



Safety and immunogenicity of a Zika purified inactivated virus vaccine given via standard, accelerated, or shortened schedules: a single-centre, double-blind, sequential-group, randomised, placebo-controlled, phase 1 trial

Kathryn E Stephenson, Chen Sabrina Tan, Stephen R Walsh, Andrew Hale, Jessica L Ansel, Diane G Kanjilal, Kate Jaegle, Lauren Peter, Erica N Borducchi, Joseph P Nkolola, Tatenda Makoni, Rachel Fogel, Connor Bradshaw, Anna Tyler, Edward Moseley, Abishek Chandrashekar, Katherine E Yanosick, Michael S Seaman, Kenneth H Eckels, Rafael A De La Barrera, Jason Thompson, Peter Dawson, Stephen J Thomas, Nelson L Michael, Kayvon Modjarrad, Dan H Barouch

Summary

Background The development of an effective vaccine against Zika virus remains a public health priority. A Zika purified inactivated virus (ZPIV) vaccine candidate has been shown to protect animals against Zika virus challenge and to be well tolerated and immunogenic in humans up to 8 weeks of follow-up. We aimed to assess the safety and immunogenicity of ZPIV in humans up to 52 weeks of follow-up when given via standard or accelerated vaccination schedules.

Methods We did a single-centre, double-blind, randomised controlled, phase 1 trial in healthy adults aged 18–50 years with no known history of flavivirus vaccination or infection at Beth Israel Deaconess Medical Center in Boston, MA, USA. Participants were sequentially enrolled into one of three groups: ZPIV given at weeks 0 and 4 (standard regimen), weeks 0 and 2 (accelerated regimen), or week 0 alone (single-dose regimen). Within each group, participants were randomly assigned using a computer-generated randomisation schedule to receive an intramuscular injection of 5 µg ZPIV or saline placebo, in a ratio of 5:1. The sponsor, clinical staff, investigators, participants, and laboratory personnel were masked to treatment assignment. The primary endpoint was safety up to day 364 after final dose administration, and secondary endpoints were proportion of participants with positive humoral immune responses (50% microneutralisation titre [MN_{50}] ≥ 100) and geometric mean MN_{50} at observed peak response (ie, the highest neutralising antibody level observed for an individual participant across all timepoints) and week 28. All participants who received at least one dose of ZPIV or placebo were included in the safety population; the analysis of immunogenicity at observed peak included all participants who received at least one dose of ZPIV or placebo and had any adverse events or immunogenicity data after dosing. The week 28 immunogenicity analysis population consisted of all participants who received ZPIV or placebo and had immunogenicity data available at week 28. This trial is registered with ClinicalTrials.gov, NCT02937233.

Findings Between Dec 8, 2016, and May 17, 2017, 12 participants were enrolled into each group and then randomly assigned to vaccine (n=10) or placebo (n=2). There were no serious or grade 3 treatment-related adverse events. The most common reactions among the 30 participants who received the vaccine were injection-site pain (24 [80%]), fatigue (16 [53%]), and headache (14 [46%]). A positive response at observed peak titre was detected in all participants who received ZPIV via the standard regimen, in eight (80%) of ten participants who received ZPIV via the accelerated regimen, and in none of the ten participants who received ZPIV via the single-dose regimen. The geometric mean of all individual participants' observed peak values was 1153.9 (95% CI 455.2–2925.2) in the standard regimen group, 517.7 (142.9–1875.6) in the accelerated regimen group, and 6.3 (3.7–10.8) in the single-dose regimen group. At week 28, a positive response was observed in one (13%) of eight participants who received ZPIV via the standard regimen and in no participant who received ZPIV via the accelerated (n=7) or single-dose (n=10) regimens. The geometric mean titre (GMT) at this timepoint was 13.9 (95% CI 3.5–55.1) in the standard regimen group and 6.9 (4.0–11.9) in the accelerated regimen group; antibody titres were undetectable at 28 weeks in participants who received ZPIV via the single-dose regimen. For all vaccine schedules, GMTs peaked 2 weeks after the final vaccination and declined to less than 100 by study week 16. There was no difference in observed peak GMTs between the standard 4-week and the accelerated 2-week boosting regimens (p=0.4494).

Interpretation ZPIV was safe and well tolerated in humans up to 52 weeks of follow-up. ZPIV immunogenicity required two doses and was not durable. Additional studies of ZPIV to optimise dosing schedules are ongoing.

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Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA, USA (K E Stephenson MD, C S Tan MD, S R Walsh MD, J L Ansel BSN, D G Kanjilal BSN, K Jaegle BSN, L Peter BA, E N Borducchi PhD, J P Nkolola PhD, T Makoni BS, R Fogel BA, C Bradshaw BS, A Tyler BS, E Moseley BA, A Chandrashekar MS, K E Yanosick BS, M S Seaman PhD, Prof D H Barouch MD); Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard, Cambridge, MA, USA (K E Stephenson, Prof D H Barouch); Walter Reed Army Institute of Research, Silver Spring, MD, USA (K H Eckels PhD, R A De La Barrera MS, Prof S J Thomas MD, Prof N L Michael MD, K Modjarrad MD); Emmes, Rockville, MD, USA (J Thompson MS, P Dawson PhD); University of Vermont Medical Center, Burlington, VT, USA (A Hale MD); and Lerner College of Medicine, Burlington, VT, USA (A Hale MD)

Correspondence to:
Prof Dan H Barouch, Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA
dbarouch@bidmc.harvard.edu

