ORIGINAL ARTICLE

Safety and Immunogenicity of an Anti–Zika Virus DNA Vaccine

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ABSTRACT

BACKGROUND

Although Zika virus (ZIKV) infection is typically self-limiting, other associated complications such as congenital birth defects and the Guillain–Barré syndrome are well described. There are no approved vaccines against ZIKV infection.

METHODS

In this phase 1, open-label clinical trial, we evaluated the safety and immunogenicity of a synthetic, consensus DNA vaccine (GLS-5700) encoding the ZIKV premembrane and envelope proteins in two groups of 20 participants each. The participants received either 1 mg or 2 mg of vaccine intradermally, with each injection followed by electroporation (the use of a pulsed electric field to introduce the DNA sequence into cells) at baseline, 4 weeks, and 12 weeks.

RESULTS

The median age of the participants was 38 years, and 60% were women; 78% were White and 22% Black; in addition, 30% were Hispanic. At the interim analysis at 14 weeks (i.e., after the third dose of vaccine), no serious adverse events were reported. Local reactions at the vaccination site (e.g., injection-site pain, redness, swelling, and itching) occurred in approximately 50% of the participants. After the third dose of vaccine, binding antibodies (as measured on enzyme-linked immunosorbent assay) were detected in all the participants, with geometric mean titers of 1642 and 2871 in recipients of 1 mg and 2 mg of vaccine, respectively. Neutralizing antibodies developed in 62% of the samples on Vero-cell assay. On neuronal-cell assay, there was 90% inhibition of ZIKV infection in 70% of the serum samples and 50% inhibition in 95% of the samples. The intraperitoneal injection of postvaccination serum protected 103 of 112 IFNAR knockout mice (bred with deletion of genes encoding interferon-α and interferon-β receptors) (92%) that were challenged with a lethal dose of ZIKV-PR209 strain; none of the mice receiving baseline serum survived the challenge. Survival was independent of the neutralization titer.

CONCLUSIONS

In this phase 1, open-label clinical trial, a DNA vaccine elicited anti-ZIKV immune responses. Further studies are needed to better evaluate the safety and efficacy of the vaccine. (Funded by GeneOne Life Science and others; ZIKA-001 ClinicalTrials.gov number, NCT02809443.)

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Zika virus (ZIKV) is a flavivirus that was originally discovered in a sentinel rhesus macaque in Uganda in 1947[1] and is endemic in Africa and Asia. After outbreaks in Yap Island and French Polynesia,[2,3] ZIKV infection was identified in Brazil in 2015[4] and has spread rapidly throughout the Americas.[5] ZIKV infection is typically self-limiting and manifests as fever, rash, conjunctivitis, arthralgias,[6] and, uncommonly, neurologic syndromes such as the Guillain-Barré syndrome.[7] ZIKV infection during pregnancy has been associated with severe congenital birth defects.[8] ZIKV can persist in bodily fluids, particularly semen, for up to 6 months after infection.[9]

ZIKV is generally transmitted by the bite of infected mosquitoes (*Aedes aegypti* and other members of the family). ZIKV has also been transmitted by means of sexual contact,[10,11] blood transfusion,[12,13] and laboratory exposures.[14] There are no approved ZIKV-specific therapies or vaccines.

In preclinical studies, a synthetic DNA vaccine that targets the ZIKV premembrane and envelope proteins and that is delivered by the CELLECTRA-3P electroporation device has been shown to generate cellular and humoral immune responses, including the production of neutralizing antibodies, in mice and nonhuman primates. The vaccine has also been shown to protect against infection in IFNAR knockout mice (bred with deletions of genes encoding interferon-α and interferon-β receptors) and to protect nonhuman primates from challenge.[15] Here, we report the results of a phase 1, dose-ranging, open-label study, called ZIKA-001, to evaluate the safety and immunogenicity of a ZIKV DNA vaccine, GLS-5700,[15] delivered by intradermal injection followed by electroporation.

METHODS

STUDY DESIGN AND PARTICIPANTS

From August 2016 through September 2016, we enrolled participants at three locations in the United States and Canada: the University of Pennsylvania Clinical Trials Unit in Philadelphia, QPS–Miami Research Associates in Miami, and Université Laval in Quebec. Eligible participants were healthy adults between the ages of 18 and 65 years who had negative results on testing for dengue virus infection. (Details regarding the inclusion and exclusion criteria and the schedule of events are provided in the protocol, available with the full text of this article at NEJM.org.)

