

Safety and immunogenicity of AGS-v PLUS, a mosquito saliva peptide vaccine against arboviral diseases: A randomized, double-blind, placebo-controlled Phase 1 trial



DeAnna J. Friedman-Klabanoff,^a Megan Birkhold,^a Mara T. Short,^b Timothy R. Wilson,^b Claudio R. Meneses,^b Joshua R. Lacsina,^b Fabiano Oliveira,^b Shaden Kamhawi,^b Jesus G. Valenzuela,^b Sally Hunsberger,^c Allyson Mateja,^d Gregory Stoloff,^e Olga Pleguezuelos,^f Matthew J. Memoli,^g and Matthew B. Laurens^{a,*}



^aCenter for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, USA

^bLaboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA

^cBiostatistics Research Branch, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, USA

^dClinical Monitoring Research Program Directorate, Frederick National Laboratory for Cancer Research, Frederick, MD, USA

^ePepTcell (t/a SEEK), London, United Kingdom

^fConserV Bioscience, Oxfordshire, United Kingdom

^gClinical Studies Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Summary

Background Immunity to mosquito salivary proteins could provide protection against multiple mosquito-borne diseases and significantly impact public health. We evaluated the safety and immunogenicity of AGS-v PLUS, a mosquito salivary peptide vaccine, in healthy adults 18–50 years old.

Methods We conducted a randomized, double-blind, placebo-controlled Phase 1 study of AGS-v PLUS administered subcutaneously on Days 1 and 22 at the Center for Vaccine Development and Global Health, Baltimore, MD, USA. Participants were block randomized 1:1:1:1 to two doses saline placebo, two doses AGS-v PLUS, AGS-v PLUS/ISA-51 and saline placebo, two doses AGS-v PLUS/ISA-51, or two doses AGS-v PLUS/Alhydrogel. Primary endpoints were safety (all participants receiving ≥ 1 injection) and antibody and cytokine responses (all participants with day 43 samples), analysed by intention to treat.

Findings Between 26 August 2019 and 25 February 2020, 51 participants were enrolled and randomized, 11 into the single dose AGS-v PLUS/ISA-51 group and ten in other groups. Due to COVID-19, 15 participants did not return for day 43 samplings. Participants experienced no treatment-emergent or serious adverse events. All solicited symptoms in 2/10 placebo recipients and 22/41 AGS-v PLUS recipients after dose one and 1/10 placebo recipients and 22/41 AGS-v PLUS recipients after dose two were mild/moderate except for one severe fever the day after vaccination (placebo group). Only injection site pain was more common in vaccine groups (15/51 after dose 1 and 11/51 after dose 2) versus placebo. Compared to placebo, all vaccine groups had significantly greater fold change in anti-AGS-v PLUS IgG and IFN- γ from baseline.

Interpretation AGS-v PLUS had favourable safety profile and induced robust immune responses. Next steps will determine if findings translate into clinical efficacy against mosquito-borne diseases.

Funding UK Department of Health and Social Care.

Copyright © 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: Mosquito-borne diseases; Vector-borne viruses; Mosquito-borne disease vaccine

eBioMedicine
2022;86: 104375
Published Online xxx
<https://doi.org/10.1016/j.ebiom.2022.104375>

*Corresponding author. Center for Vaccine Development and Global Health, University of Maryland School of Medicine, 685 W. Baltimore St, Rm 480, Baltimore, MD, 21201, USA.

E-mail address: mllaurens@som.umaryland.edu (M.B. Laurens).

Trial registration: [ClinicalTrials.gov](https://clinicaltrials.gov) NCT04009824.

Research in context

Evidence before this study

On November 9, 2021, we searched PubMed for publications since inception using the terms “vector saliva” OR “mosquito saliva” AND (pathogenesis OR immunization OR vaccine). After excluding papers related to the pathogenesis of allergies to mosquito bites, methods of collecting mosquito saliva samples, vector competence, and other unrelated papers, we identified 134 papers describing vector-saliva mediated pathogenesis of transmission or vector-saliva immunization. When restricting to clinical trials and human species, two studies were identified: the Phase 1 trial of AGS-v and a study protocol describing a longitudinal paediatric cohort study that will evaluate the burden of dengue virus infection and the immune response to *Aedes aegypti* salivary proteins. The only study describing human immunization with arthropod vector saliva or synthetic saliva derivatives was the Phase 1 trial of AGS-v.