Research in context

Evidence before this study

The 2015–16 Zika virus epidemic in the Americas prompted an urgent international effort to develop a safe and effective Zika vaccine. In 2016, the Walter Reed Army Institute of Research, Silver Spring, MD, USA, designed and manufactured a Zika purified inactivated virus (ZPIV) vaccine to be administered with aluminium hydroxide gel adjuvant. In preclinical studies, ZPIV was shown to protect mice and monkeys from Zika virus challenge, particularly when Zika-specific neutralising antibody titres were greater than 1/100. Three ZPIV clinical trials commenced in November, 2016, all of which included a two-dose, prime-boost regimen with vaccine given on week 0 and week 4. Interim results for the two-dose regimen from all three studies were published in December, 2017, and showed that ZPIV was safe and immunogenic in humans up to 8 weeks of follow-up. To search the literature for Zika virus vaccine candidates other than ZPIV, we searched PubMed for articles published in English up to Jan 3, 2020, using the terms “Zika”, “vaccine”, and “Zika vaccine”. We found two articles reporting the interim results of Zika vaccine clinical trials in humans. In these reports, three different DNA vaccines expressing pre-membrane and envelope Zika virus proteins were shown to be safe and immunogenic up to 24 weeks of follow-up. To date, there are no published reports of any completed Zika vaccine trial in humans (using ZPIV, DNA, or other platforms), nor any published reports of the safety and immunogenicity of alternative vaccine schedules of ZPIV in humans.

Added value of this study

We present the final results of the ZPIV clinical trial done at Beth Israel Deaconess Medical Center, Boston, MA, USA. In addition to testing a two-dose ZPIV schedule at week 0 and week 4, we examined the safety and immunogenicity of a one-dose vaccine schedule (at week 0) and a two-dose schedule at week 0 and week 2. The aim of our study was to ascertain whether an accelerated ZPIV schedule could induce Zika virus-specific immune responses more rapidly than could the standard vaccine schedule, without loss of magnitude or durability. We found that ZPIV was safe and well tolerated across regimens up to 52 weeks of follow-up. Vaccine immunogenicity required two doses, and the immunogenicity of the two boosting intervals was similar, with nearly all participants developing neutralising antibody titres higher than 1/100 at peak concentrations. The accelerated two-dose regimen appeared to have faster antibody decline than the standard regimen; however, by week 28, all groups had near baseline titres.

Implications of all the available evidence

The evidence suggests that ZPIV is safe and well tolerated in humans, and when given in a two-dose regimen can elicit Zika virus-specific antibody titres that are predicted to be protective on the basis of animal models. An accelerated schedule with a 2-week boost could be a feasible option in an outbreak setting if rapid induction of immune responses is desired. However, a single dose of ZPIV would not be sufficient to elicit protective immunity. Additional boosts or higher doses of ZPIV than tested in this study will probably be required to improve durability.

Introduction

Mosquito-borne flaviviruses such as Zika virus, dengue virus, and yellow fever virus cause repeated outbreaks with substantial morbidity and mortality.^{1–3} The 2015–16 Zika virus epidemic in the Americas was particularly notable for congenital Zika syndrome, in which thousands of children were born with severe developmental problems as a result of prenatal Zika virus infection.^{4–6} The development of a safe and effective Zika vaccine is therefore a global public health priority.^{7,8} After the epidemic began, several vaccine candidates were rapidly moved into clinical trials, including a Zika purified inactivated virus (ZPIV) vaccine, which is a formalin-inactivated whole virus vaccine produced by Walter Reed Army Institute of Research, Silver Spring, MD, USA. Three trials of ZPIV were initiated to address unique questions (NCT02952833, NCT02937233, NCT02963909), with all three including a two-dose, prime-boost regimen with ZPIV given at weeks 0 and 4. Interim results for this two-dose regimen from all three studies were combined and published together in December, 2017; these results showed that ZPIV is safe and immunogenic up to 8 weeks of follow-up.⁹

Here, we present the final results of the ZPIV clinical trial done at Beth Israel Deaconess Medical Center,

Boston, MA, USA. The focus of this study was to explore the ZPIV vaccination schedule. In addition to testing a two-dose ZPIV schedule at weeks 0 and 4, which is a standard regimen for purified inactivated flavivirus vaccines,^{10–12} we also examined the safety and immunogenicity of a single-dose regimen and an accelerated two-dose regimen with ZPIV given at weeks 0 and 2. We aimed to ascertain whether the accelerated ZPIV schedule could induce Zika virus-specific immune responses more rapidly than could the standard vaccine schedule, without loss of magnitude or durability. In an outbreak setting, it is essential to elicit protective immunity as quickly as possible; thus, an accelerated vaccine schedule could be beneficial to the general population living in a Zika virus outbreak, as well as to first responders who are deployed to those areas. A single-dose regimen would have the added benefit of not requiring any follow-up visits.

Methods

Study design and participants

We did a single-centre, randomised, double-blind, sequential-group, placebo-controlled trial at Beth Israel Deaconess Medical Center in Boston, MA, USA. Eligible participants were healthy, aged 18–50 years, and had no

known history of flavivirus infection or previous receipt of flavivirus vaccine. Full eligibility criteria are described in the appendix (pp 220–22). All participants gave written informed consent and successfully completed a test of understanding before the initiation of study procedures. The protocol was approved by the Beth Israel Deaconess Medical Center Institutional Review Board and the Human Research Protection Office of the US Army Medical Research and Materiel Command.