The study was reviewed and approved by the institutional review board at each study center. All the participants provided written informed consent before enrollment. The studies in animals were approved by the Wistar Institutional Animal Care and Use Committee (IACUC). The study was sponsored by GeneOne Life Science and was codeveloped with Inovio Pharmaceuticals and the investigators. The investigators and representatives of GeneOne Life Science collected the study data. Immunogenicity testing was performed at the Wistar Institute. Immunology analyses were performed and interpreted by Wistar scientists and by representatives of Inovio and GeneOne Life Science, with additional interpretation by the lead author. The authors had unrestricted access to the data and were involved in data analysis. The authors wrote the first and subsequent drafts of the manuscript and made the decision to submit the manuscript for publication. All the authors attest to the integrity of the trial, the completeness and accuracy of the data, and the fidelity of the trial to the protocol.

DNA VACCINE

The vaccine was produced by DNA-plasmid manufacturer VGXI according to current Good Manufacturing Practices. GLS-5700 contains plasmid pGX7201 at a concentration of 10 mg per milliliter of a sodium salt citrate buffer. Plasmid pGX7201 encodes ZIKV premembrane and envelope proteins, which were generated as a consensus of pre-2016 human infectious ZIKV strain sequences available in GenBank and cloned into a modified pVax1 expression vector, pGX0001.[15]

STUDY PROCEDURES

A total of 40 participants (20 in each of two groups) received GLS-5700 in a 1-mg or 2-mg dose. The vaccine was administered in 0.1-ml intradermal injections followed by electroporation at the site of inoculation, in order to increase the immunogenicity of the vaccine.[16,17] Participants received one or two injections into the deltoid region during vaccinations at baseline, 4 weeks, and 12 weeks. Electroporation was performed by means of CELLECTRA-3P with four 52-msec pulses at 0.2 A (40 to 200 V, depending on tissue resistance) per session. The first two pulses were spaced 0.2 seconds apart, followed

by a 3-second pause before the final two pulses, which were spaced 0.2 seconds apart. Electroporation needle arrays were 3 mm long. Dose escalation from 1 mg to 2 mg and recruitment of the remaining 1-mg cohort occurred after a review by the data and safety monitoring committee following the administration of the first dose of vaccine in the first five participants in the 1-mg vaccine group (Fig. 1).

SAFETY EVALUATIONS

Participants recorded any local or systemic reactions with the use of a Post-Vaccination Memory Aid for 7 days after each dose. Local injection-site reactions and systemic events that were recorded in the participant's memory aid were collected by study staff members on clinical report forms. Adverse events were graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines that were issued by the Food and Drug Administration in September 2007. Screening and safety laboratory evaluations included complete blood counts, comprehensive metabolic panels, and measures of levels of aspartate aminotransferase, alanine aminotransferase, and creatine kinase. Participants with an abnormal baseline electrocardiogram were excluded from the study because of a theoretical arrhythmogenicity associated with electroporation, a procedure that generates a small electric impulse. However, electroporation-associated arrhythmias have not been observed in trials.[17]

ASSESSMENT OF ZIKV-SPECIFIC ANTIBODY RESPONSES

The collection of blood samples to measure vaccine-specific immune responses was scheduled to occur at baseline and at weeks 1, 4, 8, 12, 14, 20, 36, and 60. Serum samples were analyzed on enzyme-linked immunosorbent assay (ELISA) to measure binding-antibody responses to recombinant vaccine–matched ZIKV envelope (rZIKV-E) protein[15] and reported as the end-point titer. We used two different assays to measure neutralizing antibody against ZIKV in serum samples from participants. First, we tested for antibody on a 50% microneutralization assay in Vero cells. (Details regarding this assay are provided in Table S1 in the Supplementary Appendix, available at NEJM.org.) Second, we performed an immunofluorescence-based neutralization assay using human glioblastoma cells (U87MG), a model for ZIKV infection of neural progenitor cells.[18] For this assay, ZIKV-MR766 was preincubated for 1.5 hours with a single 1:25 dilution of serum obtained either at baseline or at week 14 and then added to monolayers of U87MG cells. Four days postinfection, cells were fixed and

