

Original Article

A randomized, placebo-controlled, blinded phase 1 study investigating a novel inactivated, Vero cell-culture derived Zika virus vaccine

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Abstract

Background: Zika virus (ZIKV) is an emerging public health threat, rendering development of a safe and effective vaccine against the virus a high priority to face this unmet medical need. Our vaccine candidate has been developed on the same platform used for the licensed vaccine IXIARO[®], a vaccine against Japanese Encephalitis virus, another closely related member of the *Flaviviridae* family.

Methods: Between 24 February 2018 and 16 November 2018, we conducted a randomized, observer-blinded, placebo controlled, single center phase 1 study to assess the safety and immunogenicity of an adjuvanted, inactivated, purified whole-virus Zika vaccine candidate in the USA. A total of 67 healthy flavivirus-naïve adults aged 18–49 years were randomly assigned to one of five study arms to receive two immunizations of either high dose or low dose (6 antigen units or 3 antigen units) with both dose levels applied in two different immunization regimens or placebo as control.

Results: Our vaccine candidate showed an excellent safety profile independent of dose and vaccination regimen with predominantly mild adverse events (AEs). No serious AE has been reported. The ZIKV vaccine induced neutralizing antibodies in all tested doses and regimens with seroconversion rates up to 85.7% (high dose), which remained up to 40% (high dose) at 6 months follow-up. Of note, the rapid regimen triggered a substantial immune response within days.

Conclusions: The rapid development and production of a ZIKV vaccine candidate building on a commercial Vero-cell manufacturing platform resulted in a safe and immunogenic vaccine suitable for further clinical development. To optimize antibody persistence, higher doses and a booster administration might be considered.

Key words: Zika, phase-1, vaccine, Vero-cell platform

Introduction

Zika virus (ZIKV) is a flavivirus that is phylogenetically related to other well-known flaviviruses, such as Japanese encephalitis virus (JEV), dengue virus (DENV), West Nile virus (WNV) or Yellow fever virus (YFV). ZIKV is now listed on the WHO R&D Blueprint for pathogens with epidemic/pandemic potential.¹ With recent infections reported from Brazil, India, Thailand and Angola, the virus may readily re-emerge beyond the Americas.^{2–5} Considering the global travelling activities, the appearance of the ZIKV in regions outside endemic areas can hardly be predicted.^{6–8} It is primarily transmitted by infected *Aedes* mosquito species, which can also efficiently transmit DENV, YFV and chikungunya virus.⁹ Most common symptoms due to ZIKV occurring in ~20% of infected people include fever, rash, headache, joint pain, conjunctivitis and muscle pain lasting for several days to a week.¹⁰ In a recent meta-analysis focusing on patients with confirmed ZIKV infection, however, symptom frequencies were substantially higher with fever and arthralgia reported in more than 60% of subjects.¹¹ In pregnant women, ZIKV is able to cross the placenta and cause neuropathology in developing fetuses, resulting in the dreaded congenital Zika syndrome characterized by malformation and microcephaly in neonates.^{12,13} Additionally, ZIKV has been associated with neurological disorders such as Guillain-Barré syndrome in adults.¹⁴

Due to severe disease outcomes such as the congenital Zika syndrome, the development of a safe and effective ZIKV vaccine remains a unmet medical need.¹⁵ In a pharmaco-economic analysis, the value of a Zika vaccine defined as cost savings in combination with beneficial health effects was established at an infection risk as low as 0.1% for a vaccine efficacy of 75% providing a meaningful target for vaccine development.¹⁶ Inactivated vaccines have a long-established safety profile and would be indicated for use during pregnancy or in lactating women. Our vaccine is composed of Vero cell-culture derived, purified, inactivated whole ZIKV particles, manufactured on the commercial JEV (IXIARO®) platform.¹⁷ In unpublished preclinical studies, the vaccine candidate was able to induce substantial levels of neutralizing antibodies in a dose-dependent manner against both homologous and heterologous ZIKV strains after a single immunization in CD-1 mice.

Here, we report the outcome of a first-in-human clinical study to evaluate the safety and immunogenicity of two different dose levels and immunization regimens of an aluminium-hydroxide (alum) adjuvanted, inactivated, purified whole virus ZIKV vaccine candidate in healthy individuals.

Methods

Study design

This was a randomized, observer-blind, placebo controlled, single center phase 1 study conducted in the US to assess the safety and immunogenicity of two different dose levels of an alum adjuvanted formalin-inactivated, purified whole ZIKV vaccine candidate in comparison to placebo. Doses were administered intramuscularly into the deltoid muscle investigating two vaccination regimens, with vaccinations on Days 0 and 28 (standard regimen) or on Days 0 and 7 (rapid regimen). Investigating the rapid schedule with two immunizations seven days apart was

intended to serve as quick intervention in an outbreak situation, but also as a precautionary measure in case of urgent travelling requirements. The study obtained approval from the Chesapeake IRB as responsible ethics committee and was conducted between 24 February 2018 and 16 November 2018 in compliance with the current International Conference on Harmonization/Good Clinical Practice guidelines and the Declaration of Helsinki. Throughout the study, a Data Safety Monitoring Board (DSMB) consisting of four independent medical experts performed periodic reviews of accruing safety information. The study is registered under [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03425149), NCT #03425149.