Randomisation and masking

Participants were enrolled sequentially into a group that received the standard regimen, a group that received the accelerated regimen, and a group that received the single-dose regimen. Within each group, participants were randomly assigned in a ratio of 5:1 to a subgroup vaccine or placebo using a computer-generated randomisation schedule that was prepared before the study. Once patient eligibility for the trial was confirmed, each participant was randomly assigned in the electronic database using randomised treatment assignments generated by the Data Management Center (Emmes, Rockville, MD, USA). After successful randomisation, a masked allocation number was provided. The Data Management Center created the randomisation table and the sponsor monitored the implementation of this process. The masked allocation number was referenced against a confidential list provided to an unmasked on-site pharmacist to determine the treatment allocation. The sponsor, clinical staff, investigators, participants, and laboratory personnel were masked to subgroup assignment until study completion. There was no masking regarding schedule assignment. The pharmacist with primary responsibility for study product preparation and dispensing was not masked to the schedule or subgroup assignment. To preserve blinding, the pharmacist placed an overlay on the syringes.

Procedures

ZPIV contains a formalin-inactivated Zika virus strain (PRVABC59) purified on a chromatographic column that includes all Zika virus structural proteins and genomic RNA. Participants assigned to the standard regimen received two doses of vaccine or placebo, one each at weeks 0 and 4; those assigned to the accelerated regimen received two doses of vaccine or placebo, one each at weeks 0 and 2; and those assigned to the single-dose regimen received one dose of vaccine or placebo at week 0. The dose was 5 µg ZPIV and 500 µg aluminium hydroxide adjuvant (Alhydrogel; Brentag Biosector, Frederikssund, Denmark) per 0.5 mL injection. Placebo consisted of 0.9% saline per 0.5 mL injection. All study products were given as intramuscular injections into the deltoid (left or right). No local or topical anaesthetic was used before the injections.

Local and systemic reactogenicity safety data were collected for 7 days after each vaccine or placebo administration (schedule of procedures in appendix pp 262–64).

Data on unsolicited adverse events were collected after the first vaccination until 28 days after the last vaccination. Neurological and neuroinflammatory disorders (eg, Guillain-Barré syndrome) were considered adverse events of special interest. Data on adverse events of special interest and serious adverse events were collected up to study day 364. Blood samples for serum chemistry and haematology analyses were collected at nine to 12 timepoints throughout the study (depending on schedule assignment), as were urine samples for pregnancy testing and urinalysis (appendix pp 262–64). A protocol safety review team, an independent safety monitoring committee, and an independent research monitor appointed by the US Department of Defense monitored the safety data. Local and systemic adverse events were graded with the adult toxicity table from the National Institute of Allergy and Infectious Diseases' Division of Microbiology and Infectious Diseases.¹³

Approximately 85–100 mL of peripheral blood was collected to measure Zika virus-specific immune responses at baseline, 2 and 4 weeks after each vaccination, and at study weeks 16, 28, 40, and 52. Serum neutralising antibody titres against Zika virus were measured with a ZPIV microneutralisation assay using the vaccine-matched Zika virus Puerto Rico PRVABC59 strain, as previously described.^{9,14,15} Neutralisation titres are expressed as 50% microneutralisation titres (MN₅₀), which is the reciprocal of the serum dilution capable of neutralising 50% of the test virus dose. Serum collected at baseline (day 1) was retrospectively screened for Zika virus, dengue virus, yellow fever virus, Japanese encephalitis virus, and West Nile virus. Serum IgG antibodies to Zika virus Env and NS1 were measured with commercially available ELISAs (Alpha Diagnostics, San Antonio, TX, USA), as previously described,¹⁶ as well as in-house at Beth Israel Deaconess Medical Center using Env and NS1 proteins from the SPH2015 strain provided by Alpha Diagnostics at 2 and 4 weeks after each immunisation. T-cell responses specific to Zika virus were measured with interferon γ enzyme-linked immunospot assays, as previously described,¹⁷ using pools of Zika virus Cap, ENV, NS1, and prM peptides (JPT Peptide Technologies, Berlin, Germany). The threshold for a positive enzyme-linked immunospot assay response was the mean plus two SDs of a Zika virus-negative sample set obtained from Research Blood Components, Watertown, MA, USA. For a full description of the immunogenicity assays, see appendix pp 239–40.

Outcomes

The primary endpoints were incidence, intensity, and relationship to vaccination of solicited local and systemic adverse events during the 7-day follow-up period (days 1–7) after each ZPIV dose; incidence, intensity, and relationship to vaccination of unsolicited adverse events during the 28-day follow-up period (days 1–29) after each ZPIV dose; grade 2 and grade 3 laboratory abnormalities on day 8 after each vaccine dose; and incidence of serious adverse events

See Online for appendix

(including adverse events of special interest) and treatment-related adverse events from day 1 to day 364. The secondary endpoints were proportion of participants per dose subgroup with positive humoral immune responses and geometric mean MN_{50} titres at observed peak immunogenicity (ie, the highest neutralising antibody concentration observed for an individual participant across all timepoints) and at 6 months from day 1, and Zika Env-specific and NS1-specific ELISA titres at observed peak immunogenicity and at 6 months from day 1. At study initiation, analysis of the endpoints at 6 months was planned to take place on day 180 (week 26), but this timepoint was amended on Feb 27, 2017, to day 196 (week 28) to harmonise timepoints with other concurrent trials on the ZPIV vaccine (NCT02952833, NCT02963909). A positive response was defined as an MN_{50} of 100 or more; this titre has been shown to be the threshold for complete protection in previous animal studies in mice and non-human primates in which adoptive transfer of vaccine-elicited antibodies at an MN_{50} of 60 protected against Zika viraemia.^{8,14,15}

Additional endpoints included plaque reduction neutralisation test titres, durability and kinetics of humoral immune responses (up to day 364), and cellular immunogenicity.