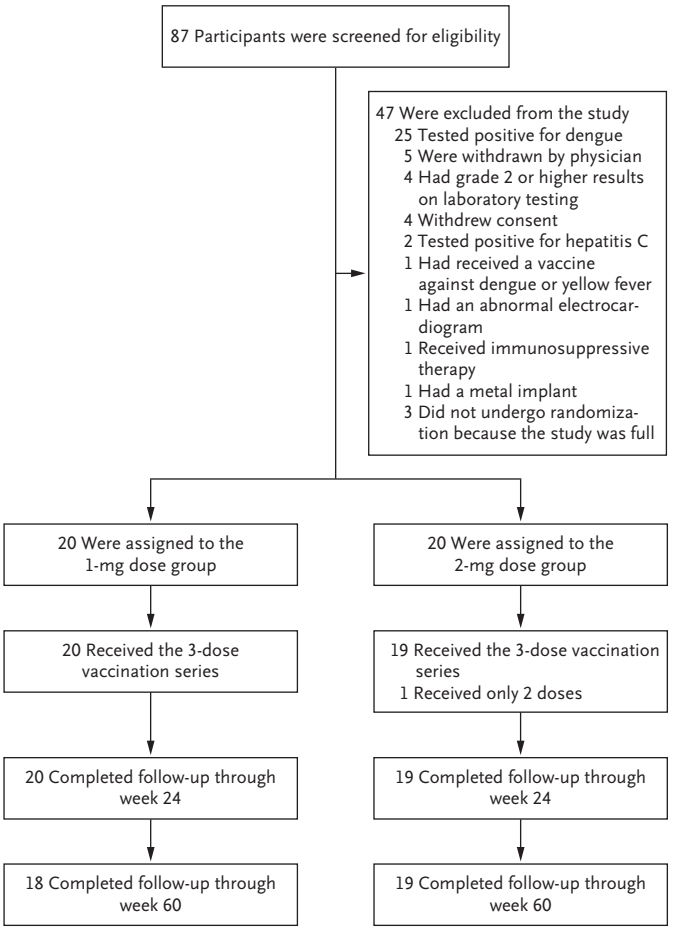

Figure 1. Screening, Enrollment, Vaccinations, and Follow-up.

Participants were enrolled sequentially according to a dose-escalation protocol to receive either 1 mg or 2 mg of the GLS-5700 vaccine against Zika virus (ZIKV) infection. After the first 5 participants received the 1-mg dose, a safety committee reviewed the side-effect profile and agreed to continue with enrollment at the 1-mg dose level and to proceed with enrollment for the 2-mg dose escalation. The rest of the participants were assigned to receive either the 1-mg dose or the 2-mg dose sequentially at each site. All available study data and samples were used for the study analyses. All the participants but 1 completed the study-injection regimen and 24 weeks of follow-up; 1 participant was lost to follow-up after the second dose of the vaccine. Follow-up at 60 weeks was completed by 18 participants in the 1-mg dose group and 19 participants in the 2-mg dose group.

subjected to indirect immunofluorescence with a pan-flavivirus antibody to detect virus-infected cells. Two independent reviewers quantified the proportions and 95% confidence intervals of the week 14 serum samples that inhibited infection relative to baseline by 50% and 90%.

ASSESSMENT OF ZIKV-SPECIFIC T-CELL RESPONSES

Whole blood was processed to obtain peripheral-blood mononuclear cells (PBMCs), which were frozen for subsequent enzyme-linked immunospot (ELISPOT) assay. (Details regarding this assay are provided in Table S1 in the Supplementary Appendix.) The average number of spot-forming units that were counted in media control wells was subtracted from the average in individual ZIKV peptide wells and then adjusted to 1×10^6 PBMCs for each ZIKV peptide pool. The total ZIKV-specific response is the sum of the responses in the three individual peptide pools.

PROTECTION OF POSTVACCINATION SERUM IN MICE

We evaluated the induction of protective capability of vaccine-induced antibodies against ZIKV infection in the IFNAR knockout murine model.[19] Immunocompetent mice contract a nonlethal, short-lived ZIKV infection,[20] whereas IFNAR knockout mice contract an infection that is lethal in 6 to 7 days.[19] IFNAR knockout mice received 0.1 ml of either phosphate-buffered saline as a control or serum collected from participants at baseline or week 14 (after the third dose of the vaccine) as an intraperitoneal injection. One hour later, the mice received an intraperitoneal injection of 1×10^5 plaque-forming units of Puerto Rico ZIKV clinical strain PR209. The animals were followed for clinical signs of disease twice daily for up to 14 days.[15] All the challenge studies in the mice were conducted in accordance with the Wistar IACUC guidelines.