Participants

In this study, 67 generally healthy flavivirus-naïve adults of either gender aged 18–49 years were enrolled and provided their written informed consent prior to any study-related procedures. Exclusion criteria included a history of flavivirus infection or vaccination with a licensed or investigational flavivirus vaccine, or any inactivated vaccine within 2 weeks or live vaccine within 4 weeks prior to vaccination in this study. Woman of childbearing potential had to present a negative serum pregnancy test at screening and agree to employ adequate birth control measures for the duration of the study.

Randomization and masking

As a safety precaution, the study began with enrollment of two sentinels, who received 6 antigen units (AU) on Days 0 and 7 in an open-label fashion. Both sentinels were followed-up for safety by a safety phone call on the next day and returned to the study site at Day 7 for safety follow-up. Available data of these two sentinels were reviewed by the DSMB prior to continuation with the blinded, randomized part of the study. The remaining 65 individuals were randomized into five study arms at a ratio of 3:3:3:3:1 (four vaccine study arms and one placebo study arm). Participants and site staff performing the safety assessment were blinded. Eligible subjects were assigned to a study arm, using randomization envelopes according to a randomization list generated by the study statistician. Randomization of participants as well as preparation and administration of the vaccine were performed by unblinded study staff not otherwise involved in study-related activities. Dosing was adjusted by injection volume, dispensing 0.5 mL for the 6 AU or 0.25 mL for the 3 AU dose. To maintain blinding with regard to the vaccination regimen, three vaccinations were administered to all participants: two active immunizations and one placebo vaccination for those enrolled in one of the Zika vaccine arms and three vaccinations of 0.5-mL phosphate buffered saline in the placebo arm.

Procedures and outcomes

Participants were to return to the study site on Days 14, 28, 35, 56 and 208 for safety and immunogenicity evaluations. Participants' diaries were used for the collection of daily oral body temperature, solicited injection and systemic reactions, and other adverse events (AEs). Additionally, safety laboratory assessments were performed. Solicited AEs and safety assessments were