Statistical analysis

The sample size was not determined by formal hypothesis testing; rather, it was selected to provide an assessment of the safety and tolerability of the different vaccine or placebo regimens. The statistical analysis of safety data included all randomised participants who received at least one dose of vaccine or placebo. The analysis of immunogenicity at observed peak antibody titres included all participants who received at least one dose of ZPIV or placebo and had any adverse events or immunogenicity data after dosing. The week 28 immunogenicity analysis population consisted of all participants who received ZPIV or placebo and had immunogenicity data available at week 28.

For each vaccine or placebo regimen, the number and proportion of participants who had adverse events, serious adverse events, or laboratory abnormalities were tabulated and summarised by individual and pooled subgroups, with the denominator including only participants with data available. Immunogenicity outcomes were described using geometric mean titres (GMTs) and 95% CIs. Placebo recipients across all groups were pooled into a single placebo subgroup for analyses.

Peak responses after vaccination were compared between the three vaccine subgroups and the collapsed placebo subgroup using Kruskal-Wallis tests (significance level 0.05); pairwise subgroup comparisons were done using Wilcoxon rank sum tests, without multiplicity adjustments. Missing data were not imputed. SAS (version 9.4) was used for the final statistical analysis.

Two prespecified, blinded interim analyses of safety and immunogenicity (including assay feasibility) were done for each group once data had been collected 28 days and

6 months after the final vaccination. The results of the interim analyses were reported by the Data Management Center to the sponsor on April 25, 2017 (immunogenicity data for the standard regimen), May 5, 2017 (safety data for the standard regimen), Sept 14, 2017 (safety data for the accelerated and single-dose regimens), and May 8, 2018 (safety and immunogenicity data for all groups). A final analysis was done when day 364 data had been collected (including some delayed 6-month immunogenicity data for the single-group regimen) and reported on Sept 28, 2018. Data up to 8 weeks of follow-up for the standard regimen were included in a preliminary report published on Dec 4, 2017.⁹ This study is registered with ClinicalTrials.gov, NCT02937233.

Role of the funding source

The funders were involved in the study design, study operations, data collection, data analysis, data interpretation, and review and edit of the manuscript. The ZPIV programme leads (KM and NLM), Beth Israel Deaconess Medical Center study leads (KES and DHB), and Emmes data and statistical leads (JT and PD) had access to all the data. The Beth Israel Deaconess Medical Center study leads had final responsibility for the decision to submit for publication.

Results

Enrolment took place between Dec 8, 2016, and May 17, 2017, and the last study visit was completed on June 4, 2018. 73 individuals were screened for eligibility, of whom 32 were ineligible and five were eligible but could not be included because they were lost to follow-up (figure 1). The most common reason for ineligibility was that individuals were not available for the duration of the trial (appendix p 2). 36 participants were eligible and 12 were enrolled into each study group and randomly assigned to receive vaccine or placebo (figure 1). One participant in the single-dose group who received placebo voluntarily withdrew on day 7 and did not contribute any immunogenicity data after vaccination. Baseline characteristics for that participant are reported, but the individual was excluded from timepoints and peak calculations after vaccination.

Demographics of the study population are described in table 1. Age, ethnicity, and race were similar across groups. Four participants were seropositive for flavivirus infection on retrospective serological testing. One vaccine recipient in the standard regimen group was positive for all four serotypes of dengue virus, as well as for West Nile virus. Two vaccine recipients in the accelerated regimen group were positive for dengue virus serotype 2 (n=1) or yellow fever virus (n=1). One participant in the standard schedule who received placebo was positive for Japanese encephalitis virus.

Data for one vaccine recipient in the single-dose group was unblinded on June 12, 2017, because of public health concerns. The participant donated