STATISTICAL ANALYSIS

The antibody-binding response that was assessed on ELISA is reported as the proportion of participants in whom an antibody response developed at a given time point and as the geometric mean titer (both with 95% confidence intervals). We used Fisher's exact test to determine positive response rates and Student's t-test to compare the magnitude of the log-transformed antibody response between the two dose groups and within individuals as the change from baseline. Spearman's correlation was used to evaluate the correlation between titers for binding-antibody and neutralizing-antibody responses. The Mann–Whitney test was used for the comparison of the magnitude of the T-cell response between the two dose groups. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

STUDY PARTICIPANTS

The 40 study participants were enrolled at the three clinical research sites (Fig. 1). The demographic characteristics of the participants are summarized in Table S2 in the Supplementary Appendix. The median age was 38 years (interquartile range, 30 to 54). A total of 60% of the participants were women; 78% were White and 22% Black; in addition, 30% were Hispanic.

VACCINE SAFETY

All but one participant completed the three-injection series; one participant in the 2-mg dose group was lost to follow-up after the second dose of vaccine. No serious adverse events were reported. The investigators asked participants about any injection-related adverse events; the most frequently reported events were injection-site pain (any level), redness, swelling, and itching, which occurred in approximately 50% of the participants (Fig. 2, and Table S3 in the Supplementary Appendix). Systemic adverse events were uncommon and included headache, myalgias, upper respiratory infection, fatigue, and nausea. Except for injection-site reactions, the local investigators considered that 58% of the adverse events were unrelated to vaccine administration. Transient laboratory abnormalities included one case of grade 4 hyperkalemia, one case of grade 3 hypoglycemia, two cases of grade 1 neutropenia, and one case of grade 1 anemia, which totaled five events in four participants.

ANTIBODY RESPONSES

At baseline, none of the participants had measurable antibody responses against ZIKV on ELISA. Four weeks after the first dose, 41% of the participants had detectable binding-antibody responses, with rates of 25% in the 1-mg dose group and 60% in the 2-mg dose group. At week 6 (2 weeks after the second dose), the antibody response was 74% overall: 65% in the 1-mg dose

