improve.

Contributors

MRG is the principal investigator of VRC 319. GLC is the principle investigator of VRC 320. CSH, LAH, SE, MAM, NGR, KEL, GEC, and BSG were study investigators. ZH did the statistical analysis. GY, RSR, NB, IJG, JHC, SS, CL, RMS, JRM, BSG, TCP, JEL, and GLC designed the clinical trials. MRG, FM, JGS, LN, CSH, LAH, IJG, SE, MAM, NGR, KEL, and GEC collected data. MRG, KVH, KMM, JHC, RTB, BMF, KB, RSP, DNG, CRD, KAD, JRM, BSG, TCP, JEL, and GLC analysed and interpreted the data. All authors contributed to the writing of the report and approved the final version.

Declaration of interests

We declare no competing interests.

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