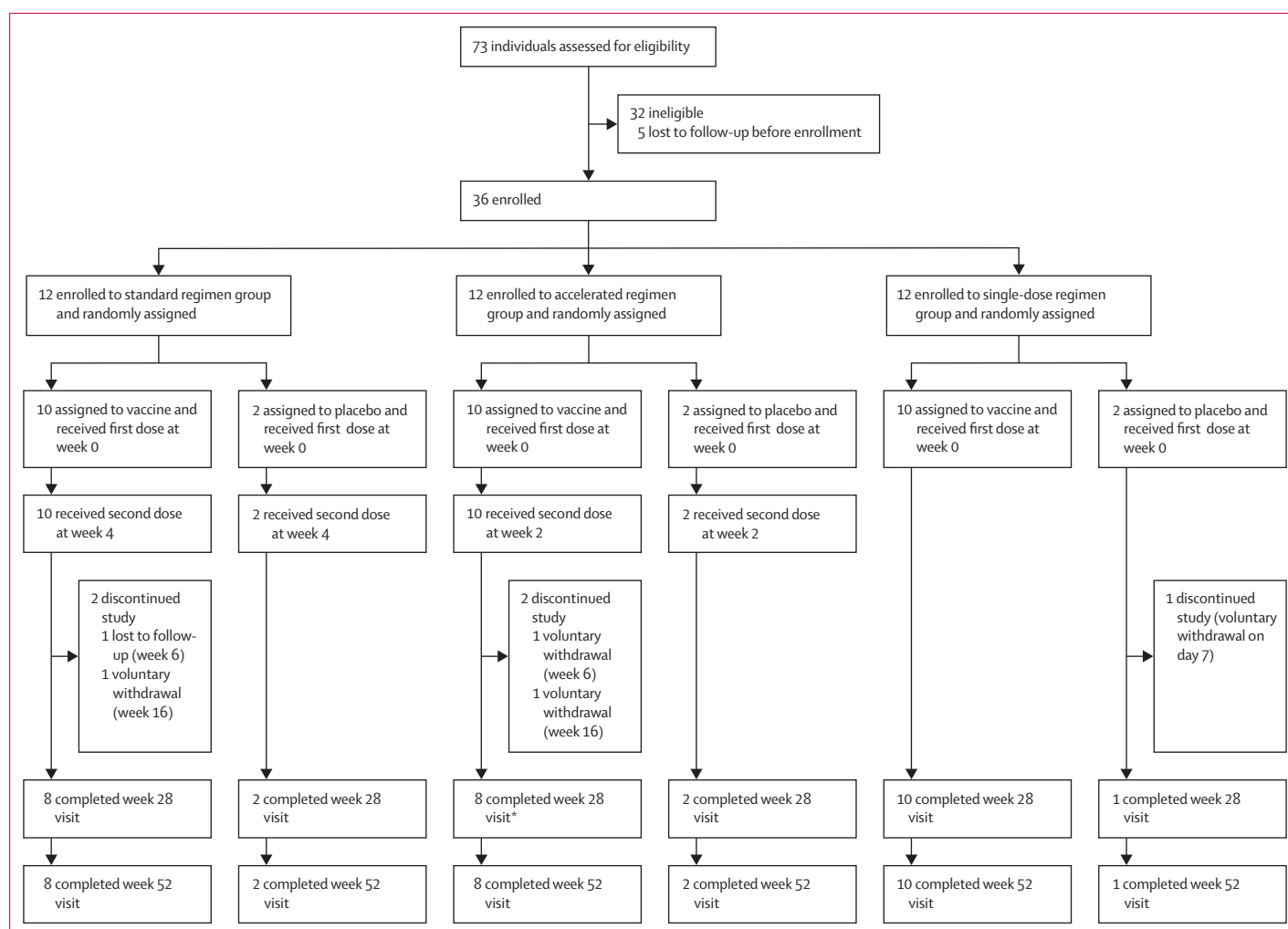


Figure 1: Trial profile

*Seven completed week 28 visit, one missed visit but did not discontinue study.

platelets 6 h after ZPIV administration, and the American Red Cross detected Zika virus RNA in the donated sample using the Hologic Procleix Zika Virus Assay (an investigational ultrasensitive nucleic acid test).¹⁸ The study team was made aware of the donation by the Blood Safety Taskforce at the US Centers for Disease Control and Prevention after an investigation by the Massachusetts Department of Public Health into the donor's potential Zika exposures. The consent form the participant had signed stipulated that no blood or other body tissues should be donated during the study. Unblinding was approved by the study principal investigator and collaborators at Walter Reed Army Institute of Research, and the unblinded treatment assignment was provided to the sponsor in a memorandum dated June 12, 2017, confirming that the participant had received ZPIV. The study team and collaborators at Walter Reed Army Institute of Research concurred that vaccine administration was the most

likely cause of the positive Zika virus nucleic acid test, presumably due to detection of Zika virus genomic RNA released from damaged inactivated virions. A similar phenomenon of transient antigenaemia detectable in blood donors has been well described after hepatitis B virus vaccination.^{19,20} Further investigation was curtailed by the Massachusetts State Department of Public Health. The incident was reported to the safety monitoring committee and the US Food and Drug Administration. The data for this participant were included in the analysis.

No serious adverse events, adverse events of special interest, or treatment-related unsolicited adverse events were reported during the study (appendix p 3). No participants withdrew due to adverse events. No pregnancies were reported during study follow-up. There was one unrelated severe event reported for a participant in the standard regimen group who had a concussion after a sledding accident. No severe

	Standard regimen (n=10)	Accelerated regimen (n=10)	Single-dose regimen (n=10)	Placebo (n=6)
Age, years				
Mean (SD)	28.8 (6.6)	25.7 (8.3)	25.1 (7.2)	23.2 (5.6)
Range	18–38	19–46	19–42	19–33
Sex				
Female	5 (50%)	7 (70%)	8 (80%)	5 (83.3)
Male	5 (50%)	3 (30%)	2 (20%)	1 (16.7%)
Race				
White	7 (70%)	5 (50%)	7 (70%)	3 (50%)
Black or African American	1 (10%)	1 (10%)	0	1 (16.7%)
Asian	0	2 (20%)	2 (20%)	2 (33.3%)
Other	0	2 (20%)	1 (10%)	0
Mixed	2 (20%)	0	0	0
Ethnicity				
Hispanic or Latino	1 (10%)	0	1 (10%)	0
Not Hispanic or Latino	9 (90%)	10 (100%)	9 (90%)	6 (100%)
Flavivirus positive				
Yes	1 (10%)	2 (20%)	0	1 (17%)
No	9 (90%)	8 (80%)	10 (100%)	5 (83%)

Data are n (%), unless otherwise stated.