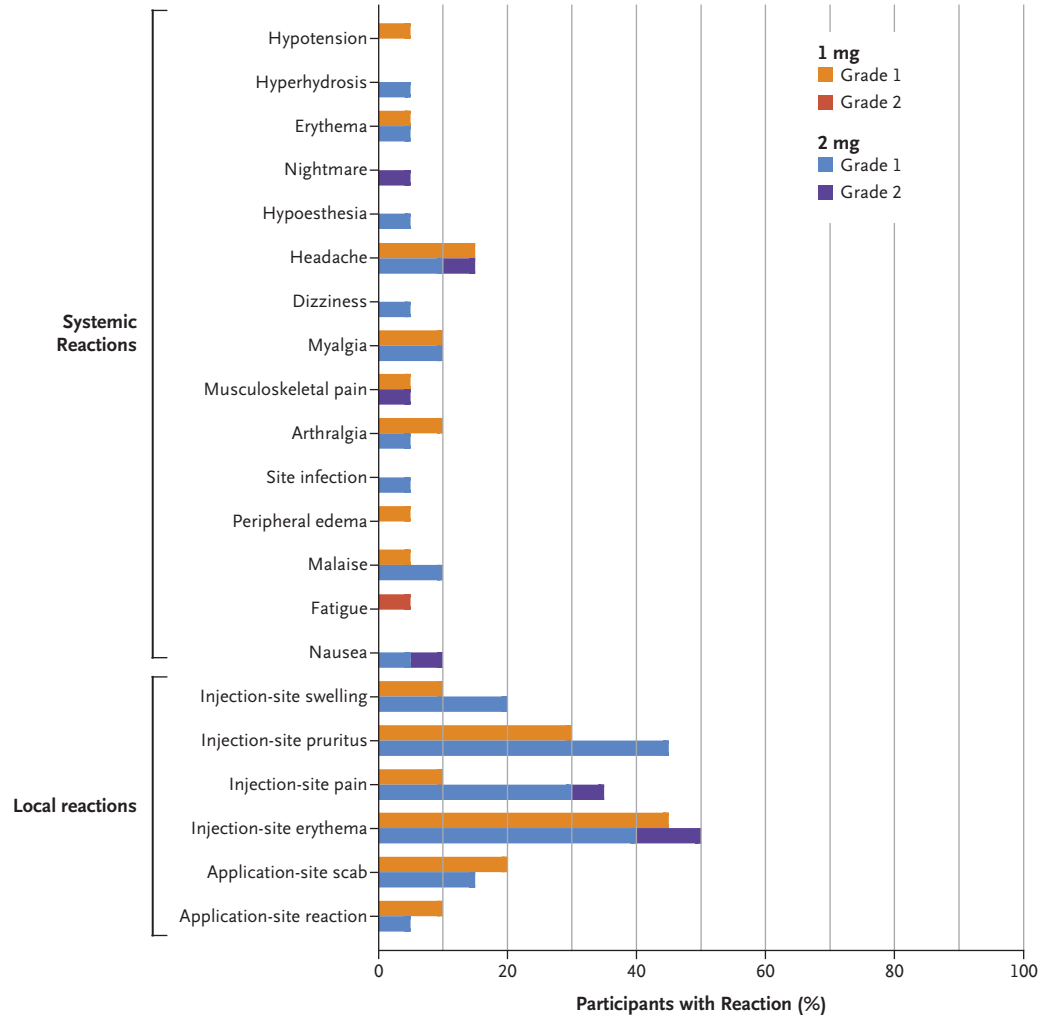

Figure 2. Adverse Events during Vaccine Administration.

Listed are all the local and systemic adverse events that were reported by study participants who were asked about any side effects during the period of vaccine administration. A list of all adverse events that occurred during treatment is provided in Table S3 in the Supplementary Appendix.

group and 84% in the 2-mg dose group. At week 12 (just before the third dose of vaccine), the antibody response increased to 70% and 95%, respectively, in each group. By week 14 (2 weeks after the third dose), ZIKV-specific binding antibodies had developed in all the participants, with geometric mean titers of 1642 in the 1-mg dose group and 2871 in the 2-mg dose group (Fig. 3A).[21] Geometric mean antibody titers on ELISA were higher in the 2-mg dose group than in the 1-mg dose group at all time points, but the between-group difference was significant only at week 6 (P=0.04). The antibody responses declined at weeks 36 and 60, but most participants had persistent binding antibodies (61% in the 1-mg dose group and 84% in the 2-mg dose group) (Fig. 3A, and Table S4 in the Supplementary Appendix).

At the end of the vaccination period, neutralizing-antibody titers against ZIKV had developed in approximately 62% of the participants on Vero-cell assay. Such titers ranged from 1:18 to 1:317 and did not correlate with the vaccine dose (Fig. 3B). In addition, there was no significant correlation between the titers of binding and neutralizing antibodies. Serum samples that were