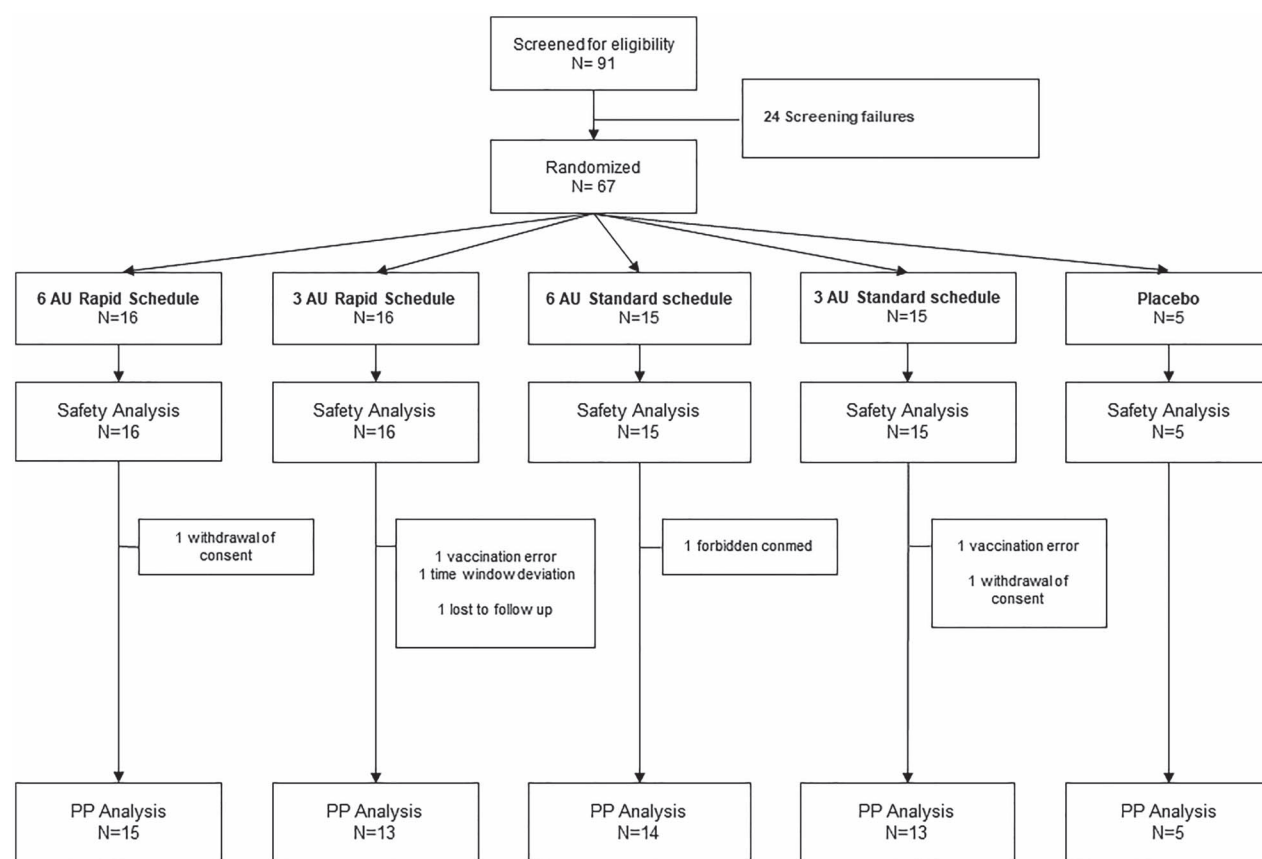


Figure 1. Trial profile. CONSORT diagram; abbreviations: Per-Protocol (PP) Population, concomitant medication (conmed), number of participants (N).

evaluated using FDA's toxicity grading scale.¹⁸ Immunogenicity was assessed by a standard Plaque Reduction Neutralization Test (PRNT).¹⁹ The neutralizing titre was determined as the serum dilution causing 50% plaque count reduction (PRNT₅₀) compared with the virus control lacking neutralizing antibody. A PRNT₅₀ titre of $\geq 1:10$ was defined as seroconverted. At baseline, participants were screened for several flaviviruses with commercially available IgG ELISA tests (Euroimmun [DENV and Zika], DiaSorin [WNV], InBioS [JEV]) and if positive were excluded from participation.

The primary objective of this study was to assess the safety and tolerability of the vaccine candidate. Secondary objectives included the evaluation of the immunogenicity, identification of the optimal dose level and the optimal vaccination regimen, and antibody persistence. Long-term safety was addressed by closely monitoring adverse events of special interest (AESIs), i.e. Guillain-Barré syndrome and severe flavivirus diseases.

Statistical analysis

The sample size of 62 participants receiving the vaccine candidate allowed for the detection of AE with a true underlying prevalence of 2.5% with a probability of 79%. All participants who received at least one vaccination were included in the safety analysis (Safety Population). The frequency of solicited injection site and systemic reactions up to 7 days after each vaccination, and unsolicited AEs, SAEs and AESIs were presented for each study arm including 95% confidence intervals according to Altman.

The Per-Protocol (PP) population contained all participants who completed the study without major protocol violations. The immunogenicity analysis compared the geometric mean titers (GMTs), the geometric mean fold increases (GMFIs) and seroconversion rates (SCRs) between the study arms at 7 and 28 days after the second vaccine dose by analysis of variance (ANOVA; factor study arm) and 208 days after the first vaccination. For pair-wise comparisons, Tukey's honest significant difference test was performed.

Results

Sixty-seven subjects were assigned to five study arms receiving either two active vaccinations ($n = 62$) or placebo ($n = 5$). Three participants terminated the study before Day 208, but none due to AEs (Figure 1). After flavivirus screening at baseline, two participants were excluded due to positive results (one positive for DENV and the other for DENV and WNV). Overall, the baseline characteristics of the study participants were balanced across study arms, except slightly more females enrolled (56.5%). Participants receiving the ZIKA vaccine were predominantly Caucasian (87.1%) with a mean age of 33.6 years (range from 19 to 49 years old) (Supplementary Table S1, Supplementary data are available at *JTM* online).

Vaccinations with the low (3 AU) or the high dose (6 AU) were generally safe and well tolerated irrespective of the vaccination regimen (standard or rapid schedule). No SAEs, AESIs or AEs leading to withdrawal from the study or from further vaccination

Table 1. Summary table of solicited and unsolicited AEs (safety population)

Parameter	Statistic	6 AU rapid N = 16	3 AU rapid N = 16	6 AU standard N = 15	3 AU standard N = 15	Placebo N = 5
Any AE	<i>n</i> (%) Obs [95% CI]	12 (75.0) 60 [50.5, 89.8]	9 (56.3) 42 [33.2, 76.9]	12 (80.0) 37 [54.8, 93.0]	9 (60.0) 27 [35.7, 80.2]	5 (100) 23 [56.6, 100]
Any related AE	<i>n</i> (%) Obs [95% CI]	11 (68.8) 49 [44.4, 85.8]	9 (56.3) 36 [33.2, 76.9]	11 (73.3) 25 [48.0, 89.1]	7 (46.7) 21 [24.8, 69.9]	5 (100) 15 [56.6, 100]
Any severe AE	<i>n</i> (%) Obs [95% CI]	1 (6.3) 1 [1.1, 28.3]	0 (0.0) 0 [0.0, 19.4]	1 (6.7) 2 [1.2, 29.8]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
Any related severe AE	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	1 (6.7) 2 [1.2, 29.8]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
Any SAE	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
Any AESI	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
Any related medically attended AE	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	1 (6.3) 2 [1.1, 28.3]	1 (6.7) 2 [1.2, 29.8]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
Any solicited AE	<i>n</i> (%) Obs [95% CI]	11 (68.8) 46 [44.4, 85.8]	8 (50.0) 34 [28.0, 72.0]	11 (73.3) 24 [48.0, 89.1]	6 (40.0) 20 [19.8, 64.3]	5 (100) 15 [56.6, 100]
Any severe solicited AE	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	1 (6.7) 2 [1.2, 29.8]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
Any injection site reaction	<i>n</i> (%) Obs [95% CI]	10 (62.5) 26 [38.6, 81.5]	5 (31.3) 12 [14.2, 55.6]	4 (26.7) 5 [10.9, 52.0]	1 (6.7) 1 [1.2, 29.8]	1 (20.0) 2 [3.6, 62.4]
Any solicited systemic AE	<i>n</i> (%) Obs [95% CI]	10 (62.5) 20 [38.6, 81.5]	5 (31.3) 12 [14.2, 55.6]	9 (60.0) 19 [35.7, 80.2]	6 (40.0) 19 [19.8, 64.3]	5 (100) 13 [56.6, 100]
Any related unsolicited AE	<i>n</i> (%) Obs [95% CI]	2 (12.5) 3 [3.5, 36.0]	1 (6.3) 2 [1.1, 28.3]	1 (6.7) 1 [1.2, 29.8]	1 (6.7) 1 [1.2, 29.8]	0 (0.0) 0 [0.0, 43.4]
Any related severe unsolicited AE	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]