Added value of this study

We conducted a randomized, double-blinded, placebo-controlled Phase 1 clinical trial to evaluate the safety, immunogenicity, and *in vitro* anti-Zika virus effects of AGS-v PLUS which combines the four synthetic peptides (AGS-v) previously tested in a Phase 1 study with a fifth peptide found in the saliva of many mosquitoes, increasing potential breadth of protection. Geometric mean concentration and fold increase in AGS-v PLUS-specific IgG, IgM, and IgE and interferon- γ and interleukin-4 responses to AGS-v PLUS and to saliva from *A. aegypti*, *Aedes albopictus*, and *Anopheles gambiae* were measured on days 1, 43 and 50. *In vitro* anti-Zika virus effect was evaluated by recreating the environment at the mosquito bite site by *in vitro* mixing Zika virus (ZIKV) with mosquito saliva and exposing this mix to serum and PBMCs from vaccinated participants. Infectivity of the virus

was measured after 24 h incubation. Compared to the AGS-v study, this study also included another adjuvant, Alhydrogel, as comparison. Participants were randomized 1:1:1:1:1 to receive one of five dosing regimens: (i) two doses saline placebo; (ii) two doses non-adjuvanted AGS-v PLUS; (iii) one dose ISA-51-adjuvanted AGS-v PLUS followed by saline placebo; (iv) two doses ISA-51-adjuvanted AGS-v PLUS; and (v) two doses Alhydrogel-adjuvanted AGS-v PLUS. The two vaccinations were separated by three weeks. Three weeks after second vaccination, participants underwent uninfected mosquito feeding with *A. aegypti* and *A. albopictus* and had redness and swelling at the bite sites assessed 30 min, 2 days, and 5 days after feeding. To our knowledge, this is the second clinical trial evaluating a vector saliva vaccine and the first to address the cytopathologic effect of the vaccine against ZIKV, an arbovirus, *in vitro*. We found no difference in solicited reactogenicity between the groups except for injection site pain, which was less common in the placebo group. All formulations of AGS-v PLUS induced robust AGS-v PLUS-specific IgG and IFN- γ fold change responses, similar to the Phase 1 trial of AGS-v. No significant increase in redness or swelling after bites from *A. aegypti* or *A. albopictus* mosquitoes occurred in any group. Single dose AGS-v PLUS with ISA-51 induced immune responses that led to increased survival of Vero cells *in vitro* due to decreased infectivity of ZIKV.

Implications of all the available evidence

Study results demonstrate that vector-targeted vaccines can induce robust immune responses in a small number of participants without significant adverse effects. Results of the *in vitro* effects on Zika virus hold promise for clinical efficacy. Advancing clinical development of AGS-v PLUS is warranted.

Introduction

Mosquito-borne diseases cause ~360 million cases of illness and >600,000 deaths annually, although the true burden is likely underestimated.¹ Among these, malaria causes the greatest morbidity and mortality, but arboviruses such as dengue, yellow fever, West Nile virus, chikungunya, Japanese encephalitis and Zika virus also affect communities and many have no effective pathogen-specific preventative treatments. Symptoms can be absent or mild in some cases, but acute and severe complications such as haemorrhagic fever and death can occur. Although vaccines currently exist for yellow fever and Japanese encephalitis, approximately 109,000 cases and 51,000 deaths due to yellow fever occurred in 2018, and approximately 57,000 cases and 21,000 deaths due to Japanese encephalitis occurred in

2019.^{2,3} A vaccine for dengue fever has recently become available, but has a limited indication for those with a history of laboratory-confirmed illness, due to vaccine-associated increased risk of severe disease in dengue naïve individuals.⁴ RTS,S, a vaccine for malaria with modest efficacy, has also recently been recommended for widespread use by the World Health Organization, but the schedule is logistically challenging (primary series of three vaccines monthly then a booster dose 18 months after the last vaccine), which may limit access.^{5,6} Arboviruses can also cause devastating and difficult to control epidemics, like the 2015–2016 Zika virus epidemic.⁷ Vector control and, in the case of malaria, prompt treatment, have been the main strategy to try to decrease the burden of mosquito-borne diseases. However, these programs require continuous attention and

collaboration between international organizations, local governments, healthcare systems, and people living in the community. Even with these measures in place, the number of cases and deaths from malaria has not significantly decreased since 2015.^{8,9} Additional strategies to combat mosquito-borne diseases are needed to address these challenges.