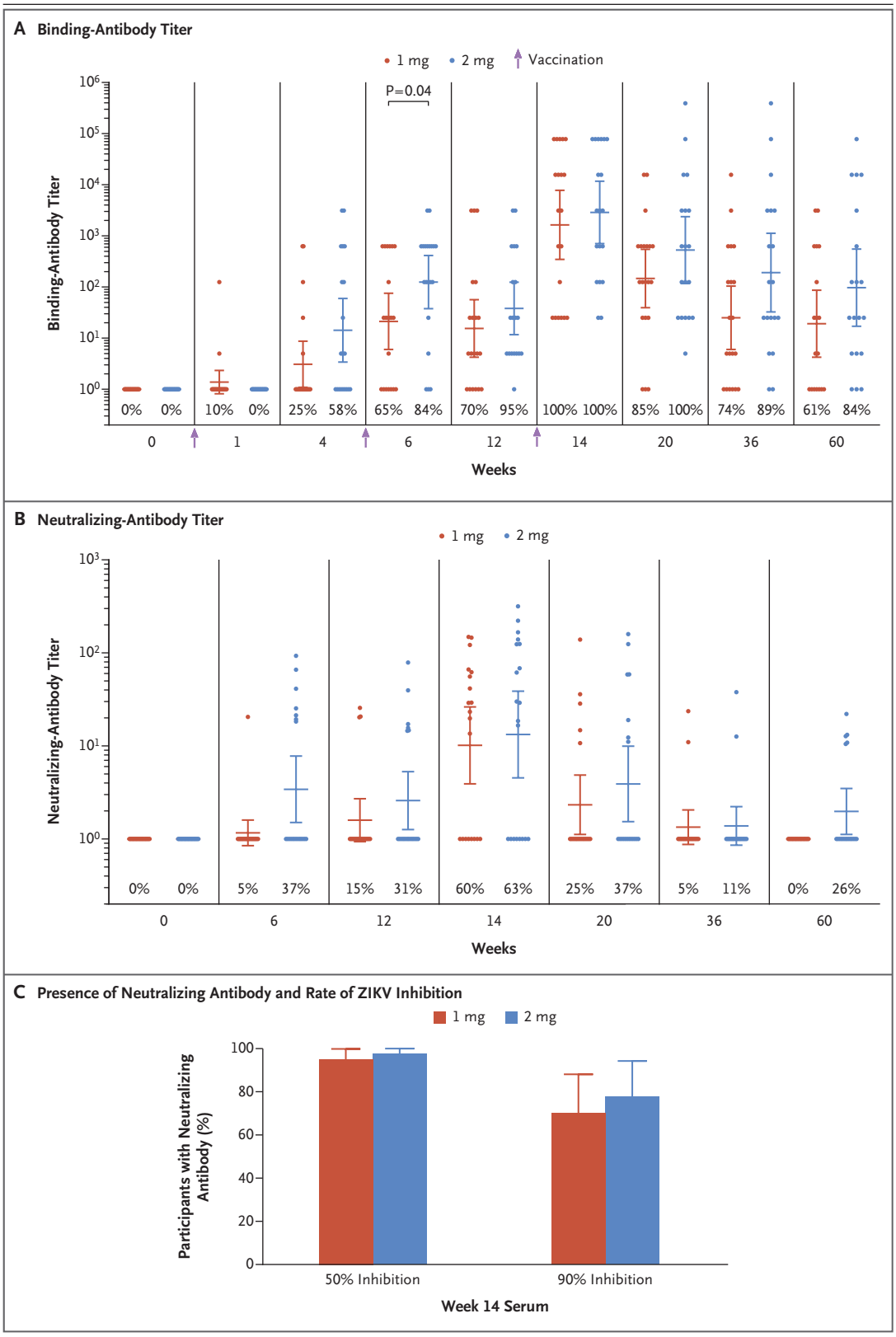

Figure 3 (facing page). Antibody Response.
Shown are geometric mean titers of binding antibody (Panel A) and neutralizing antibody (Panel B) at baseline and at weeks 1, 4, 6, 12, 14, 20, 36, and 60 among the study participants, according to dose group (1 mg or 2 mg). Binding antibody was measured on enzyme-linked immunosorbent assay, and neutralizing antibody on a standard 50% microneutralization assay in Vero cells.[21] The between-group difference in the binding-antibody response was significant only at week 6 (P=0.04), which was 2 weeks after the second vaccine dose. In Panels A and B, shown under the data points are the percentages of participants with a positive response. Also shown is the proportion of serum samples (1:25 dilution) obtained from participants at week 14 that produced 50% and 90% inhibition of cell fluorescence quantified by two independent analyses in glioblastoma cells (U87MG) (Panel C). In Panels A and B, the I bars indicate 95% confidence intervals; in Panel C, the T bars indicate the upper limits of the 95% confidence intervals.

obtained from more than 95% of the participants at week 14 (diluted 1:25) neutralized infection of glioblastoma cells (U87MG) by 50%, whereas more than 70% of the samples neutralized infection of U87MG cells by 90% (Fig. 3C). Neutralizing antibody responses declined at weeks 36 and 60 on the Vero-cell microneutralization assay. Only 25% of the participants maintained neutralizing titers at week 60 in the 2-mg dose group.

T-CELL RESPONSES

We compared PBMCs before immunization with those obtained at weeks 4, 6, 14, 20, 36, and 60 by means of ELISPOT to detect the production of interferon-γ–secreting cells in response to stimulation with ZIKV premembrane and envelope peptides. The median numbers of interferon-γ–secreting cells obtained per million PBMCs encompassing all the premembrane and envelope proteins in three peptide pools are shown in Figure 4, and in Table S5 in the Supplementary Appendix. Participants in the 2-mg dose group had significantly higher median responses than those in the 1-mg dose group after the second vaccine dose at weeks 6 and 36 (P=0.006 and P=0.002, respectively, by the Mann–Whitney test). The magnitude of the T-cell responses should be interpreted with caution, since we identified a shipping problem that had an effect on the viability of PBMCs. Cellular responses peaked at week 36 during follow-up, but most persisted to week 60.