Abbreviations: SAE = serious adverse event, *n* = number of subjects with AE, % = percent, Obs = observations, CI = confidence interval, N = number of subjects, AU = antigen units, NC = not calculated.

were reported (Table 1). Higher rates of solicited AEs occurred in the high-dose arm: 11 of 15 (73.3%) individuals receiving the high-dose standard regimen and 11 of 16 (68.8%) individuals receiving the rapid regimen reported any solicited AE. In contrast, participants in the low-dose arm allocated to either the standard or rapid regimen showed a lower AE incidence (6 of 15 [40.0%] or 8 of 16 [50.0%], respectively). However, there was no statistically significant difference in the frequency of solicited AEs between the study arms ($P=0.0922$, Table 1). Medically attended AEs were documented in three subjects receiving the vaccine candidate, among them two subjects for events classified as related to the vaccine (Table 1).

Solicited injection site and systemic reactions—per definition regarded as related to the vaccine—were predominantly of mild severity (Supplementary Tables S2 and S3, Supplementary data are available at *JTM* online). Only one individual reported related severe nausea and vomiting six days after the second vaccine dose, with a likelier alternative cause (subject felt she had contracted a virus while visiting a sick child at the Children's Hospital). The most common injection site reaction was tenderness, which was reported by 13 (21.0%) participants in one of the vaccination arms and one (20.0%) participant in the placebo arm ($P=0.0035$) (Table 2). The only other notable injection site reaction, injection site pain was reported by 11 (17.7%) individuals who received the vaccine candidate and by one individual (20.0%) who received placebo (Table 2; data about

the 1st vaccination: Supplementary Table S2, Supplementary data are available at *JTM* online). All episodes of tenderness and injection site pain were of mild severity. The solicited systemic AE headache, fatigue and myalgia were more frequent among participants in the high-dose arm (Table 2, Supplementary Table S3, Supplementary data are available at *JTM* online).

Related unsolicited AEs were reported by two individuals (12.5%) in the high-dose arm vaccinated according to the rapid regimen and by one participant in each remaining study arm receiving the Zika vaccine candidate (6.3–6.7%) without statistically significant differences across the study arms (Table 1, Supplementary Table S4, Supplementary data are available at *JTM* online). The majority of unsolicited AEs were of mild or moderate severity. In total, five individuals (8.1%) across all study arms reported related transient unsolicited AEs of nasal or eye pruritus, urinary tract infections, many with likelier alternative causes than the study vaccine and with none of them recurring upon successive vaccinations. Generally, rates of injection site reactions and systemic reactions decreased with successive vaccinations (Supplementary Table S4, Supplementary data are available at *JTM* online).

Both doses induced substantial ZIKV-specific neutralizing antibody titers. The highest titers were obtained with the high dose (6 AU) reaching a peak GMT of 37.68 at 7 days after the second vaccination in the standard immunization regimen (Figure 2A, Supplementary Table S5, Supplementary data are

Table 2. Solicited AEs: injection site reactions and systemic AEs after any active vaccination by symptom (safety population)