Table 1: Baseline characteristics of the intention-to-treat population

	All ZPIV (n=30)	Placebo (n=6)
Local symptoms		
Any local symptom	26 (87%)	3 (50%)
Mild	23 (77%)	3 (50%)
Moderate	3 (10%)	0
Erythema	7 (23%)	1 (17%)
Mild	6 (20%)	1 (17%)
Moderate	1 (3%)	0
Induration (severity)	4 (13%)	1 (17%)
Mild	4 (13%)	1 (17%)
Moderate	0	0
Induration (size)	4 (13%)	1 (17%)
Mild	4 (13%)	1 (17%)
Moderate	0	0
Pruritus	1 (3%)	0
Mild	1 (3%)	0
Moderate	0	0
Pain	24 (80%)	2 (33%)
Mild	21 (70%)	2 (33%)
Moderate	3 (10%)	0
Systemic symptoms		
Any systemic symptom	24 (80%)	4 (67%)
Mild	18 (60%)	3 (50%)
Moderate	6 (20%)	1 (17%)
Abdominal pain	3 (10%)	1 (17%)
Mild	2 (7%)	0
Moderate	1 (3%)	1 (17%)

(Table 2 continues in next column)

	All ZPIV (n=30)	Placebo (n=6)
(Continued from previous column)		
Arthralgia	2 (7%)	0
Mild	2 (7%)	0
Moderate	0	0
Diarrhoea	5 (17%)	0
Mild	5 (17%)	0
Moderate	0	0
Fatigue	16 (53%)	1 (17%)
Mild	12 (40%)	1 (17%)
Moderate	4 (13%)	0
Feverishness	4 (13%)	1 (17%)
Mild	3 (10%)	1 (17%)
Moderate	1 (3%)	0
Headache	14 (46%)	2 (33%)
Mild	10 (33%)	2 (33%)
Moderate	4 (13%)	0
Malaise	10 (34%)	1 (17%)
Mild	8 (27%)	1 (17%)
Moderate	2 (7%)	0
Myalgia	13 (44%)	1 (17%)
Mild	11 (37%)	1 (17%)
Moderate	2 (7%)	0
Nausea	7 (23%)	1 (17%)
Mild	6 (20%)	1 (17%)
Moderate	1 (3%)	0
Rash	1 (3%)	0
Mild	0	0
Moderate	1 (3%)	0
Fever	1 (3%)	0
Mild	0	0
Moderate	1 (3%)	0
Vomiting	2 (7%)	0
Mild	2 (7%)	0
Moderate	0	0

Data are n (%). ZPIV=Zika purified inactivated vaccine. * All participants who received the specified dose.

Table 2: Maximum reactogenicity within 7 days of vaccination in the safety population*

laboratory events or vital sign measurements were reported (appendix p 3).

Solicited local symptoms were reported by 26 (87%) of 30 vaccine recipients and three (50%) of six placebo recipients (table 2). Solicited systemic symptoms were reported by 24 (80%) vaccine recipients and four (67%) placebo recipients. The most common vaccine reactions among the vaccine recipients were injection-site pain (24 [80%]), fatigue (16 [53%]), and headache (14 [46%]). All vaccine reactions were either mild or moderate. Data on participants who experienced solicited events by dose, symptom, maximum severity, and treatment subgroup are in the appendix (pp 4, 7, 9, 12).

Zika virus-specific MN₅₀ titres in each treatment group are shown in figure 2 and the appendix (pp 14–15). Among

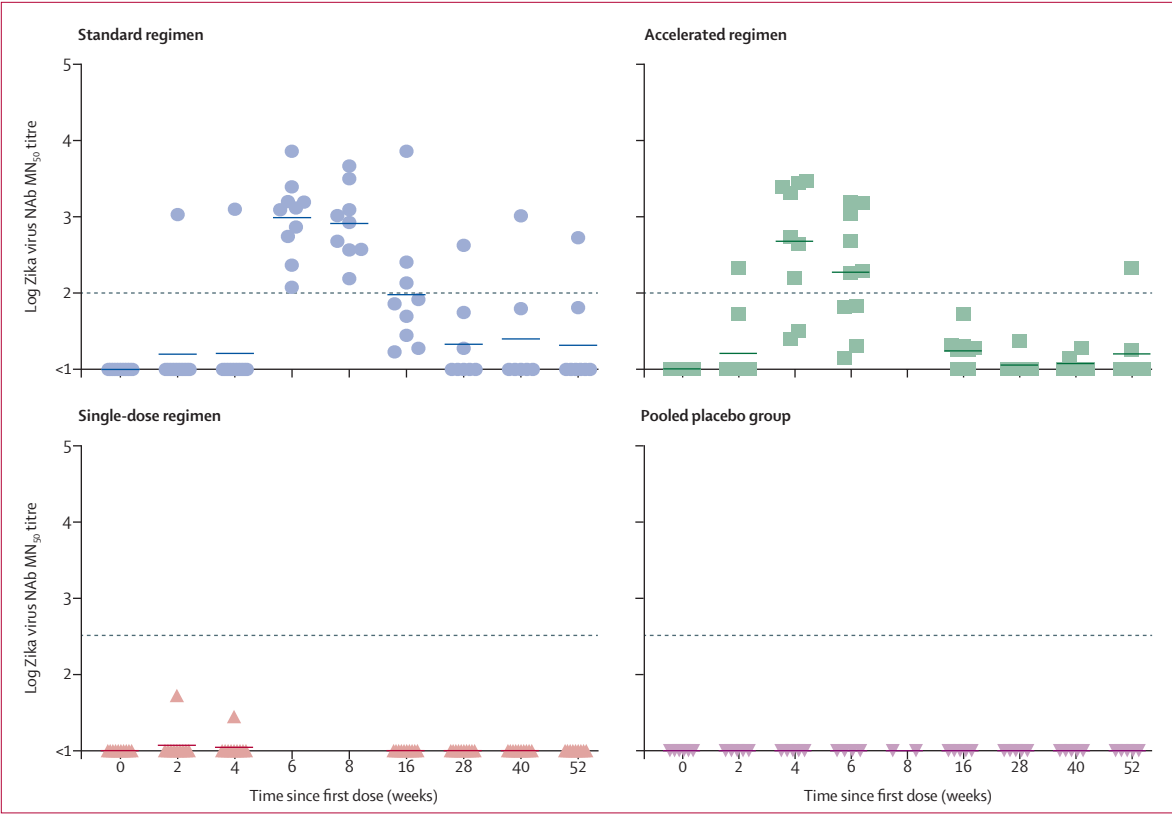


Figure 2: Zika virus-specific neutralising antibody responses over time
The graphs show log-transformed geometric mean titres by treatment group and study week. The dotted line indicates an MN₅₀ titre of 100, which is the threshold above which antibodies are expected to protect against Zika virus infection on the basis of animal studies.^{14,15,21} Solid horizontal lines reflect mean titres.