A vaccine efficacious against multiple mosquito-borne diseases could significantly impact public health with one product. AGS-v PLUS is a vaccine containing five synthetic mosquito salivary peptides, aiming to induce an immune response that blocks mosquito-borne pathogen transmission. In animal models, mosquito saliva increases viremia and morbidity associated with arboviral disease.^{10,11} Mosquito saliva triggers a non-inflammatory Type 2 T helper cell (Th2) immune response in rodents that causes the characteristic skin irritation and allergic reaction associated with mosquito bites and increases susceptibility to arboviruses.^{10–14} It also downregulates Type 1 T helper cell (Th1) responses, including interferon- γ (IFN- γ), which has been shown to be important for antiviral responses.^{11,15} With time and continuous exposure, individuals become desensitized to mosquito bites, which could result in a more effective Th1 response.¹⁶ In a mouse model, repeated exposure to bites from uninfected mosquitoes produced Th1 responses at the bite site and reduced *Plasmodium yoelii* transmission, and passive immunization of mice with antibodies against two *A. aegypti* salivary proteins resulted in enhanced survival and decreased viremia in a Zika challenge model.^{17,18} The AGS-v PLUS vaccine aims to similarly prompt a Th1 response at mosquito bite sites and prevent pathogen transmission.

Immunization with AGS-v, a first-generation construct that contained four synthetic mosquito salivary peptides based on proteins derived from *A. gambiae* salivary gland lysate, induced a strong IFN- γ and antibody response to AGS-v peptides in mice. In a mouse challenge model using *A. gambiae* to infect mice with *P. yoelii nigeriensis*, immunizing mice with AGS-v also resulted in a greater than 50% decrease in infection and death (unpublished data, Supplementary Materials with [Supplementary Fig. S1](#)). A Phase 1 clinical study testing the safety and immunogenicity of AGS-v/ISA-51 performed at the National Institutes of Health Clinical Center identified no safety concerns and showed increased AGS-v-specific immunoglobulin G (IgG) and IFN- γ responses in vaccinees.¹⁹ The AGS-v PLUS vaccine adds a fifth synthetic peptide to the four contained in the AGS-v vaccine. This fifth peptide is found in saliva of many mosquito species, including *A. gambiae* (a major vector of malaria in sub-Saharan Africa), *Anopheles darlingi* (a major malaria vector in South America), *Culex quinquefasciatus* (which is a major vector of *Wuchereria bancrofti* and contributes to the spread of West Nile virus and St. Louis encephalitis virus), *A. aegypti* and *A. albopictus* (both of which are vectors of

Zika, dengue, chikungunya and yellow fever), increasing the potential breadth of protection.^{20–22} Preclinical data showed that the fifth peptide induced strong IgG antibody titres in mice (unpublished data, Supplementary Materials with [Supplementary Figs. S2–S4](#)). We conducted a Phase 1 clinical trial in healthy adults in Baltimore, MD, to evaluate the safety, immunogenicity, and *in vitro* effect on ZIKV infectivity of AGS-v PLUS with and without adjuvant and in different dosing regimens.

Methods

Trial design and participants

We conducted a randomized, double-blind, placebo-controlled Phase 1 study of AGS-v PLUS administered on Days 1 and 22 at the University of Maryland School of Medicine's Center for Vaccine Development and Global Health (CVD) in Baltimore, MD, USA. Healthy adults aged 18–50 years old, inclusive, with a body mass index of 18–40, inclusive, who agreed to use effective contraception (as defined in the protocol) from four weeks before enrolment until 12 weeks after second vaccination and had no history of previous severe allergic reaction, recent immunosuppression, ongoing chronic skin condition other than mild eczema, or other condition that would preclude ability to participate were enrolled. Participants were recruited and enrolled without regard for sex, and we recorded participant sex based on self-report. Full trial protocol, including complete inclusion/exclusion criteria, can be accessed in the [Supplementary Materials: Clinical Trial Protocol](#).

Ethics

All participants provided written informed consent before enrolment. The University of Maryland, Baltimore, Institutional Review Board approved the study protocol (protocol number: HP-00076625, FWA00007145). The NIAID Intramural Data and Safety Monitoring Board (DSMB) provided safety oversight. The study was conducted in accordance with the International Conference on Harmonization of Good Clinical Practices and the Declaration of Helsinki. CVD investigators directed the clinical trial and conducted safety assessments. Immunogenicity assays were performed by SEEK in London, United Kingdom (UK). All statistical analyses were performed by biostatisticians at the National Institute for Allergy and Infectious Diseases (NIAID). The trial was registered at [ClinicalTrials.gov](#) under NCT04009824.