AE		Statistic	6 AU rapid N = 16	3 AU rapid N = 16	6 AU standard N = 15	3 AU standard N = 15	Placebo N = 5
Injection Site Reactions	Injection site pain	<i>n</i> (%) Obs [95% CI]	4 (25.0) 4 [10.2, 49.5]	4 (25.0) 4 [10.2, 49.5]	3 (20.0) 2 [3.7, 37.9]	0 (0.0) 0 [0.0, 20.4]	1 (20.0) 1 [3.6, 62.4]
	Itching	<i>n</i> (%) Obs [95% CI]	1 (6.3) 2 [1.1, 28.3]	1 (6.3) 1 [1.1, 28.3]	1 (6.7) 1 [1.2, 29.8]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
	Tenderness	<i>n</i> (%) Obs [95% CI]	9 (56.3) 14 [33.2, 76.9]	2 (12.5) 4 [3.5, 36.0]	1 (6.7) 1 [1.2, 29.8]	1 (6.7) 1 [1.2, 29.8]	1 (20.0) 1 [3.6, 62.4]
	Erythema/Redness	<i>n</i> (%) Obs [95% CI]	1 (6.3) 1 [1.1, 28.3]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
	Swelling	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
	Induration/Hardening	<i>n</i> (%) Obs [95% CI]	1 (6.3) 1 [1.1, 28.3]	1 (6.3) 1 [1.1, 28.3]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
	Fever	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]
	Nausea	<i>n</i> (%) Obs [95% CI]	2 (12.5) 2 [3.5, 36.0]	2 (12.5) 3 [3.5, 36.0]	3 (20.0) 3 [7.0, 45.2]	2 (13.3) 2 [3.7, 37.9]	1 (20.0) 1 [3.6, 62.4]
	Vomiting	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	2 (13.3) 2 [3.7, 37.9]	1 (6.7) 1 [1.2, 29.8]	0 (0.0) 0 [0.0, 43.4]
	Headache	<i>n</i> (%) Obs [95% CI]	6 (37.5) 7 [18.5, 61.4]	4 (25.0) 7 [10.2, 49.5]	4 (26.7) 5 [10.9, 52.0]	4 (26.7) 5 [10.9, 52.0]	3 (60.0) 4 [23.1, 88.2]
Systemic Adverse Events	Fatigue	<i>n</i> (%) Obs [95% CI]	4 (25.0) 4 [10.2, 49.5]	3 (18.8) 4 [6.6, 43.0]	3 (20.0) 3 [7.0, 45.2]	2 (13.3) 2 [3.7, 37.9]	2 (40.0) 2 [11.8, 76.9]
	Myalgia	<i>n</i> (%) Obs [95% CI]	3 (18.8) 5 [6.6, 43.0]	1 (6.3) 2 [1.1, 28.3]	1 (6.7) 1 [1.2, 29.8]	1 (6.7) 1 [1.2, 29.8]	1 (20.0) 2 [3.6, 62.4]
	Arthralgia	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	1 (6.3) 1 [1.1, 28.3]	1 (6.7) 1 [1.2, 29.8]	1 (6.7) 1 [1.2, 29.8]	0 (0.0) 0 [0.0, 43.4]
	Flu-like symptoms	<i>n</i> (%) Obs [95% CI]	1 (6.3) 1 [1.1, 28.3]	2 (12.5) 3 [3.5, 36.0]	1 (6.7) 1 [1.2, 29.8]	2 (13.3) 2 [3.7, 37.9]	0 (0.0) 0 [0.0, 43.4]
	Rash	<i>n</i> (%) Obs [95% CI]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 19.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 20.4]	0 (0.0) 0 [0.0, 43.4]

Abbreviations: *n* = number of subjects with AE, % = percent, Obs = observations, CI = confidence interval, N = number of subjects, AU = antigen units.

available at *JTM* online). Seven days after the second vaccine dose, statistically higher GMTs were measured in the standard regimen compared to the rapid immunization regimen within the corresponding dose arm (ANOVA; for high dose $P = 0.0048$ and for low dose $P = 0.0056$). Likewise, GMTs were significantly higher in the high-dose standard regimen in comparison to the low-dose rapid regimen (ANOVA $P < 0.0001$). Twenty-eight days after the second vaccine dose, the immune response in the high-dose arm (standard and rapid regimen) was significantly higher than that in the low-dose rapid regimen ($P = 0.0149$ and $P = 0.0481$, respectively) (Supplementary Table S5, Supplementary data are available at *JTM* online). At the end of the study (Day 208), there was no statistically significant difference in the GMT of high-dose standard versus rapid regimen.

The highest SCR (85.7%) [12/14] was observed in the high dose (6 AU) arm receiving the standard vaccination regimen already seven days after the second vaccine dose. At that time, the standard regimen induced significantly ($P < 0.05$) higher SCRs (high dose 85.7% [12/14], low dose 71.4% [10/14]) compared with the rapid regimen (high dose 37.5% [6/16], low dose 7.1% [1/14]) (Supplementary Table S6, Supplementary data are available at *JTM* online).

Administration of the low dose following the rapid regimen resulted in the overall lowest SCR (23.1%) [3/13] 28 days after the second vaccine dose, which declined to baseline at Day 208. During the 6-months follow-up, SCRs declined in all study arms but remained at 40.0% [6/15] in participants of the high-dose arm vaccinated according to the rapid regimen (Figure 2B, Supplementary Table S6, Supplementary data are available at *JTM* online).

Within 7 days after the second vaccine dose, the standard dosing regimen elicited a higher frequency of seroconverted individuals regardless of dose, whereas the rapid low-dose schedule showed hardly any immune response (reverse cumulative distribution curve in Figure 3A). Twenty-eight days after the second vaccine dose, the high dose of both regimens as well as the low-dose standard regimen resulted in similar proportions of vaccinees achieving high ZIKV-specific neutralizing antibody titers (Figure 3B).