participants who received ZPIV at weeks 0 and 4 (the standard regimen), geometric mean MN₅₀ neutralising antibody titres were highest for the group at the 6-week timepoint (983·3, 95% CI 425·5–2272·5). All ten vaccine recipients in the standard regimen group had an MN₅₀ of 100 or higher at their observed peak response (ie, the highest neutralising antibody level observed for an individual participant across all timepoints), with the geometric mean of all individual participants' observed peak values being 1153·9 (95% CI 455·2–2925·2; table 3). By week 28, one (13%) of eight vaccine recipients in the standard regimen group had an MN₅₀ titre of 100 or higher, and the geometric mean MN₅₀ neutralising antibody titre declined to 13·9 (3·5–55·1). Among participants who received ZPIV at weeks 0 and 2 (the accelerated regimen), geometric mean MN₅₀ neutralising antibody titres were highest for the group at the 4-week timepoint (477·4, 95% CI 111·7–2039·8), and the geometric mean of all individual participants' observed peak values was 517·7 (95% CI 142·9–1875·6; table 3). Eight (80%) of ten vaccine recipients in the accelerated regimen group had an MN₅₀ of 100 or higher at their observed peak response (appendix p 14). By week 28, none of the seven remaining participants had an MN₅₀ titre of 100 or higher, and the geometric mean MN₅₀ neutralising antibody titre was 6·9

	Standard regimen (n=10)	Accelerated regimen (n=10)	Single-dose regimen (n=10)	Placebo (n=5)	p value*
Observed peak†	1153·9 (455·2–2925·2)	517·7 (142·9–1875·6)	6·3 (3·7–10·8)	5·0‡	<0·0001
363 days after first dose	12·4 (2·8–54·2)§	9·4 (3·1–28·7)§	5·0‡	5·0‡	0·2456

Data are geometric mean titre (95% CI). NAb= neutralising antibody. MN₅₀= 50% microneutralisation titres. *p value calculated via Kruskal-Wallis test. †The highest neutralising antibody level observed across all timepoints for an individual participant. ‡95% CI not evaluable. §Eight patients had available data.

Table 3: Comparison of MN₅₀ geometric mean titres at peak immunogenicity and 1 year

(4·0–11·9). Among participants who received a single dose of ZPIV at week 0, only one participant had detectable neutralising antibodies, which peaked at 2 weeks (GMT 53), and no participant had an MN₅₀ of 100 or higher and had an observed GMT peak of 6·3 (3·7–10·8; table 3). No participant in the placebo group had an MN₅₀ titre of 10 or higher. For all vaccine schedules, geometric mean MN₅₀ neutralising antibody titres peaked 2 weeks after the final vaccination and declined to below 100 by study week 16. At the end of study follow-up (week 52), one (10%) of eight vaccine recipients in the standard regimen group and one (10%) of eight vaccine recipients in the accelerated regimen group had MN₅₀ titres of 100 or higher.

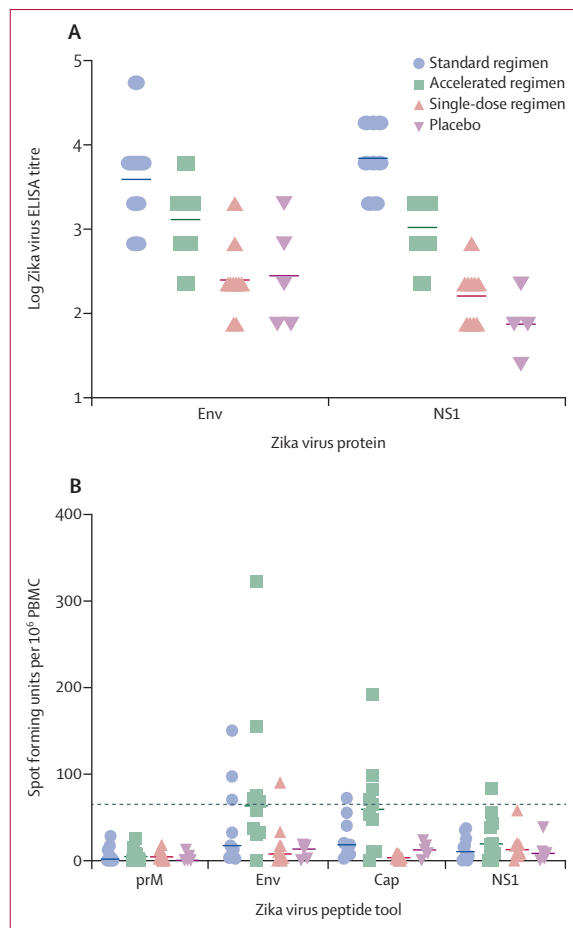


Figure 3: Peak binding antibody titres and cellular immune responses against Zika virus after vaccination

Log ELISA titres by treatment group against Zika virus Env and NS1 (SPH2015 strain) antigens. (B) Cellular immune responses by treatment group against Zika virus Env, NS1, Cap, and prM peptide pools. Dotted line is threshold for a positive response. PBMC=peripheral blood mononuclear cells.