Randomization and blinding

Participants were randomized 1:1:1:1:1 to one of five dosing groups: (i) two doses saline placebo; (ii) two doses non-adjuvanted AGS-v PLUS; (iii) one dose ISA-51-adjuvanted AGS-v PLUS and one dose saline placebo;

(iv) two doses ISA-51-adjuvanted AGS-v PLUS; and (v) two doses Alhydrogel-adjuvanted AGS-v PLUS. The planned sample size was at least 50 (ten/group) with a ceiling of 60 (twelve/group). The study statistician created the randomization scheme using block randomization with block sizes of ten and computer-generated randomization codes. The codes were sent to the unblinded pharmacist who maintained the key and prepared the appropriate product for administration. Participants and the study team were blinded to treatment assignment. All vaccination syringes were labelled with an opaque label placed over the syringe by the pharmacy team to cover contents so that blinded staff could not identify the different treatments.

The first ten participants were randomized into five arms with two in each arm and completed injections and subsequent Day 8 safety telephone calls, providing data for an interim safety analysis. The DSMB reviewed results of this analysis. No pausing or halting criteria were met (see [Supplementary Materials: Clinical Trial Protocol for list](#)), so the remaining participants were enrolled and randomized.

Procedures

The AGS-v PLUS vaccine was designed by SEEK (London, UK), and manufactured by Corden Pharma (Caponago, Italy) using Good Manufacturing Practice. Additional information on the selection and manufacture of the synthetic peptides can be found in the [Supplementary Materials: Clinical Trial Protocol](#). Lyophilized AGS-v PLUS was reconstituted before injection, resulting in a solution containing 50 nmol of each peptide. Additional details of vaccine formulation and reconstitution are in the [Supplementary Materials: Pharmacy Manual](#). The placebo group received 0.5 mL sterile saline placebo. For non-adjuvanted AGS-v PLUS, lyophilized AGS-v PLUS was reconstituted with 0.5 mL sterile water for injection (WFI). For the AGS-v PLUS/ISA-51 doses, each AGS-v PLUS vial was emulsified in 0.25 mL ISA-51 (Seppic, France) and 0.25 mL WFI to total 0.5 mL. AGS-v PLUS/Alhydrogel doses were prepared by mixing AGS-v PLUS in 0.12 mL Alhydrogel (Sergeant Adjuvants, Clifton, New Jersey, USA) and 0.38 mL sterile saline to total 0.5 mL.

Participants received their assigned study product subcutaneously in the upper arm on Days 1 and 22 and were monitored for at least 30 min after each vaccination. On day 43, participants underwent mosquito feeding using insects reared at the NIAID Laboratory of Malaria and Vector Research (LMVR). Eggs to establish this mosquito colony were donated to LMVR by Dr. Peter Armbruster (Georgetown University) from a colony established using larvae collected in Manassas, Virginia. Five starved, uninfected female *A. aegypti* and *A. albopictus* were placed in two separate containers covered with mesh, placed on the participant's right and left arms,

respectively, and allowed to feed for 10–20 min. Participants were evaluated for reactions at least 30 min after feeding and two days later, biopsies from the bite and normal skin areas were collected to evaluate mRNA expression. Redness and swelling at the bite sites were assessed by the principal investigator or designee by measuring the widest diameter of redness and induration in mm. Blood samples for immunogenicity (serum and whole blood) were collected from participants before vaccination (day 1), post-vaccination (day 43), and post-mosquito feeding (day 50). After vaccination, participants were given a memory aid to record vaccine reactivity for seven days. Participants were evaluated in clinic for adverse events and vaccine injection site reactions on days 22, 43, 45, and 50, and via telephone on days 181 and 366. Blood was collected for routine safety labs (see [Supplementary Materials: Clinical Trial Protocol for list](#)) on days 1, 22, 43, and 50.

Serum to assess AGS-v PLUS antigen specific IgG, immunoglobulin M (IgM) and immunoglobulin E (IgE) antibody responses via ELISA was collected on days 43 (primary endpoint) and 50 (secondary endpoint). 96-well plates were coated overnight with 100 µL of AGS-v PLUS antigens, each at 0.5 µM, in phosphate buffered saline (PBS). Wells were washed twice with PBS-Tween (PBS-T) and blocked with 200 µL of 1% bovine serum albumin (BSA) in PBS-T for 1 h. After washing wells five times, 100 µL of serum diluted 1:50 in 1% BSA-PBS-T were added. After 2 h, wells were washed five times and 100 µL of detection antibody (goat anti-human IgG at 1:16,000, goat anti-human IgM at 1:16,000, and goat anti-human IgE at 1:2,000, Sigma) were added for 1 h. Wells were washed five times and 100 µL of developer substrate (*p*-nitrophenyl phosphate disodium salt in diethanolamine buffer) were added. The reaction was stopped after 40 min by adding 50 µL of 3 M sodium hydroxide. Absorbance was measured at 405 nm and 620 nm (reference). Antibody concentrations were interpolated from the measured absorbance using a standard curve generated with known concentrations of human purified IgG, IgM and IgE antibodies (Sigma).