The GMFI of ZIKV-specific neutralizing antibodies was highest in the high-dose standard immunization regimen (7 days after second vaccine dose GMFI = 7.54), followed 28 days after second vaccine dose by the high-dose rapid regimen (GMFI = 5.62) and low-dose standard regimen (GMFI = 4.84) (Supplementary Table S7, Supplementary data are available at *JTM* online).

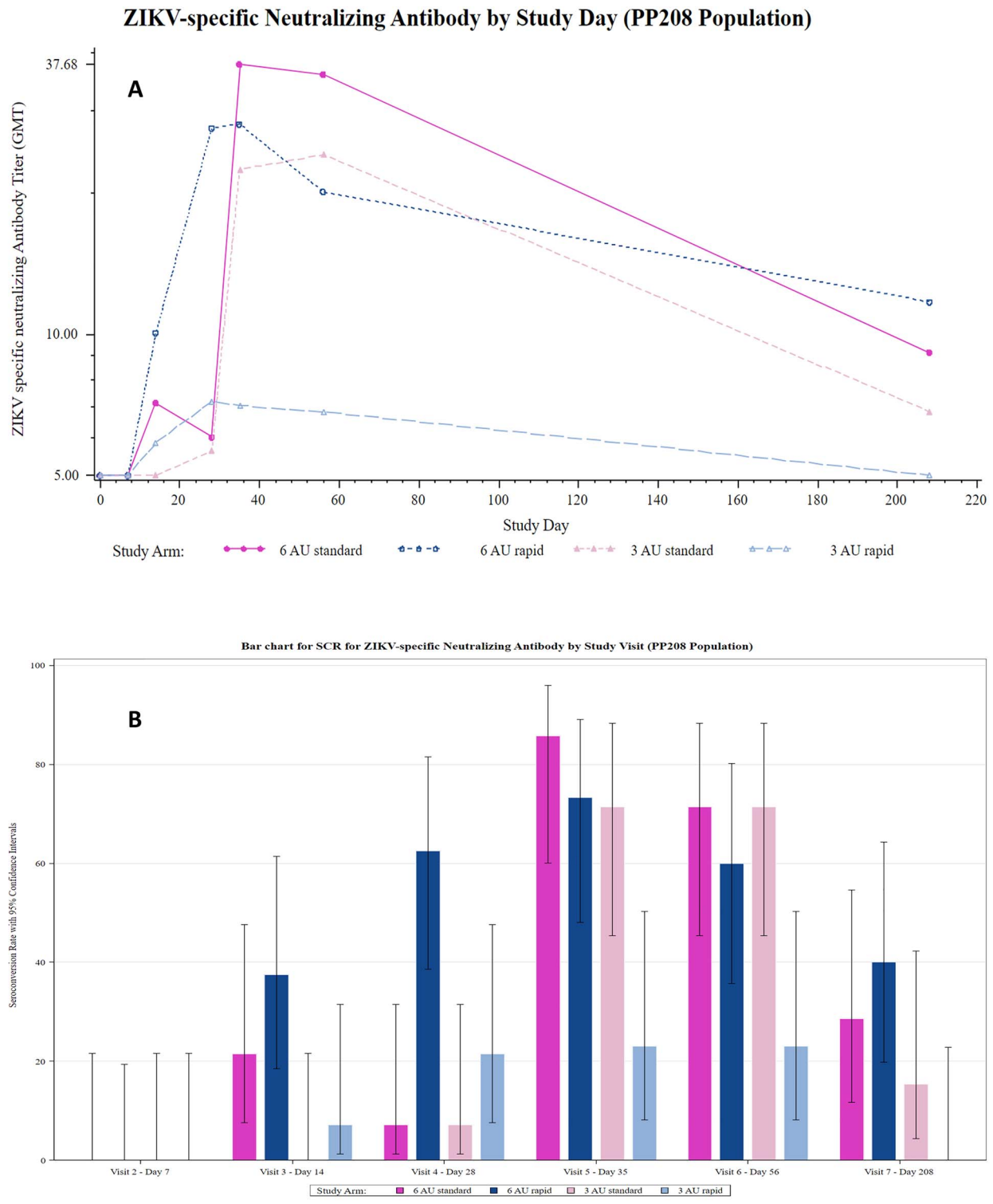


Figure 2. ZIKV-specific neutralizing antibody responses (PP Population). Neutralizing antibodies to the vaccine were evaluated from clinical specimen (human serum) using Plaque Reduction Neutralization Test (PRNT) by study arms for A: GMTs and B: SCRs.

Taken together, the Zika virus vaccine candidate was safe and immunogenic. To optimize antibody persistence, higher doses and a booster administration might be considered as no dose-limiting safety issues were observed in this study.

Discussion

In this study, we have shown that a purified, inactivated, whole virus Zika vaccine candidate adsorbed on alum, is safe, well-tolerated and immunogenic in healthy flavivirus-naïve adults.