Geometric mean MN_{50} values were significantly different between the three vaccine subgroups and the placebo group for the observed peak GMT, but no significant difference was observed at the final study visit (table 3). Pairwise group comparisons were done for the observed peak timepoint and are shown in appendix p 16. MN_{50} GMTs were significantly higher in the standard regimen and accelerated regimen groups than in the single-dose and placebo groups at that timepoint. Significant differences were not observed between the standard and accelerated regimens or between the single-dose and placebo groups. MN_{50} GMTs appeared to decline more quickly with the accelerated than with the standard regimen (appendix p 14).

Figure 3A shows the binding antibody titres against Env and NS1 Zika virus antigens at observed peak concentrations. Binding Env-specific antibody titres were detectable at low concentrations at baseline (particularly among participants with pre-existing flavivirus immunity

by microneutralisation assay), indicating substantial cross-reactivity of pre-existing binding antibodies to Zika virus antigens (appendix p 17). At observed peak concentrations, antibody titres against both Env and NS1 were approximately one log greater in the standard and accelerated regimen groups than in the single-dose and placebo groups.

Figure 3B displays cellular immune responses against prM, Env, Cap, and NS1 Zika virus antigens at observed peak concentrations. Zika virus-specific cellular immune responses were low across all groups (except for one high response to Env in the accelerated regimen group, which was an outlier), as expected for an inactivated virus vaccine.

Discussion

In this phase 1 randomised clinical trial, ZPIV was safe and well tolerated across regimens. There were no treatment-related unsolicited adverse events, serious adverse events (related or unrelated), or adverse events of special interest. The most commonly reported symptom after vaccination was local pain at the injection site, and all solicited symptoms were either mild or moderate. Two doses were required to elicit detectable humoral immune responses in nearly all participants, and the two boosting intervals had similar immunogenicity, although MN_{50} titres appeared to persist at higher concentrations for longer with the 4-week than the 2-week boosting interval. ZPIV elicited neutralising antibody titres above the protective threshold (MN_{50} titre ≥ 100) in nearly all vaccine recipients immunised with the two-dose schedule. This threshold protected mice and non-human primates against ZIKV challenge.^{14,15,21} By contrast, no participant who received the one-dose schedule developed MN_{50} titres of 100 or higher at any point in the study. These data show that a single ZPIV dose is not sufficient to induce robust MN_{50} titres and that a boost is required.

There was no significant difference between observed peak neutralising antibody titres elicited by the standard 4-week regimen versus the accelerated 2-week regimen, although the study was not formally powered to identify differences between schedules. The GMT of all individual participants' observed peak values was 517.7 in the accelerated schedule group, compared with 1153.9 in the standard schedule group. In both groups that received two doses of ZPIV, MN_{50} GMTs had declined to less than 100 by week 16, with a more rapid decline in titres noted with the accelerated regimen than with the standard regimen. These data suggest that an accelerated schedule with a 2-week boost could be a feasible option in an outbreak setting if rapid induction of immune responses is needed, but that these MN_{50} titres might be less durable.

At the final study visit, there was no significant difference in antibody titres between the three vaccine groups and placebo. This finding is consistent with the decline in immunogenicity observed at 1 year after vaccination with the Japanese encephalitis virus

purified inactivated vaccine (IXIARO).^{22–24} The short durability of ZPIV humoral immune responses is probably related to the low Zika virus-specific T-cell responses elicited by the inactivated vaccine, indicating a potential absence of CD4 T-cell helper activity, which has been shown to be important for controlling Zika virus infection in mouse models.^{25–27} Additional boosts or higher doses of ZPIV could, therefore, be required for potentially protective antibody titres to be sustained for longer than 16 weeks. For IXIARO, the US Advisory Committee on Immunization Practices recommends a boost 1 year after initial vaccination.²⁸ Ongoing studies of a late ZPIV boost (NCT02963909) and higher ZPIV doses (NCT02952833) will provide more information about whether the ZPIV regimen can be optimised further.

Our results are limited by the small sample size of the study, no randomisation between groups, the reasonably short follow-up, and the demographic characteristics of the study population (predominantly female, white, and non-Hispanic). Data from an ongoing study of ZPIV in Ponce, Puerto Rico (NCT03008122), where flaviviruses are endemic, will help to extend the generalisability of our results.

In conclusion, ZPIV was safe, well tolerated, and immunogenic when given as a two-dose regimen at either weeks 0 and 4 or weeks 0 and 2. A single dose of ZPIV was minimally immunogenic. The accelerated regimen elicited similar peak neutralising antibody titres to a standard regimen, and achieved these peak concentrations sooner. However, antibody decline appeared to be faster with the accelerated than with the standard regimen, although the durability of both regimens was low. Exploratory studies, including but not limited to epitope-specific antibody binding patterns, system serology, and the development of Zika virus-specific monoclonal antibodies, are ongoing and will be published at a future date as appropriate. Additional studies to optimise ZPIV dose and schedule are underway to extend the durability of these responses.

Contributors

KES, NLM, KM, and DHB designed the study. KES, CST, SRW, AH, JLA, DGK, KJ, TM, RF, CB, and AT conducted the clinical study. KHE, RADLB, SJT, KM, and NLM developed the vaccine. LP, ENB, JN, EM, AC, KEY, MSS, and RADLB did the immunogenicity and other laboratory analyses. JT and PD did the statistical analysis. All authors contributed to the writing and editing of the report and approved the final version.

Declaration of interests

KHE, SJT, and RADLB are co-inventors on the patent for the Zika purified inactivated vaccine. All other authors declare no competing interests.

Data sharing

The protocol and informed consent form are in the appendix, and raw data can be shared on request by contacting the corresponding author.

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