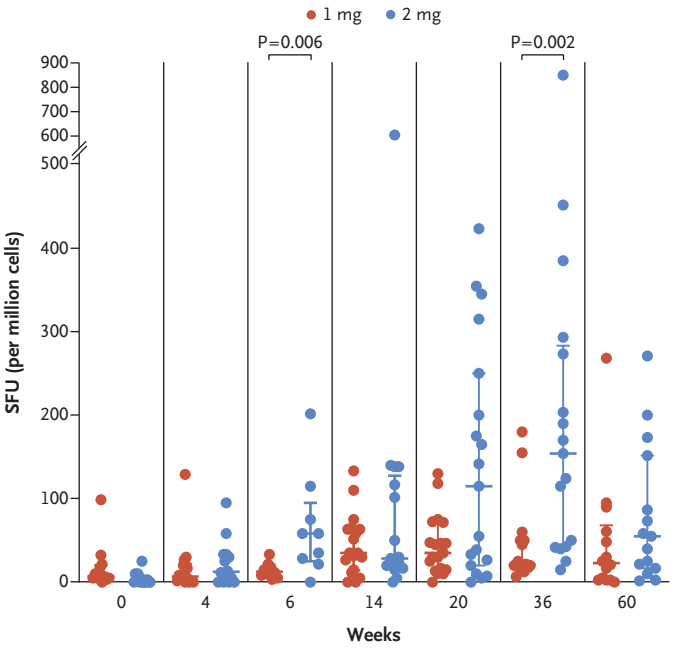

Figure 4. Cellular Response.
Peripheral-blood mononuclear cells (PBMCs) that were obtained from the participants before immunization (week 0) were compared with samples obtained at weeks 4, 6, 14, 20, 36, and 60 on enzyme-linked immunospot assay to detect the number of cells secreting interferon-γ in response to stimulation with ZIKV premembrane and envelope peptides. Shown is the median number of cells per million PBMCs, which is the sum of the responses in the three individual peptide pools encompassing all premembrane and envelope proteins. The between-group difference was significant (P=0.006) after the second vaccine dose at week 6; after the third dose, the responses were similar in the two dose groups. The I bars indicate interquartile ranges. SFU denotes spot-forming units.

IN VIVO PROTECTION AGAINST ZIKV INFECTION

The protective efficacy of postvaccination serum was evaluated in IFNAR knockout mice.[19] One hour after intraperitoneal administration of 0.1 ml of serum obtained either at baseline or at week 14 or phosphate-buffered saline (in seven mice per participant), animals were challenged with 1×10^5 plaque-forming units of ZIKV-PR209 isolate administered intraperitoneally. All the animals that were treated with phosphate-buffered saline or serum obtained at baseline died within 7 to 9 days, whereas 92% of those that were pretreated with week 14 serum survived (Fig. 5). This finding suggests that the antibody response generated by the vaccine was protective in this infection model. Protection was independent of the neutralization titer, since the mice that were inject-

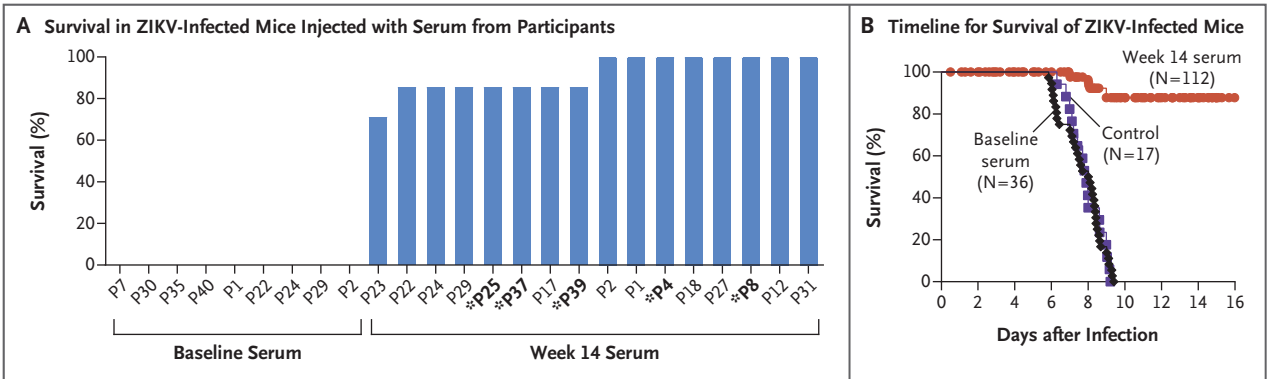

Figure 5. ZIKV Infection in IFNAR Mice Injected with Serum from Participants.