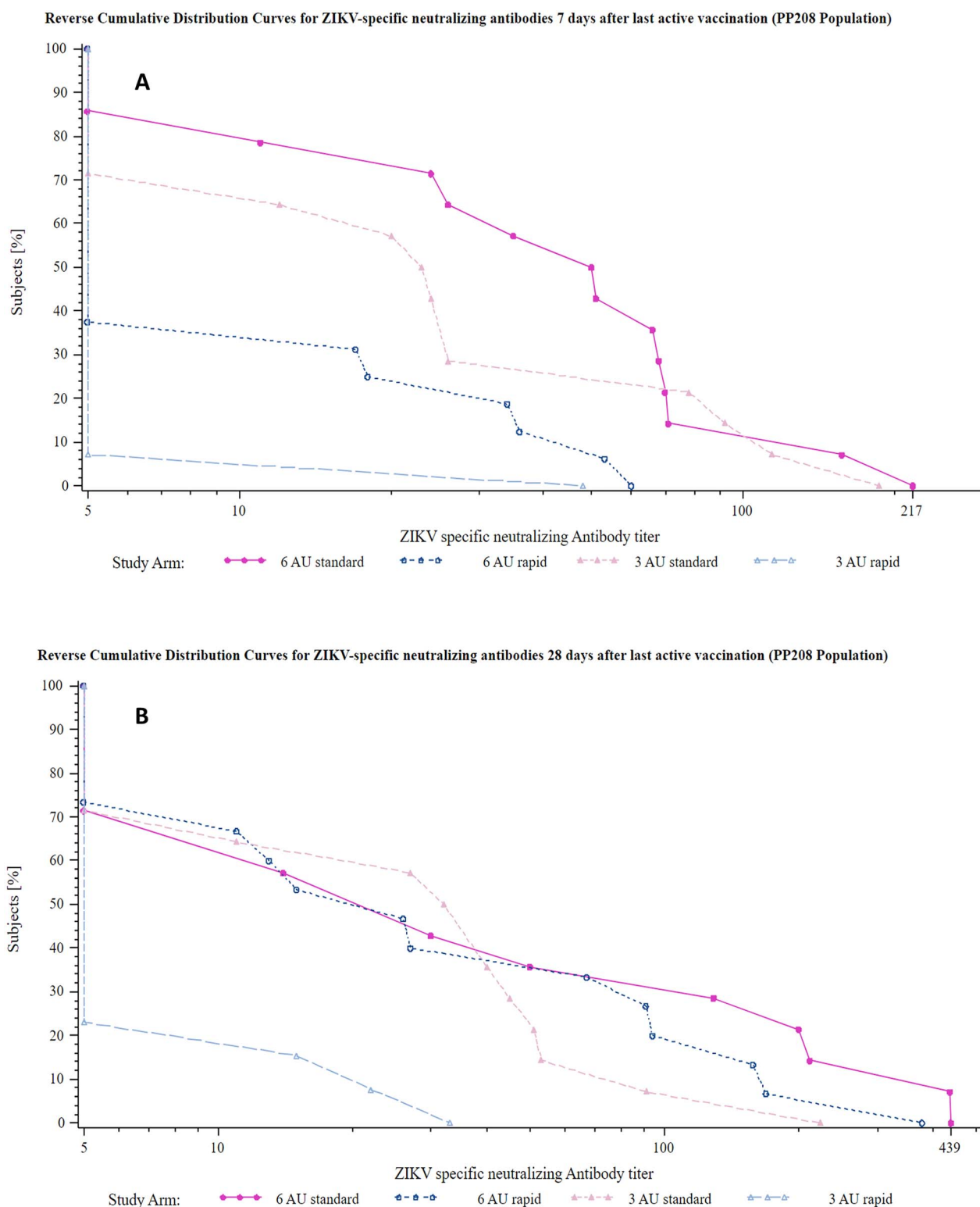


Figure 3. Reverse Cumulative Distribution Curves of ZIKV-specific neutralizing antibodies (PP Population). Percentage of individuals versus ZIKV-specific neutralizing antibody titers A: 7 days after second vaccine dose and B: 28 days after second vaccine dose.

The ZIKV vaccine-related AEs were transient, almost exclusively mild without any case of reported fever with only three individuals (4.8%) requiring medical treatment, among them two participants for related AEs.

Overall, the safety profile of the ZIKV vaccine is comparable to the safety profile of IXIARO®, which has been administered to over two million individuals and to several other ZIKV vaccines currently under development, although a direct comparison

of different vaccine types, dosing and administration routes is difficult.^{20–24} Data published recently on another inactivated Zika vaccine,²⁵ and on a different vaccine platform such as a replication-incompetent human adenovirus serotype 26 vector,²⁶ showed similar frequency and intensity of injection site pain, headache, fatigue and myalgia. In a study administering two doses of purified inactivated Zika virus vaccine, frequencies of systemic AEs were lower in contrast to local reactions with highly similar results in flavivirus naive and flavivirus primed subjects.²⁷ The favourable safety profile of our candidate supports further clinical development of the high dose and would even allow for a dose increase and/or a booster vaccination to optimize the elicited immune response.

Our inactivated ZIKA vaccine was immunogenic after two immunizations and in different regimens. Generally, the high dose resulted in slightly higher GMTs and SCRs than the low dose in the standard vaccination regimen and significantly higher titers in comparison to the low-dose rapid vaccination regimen. The standard regimen resulted in slightly higher peak antibody titers 7 days after the second vaccine dose when compared with the rapid regimen 28 days after the second vaccine dose [GMT 37.68 (SCR 85.7%), GMT 28.12 (SCR 73.3%)], respectively. Not unexpectedly, differential antibody kinetics were observed with antibody responses in the rapid regimen peaking slightly earlier than in the standard regimen. Of note, the high dose in the rapid regimen induced detectable neutralizing antibody levels in ~40% of individuals by 7 days and 60% by 14 days post-second vaccination. Therefore, the rapid regimen might still convey the benefit of a rapid onset of immune response and hence potential protection. This observation could be highly beneficial not only when urgent travel vaccinations are required but also in an outbreak situation where hygiene measures to contain infections can be completed by vaccinations within 1 week.

Comparison of immunogenicity with other ZIKV vaccine candidates currently in clinical development is hampered by substantial differences regarding vaccine production, dose, regimen and study population. These differences may explain the somewhat lower antibody titers reported in this study in comparison to Han et al. who dosed their vaccine based on μg and used different analytical methods in regards to cell culture and incubation procedures.²⁷ Thus far, no validated immunogenicity assays or reference samples to analyse and standardize neutralizing antibody production are available, further complicating direct comparisons. However, in a recent study of another purified inactivated Zika virus vaccine candidate, a similar trend in antibody kinetics was observed.²⁵

In clinical studies including flavivirus seropositive participants, a weaker, but still significant immune response in flavivirus primed subjects was observed.²⁷ In general, the protective role of neutralizing antibodies is established for multiple flavivirus vaccines. Preclinical studies in non-human primates showed that neutralizing antibodies induced against ZIKV following vaccination and upon subsequent ZIKV challenge correlated well with protection against viremia.²⁰ In addition, neutralizing antibody responses measured by PRNT for most licensed flavivirus vaccines have previously proven to directly correlate with protection,²⁸ although cellular adaptive immunity is emerging as strong contributor to the pathophysiological

mechanism of immune response and remains to be investigated during further vaccine development.

As our first-in-human study was restricted to a limited number of healthy seronegative participants, our conclusions have several limitations. Assessment of safety and long-term immunogenicity in ZIKV-positive or flavivirus seropositive volunteers will be essential early on as recent studies suggest that ZIKV vaccination in DENV-primed individuals might boost pre-existing flavivirus immunity thereby eliciting protective responses against both ZIKV and DENV.^{29,30} On the contrary, a recent finding suggests that intermediate levels of pre-existing titers of neutralizing anti-ZIKV antibodies might increase the risk of severe infection caused by dengue serotype 2.^{31,32}

Due to the self-limiting outbreak pattern, the number of people with reported ZIKA infection drops rapidly once the outbreak situation ends and remains relatively stable, but the virus incidence is probably highly underreported.³² Cases of ZIKV infection in travellers from Thailand were reported indicating ongoing transmission with asymptomatic ZIKA cases in touristic areas.⁵

The recent advancements in the development of a predictive correlate of protection³³ could significantly accelerate licensure of ZIKV vaccine candidates. Circumventing the need for large-scale field efficacy trials, which are considered unfeasible due to the unpredictable nature of the ZIKV epidemiology, could clear the path to licensure of this urgently needed vaccine.

Although the immunogenicity of our Zika virus vaccine candidate is considered encouraging, a further increase in peak antibody titers combined with prolonged antibody persistence is desirable. While the findings regarding different regimens and doses in this clinical trial should be confirmed in a larger sample size, the combination of the low dose with the rapid regimen provided an impractical immune response to be omitted from further development. Measures to optimize the primary immune response, such as a dose increase, optimizing the adjuvant to antigen concentration, investigation of alternative adjuvants such as toll-like receptor 9 (TLR9) agonist adjuvants (e.g. CpG) or a booster vaccination would merit future evaluation. Once the optimal dose has been identified, an inactivated ZIKV vaccine candidate would meet most of the desired vaccine characteristics by WHO, which include indication during pregnancy or lactation, a long-standing safety track record and the use of a platform established for licensed vaccines.

Supplementary data

Supplementary data are available at JTM online.

Data Sharing Statement

All data that underlie the results reported in this article (including study protocol) on individual participants will be made available to researchers who provide a methodologically sound proposal.

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Conflict of interest

N.W., R.H., M.S., M.O., N.B., K.L., I.C.R., K.D. and S.E.L. are Valneva employees and own stock and share options in Valneva.

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Ethics Committee Approval

Ethics committee (Chesapeake IRB) approval has been obtained prior any study related assessments occurred (approval date 22-Dec-2017).

Authors' Contributions

N.W., R.H., N.B. and K.D. contributed to study concept and design.

K.D., M.O. and S.E.L. supervised the study.

R.H., I.C.R. and K.L. did serological analyses.

N.W., R.H., M.S., M.O., K.D. and S.E.L. collected, assembled, analysed and interpreted data.

All authors contributed to the drafting and revision of the manuscript, reviewed and approved the final version.

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