Shown is the rate of survival of IFNAR knockout mice (bred with deletions of genes encoding interferon-α and interferon-β receptors) that were infected with the ZIKV-PR209 strain after peritoneal injection of serum obtained from the study participants (as identified by the numbers on the x axis) before or after receipt of the ZIKV vaccine. Panel A shows the proportion of mice surviving at 14 days after ZIKV infection, according to whether they received serum obtained from the participants at baseline (lanes 1 to 9) or at study week 14 (after the third dose of ZIKV vaccine) (lanes 10 to 25). Of the 112 mice that had received immune serum from the participants, 103 (92%) were alive at 14 days, even though five of the participants (P4, P8, P25, P37, and P39, as indicated by boldface type and an asterisk) had binding-antibody titers but no neutralizing-antibody titers at week 14. Panel B shows Kaplan–Meier curves of survival among mice that were included in the challenge study and injected with phosphate-buffered saline as a control, with baseline (prevaccination) serum, or with week 14 serum. There were no significant differences in response between male and female mice.

ed with serum from five participants who had binding-antibody responses but no neutralizing-antibody responses after vaccination had a 92% survival rate after infection. There were no significant differences in rates of survival observed between male and female mice.

DISCUSSION

Currently, there are no licensed vaccines against ZIKV infection. This clinical trial provides initial data on the safety and immunogenicity of the GLS-5700 ZIKV vaccine, which induced binding antibodies in 100% of the participants after a three-dose vaccination regimen and in 95% after two doses of vaccine. Neutralizing antibodies were found in 62% of samples obtained from the participants on Vero-cell assay and in more than 95% of the samples that were assayed on neuronal-cell targets. Immune serum that was obtained from vaccinated study participants both prevented ZIKV infection in cellular models in vitro and prevented death in an in vivo mouse model.

GLS-5700 is one of a number of ZIKV vaccines that have shown promising results in animal models.[22-24] These vaccines include those that are based on nucleic acids (DNA and messenger RNA), viral vectored vaccines, and inactivated and live-attenuated vaccines. GLS-5700 is a wholly synthetic DNA vaccine designed to express a consensus ZIKV premembrane and envelope antigens.

Synthetic DNA vaccines are appropriate for emerging infectious diseases because they allow for the rapid design of novel antigens.[25] Vaccines can be rapidly designed with the use of a common platform expressing relevant antigens from an emergent pathogen. Other platforms share this ability for rapid alteration with varying benefits and challenges.[25] The development time from initial design to initiation of this clinical trial was 7 months for GLS-5700. In preclinical and clinical studies, synthetic DNA vaccines that are administered by CELLECTRA electroporation have been shown to elicit cellular and humoral immune responses that are far greater than those elicited by simple injection of DNA alone,[16,26] a factor that can have an effect on human infection and pathogen clearance.[17,27] Our study further advances the approaches for enhancing electroporation by focusing on intradermal injection of a decreased delivery volume with a decreased energy output during electroporation. GLS-5700 was associated with rates of local and systemic side effects that were similar to those of other DNA vaccines delivered by means of electroporation.[17]

Our study was not designed to address the

efficacy of this ZIKV vaccine in humans; that will require larger randomized trials in a region where ZIKV is endemic. However, we found that GLS-5700 induced immune responses that were protective in both in vitro and in vivo models of ZIKV infection. This finding suggests that vaccine-induced antibodies may be clinically relevant to prevent infection, which is the primary criterion for ZIKV vaccine development designated by the World Health Organization.[28] The rate of protection of IFNAR knockout mice was more than 91% after injection with postvaccination serum regardless of whether the serum samples had detectable neutralizing antibodies. This phenomenon has also been observed in other flavivirus infections, such as West Nile virus, for which passive transfer of poorly neutralizing antibodies against the virus protected formerly untreated mice from challenge.[29] Our study highlights the limitations of the use of current neutralizing assays as functional measurements for the development of ZIKV or other flavivirus vaccines.

In multiple preclinical studies involving animal models, consensus DNA plasmids have been shown to provide broad protection against a number of viruses in addition to ZIKV,[15] including Ebola virus[30] and the Middle East respiratory syndrome (MERS) virus.[31] In this study, we found that the GLS-5700 vaccine generated a protective response against multiple ZIKV isolates, including the African lineage MR766 ZIKV strain in a neuronal-cell neutralization assay and the PR209 Caribbean sublineage of Asian ZIKV in a challenge model in IFNAR knockout mice.[32,33]

In conclusion, our trial shows the initial safety and immunogenicity of a DNA vaccine encoding consensus ZIKV premembrane and envelope antigens delivered by means of electroporation. Further studies will be needed to evaluate the efficacy of the vaccine and its long-term safety.

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