Peripheral blood mononuclear cells (PBMCs) were collected to evaluate Th1 (Interferon-γ) and Th2 (Interleukin-4) responses to AGS-v PLUS and to saliva from *A. aegypti*, *A. albopictus*, and *A. gambiae* by ELISA (OptEIA, BD Biosciences) on days 1, 43 and 50. PBMCs were thawed and allowed to rest for 24 h. Viable cells were counted and cell suspension adjusted to 4×10^6 cells/mL in RPMI 1640 containing 10% foetal calf serum, 100 IU/mL penicillin, 100 µg/mL streptomycin and 2 mM L-Glutamine solution. 100 µL of cells were added per well, followed by 50 µL of stimulation cocktail (2 µg/mL anti-CD-28 plus 2 µg/mL anti-CD49d) and 50 µL stimulant (mix of 4 µM of each peptide or 4 µg/mL salivary gland extracts (SGE), or 10 µg/mL concanavalin A or media alone). The SGE was prepared

by LMVR using the supernatant of sonicated salivary gland extracts from the salivary glands of each species utilized after centrifugation to maximize the use of soluble secreted proteins. Protein concentration in the SGE was measured at LMVR using a Bicinchoninic Acid (BCA) assay (Pierce) and confirmed using the BCA assay (Merck, UK) at SEEK. Cells were kept for 48 h at 37 °C 5% CO₂ when supernatants were collected to quantify IFN- γ and IL-4 cytokines following manufacturer instructions.

Whole blood samples containing PBMCs and antibodies generated against AGS-v PLUS from participants on days 1 and 43 were tested for their ability to reduce infectivity of Zika virus mixed with SGE from *A. albopictus*. PBMCs were stimulated with the vaccine antigens as done for the cytokine ELISAs. After 48 h, 50 μ L Zika virus stock (National Collection of Pathogenic Viruses, catalogue 1308258 V, strain MP1751, HPA, UK) diluted 1:10 in PBS was mixed with 20 μ L SGE in a total volume of 65 μ L for 2 h at 37 °C. Stimulated PBMCs were washed by centrifugation to remove stimulants and resuspended in RPMI medium including 20% of the subjects' own matched timepoint serum. 100 μ L of PBMCs were seeded with 100 μ L RPMI (control), 2 μ g/mL mosquito SGE in 100 μ L RPMI, Zika virus diluted 1:2000 in 100 μ L RPMI, or Zika virus in saliva in 100 μ L RPMI and were incubated for 24 h. 150 μ L of cell supernatants were then collected and added to Vero cell monolayers and incubated for four days. Cell viability was assessed by dimethylthiazole (MTT) assay.

Outcomes

Primary study endpoints included incidence and severity of treatment emergent adverse events (AEs) and serious adverse events (SAEs), geometric mean titre and fold increase in serum AGS-v PLUS specific IgG, IgM, and IgE titres from day 1 to day 43, and geometric mean concentration and fold increase in Th1 and Th2 cytokine responses after *in vitro* exposure of PBMCs with AGS-v PLUS antigens, day 1 compared with day 43. Secondary outcome measures included geometric mean titre and fold increase in AGS-v PLUS specific IgG, IgM and IgE titres seven days after mosquito feeding (day 50) compared to days 1 and 43 and geometric mean concentration and fold increase in AGS-v PLUS specific Th1 and Th2 cytokine responses in the supernatants of PBMCs (day 50 compared to days 1 and 43) after *in vitro* exposure with AGS-v PLUS. Study exploratory endpoints included measurement of mosquito bites site reactions 30 min, two days, and five days after feedings; *in vitro* viability of Zika virus mixed with mosquito SGE after incubation with serum and PBMCs from vaccinated participants; and geometric mean concentration and fold increase in Th1 and Th2 cytokine responses (from day 1 to day 43, and from day 43 to day 50) after

in vitro exposure of PBMCs to mosquito SGE from *A. aegypti*, *A. albopictus* and *A. gambiae*.

Statistics

Enrolling ten participants in each group gave at least an 80% chance of observing at least one participant with an SAE if the true underlying rate for that SAE was 0.15. By collapsing across the vaccinated arms, 40 participants receiving AGS-v PLUS gave a 95% chance of observing at least one participant with an SAE if the true rate was 0.07. This sample size also gave at least 85% power to detect a true difference in the primary immunogenicity endpoint of 0.95 standard deviations using a one-sided t-test with significance level of 0.1.

Due to the COVID-19 pandemic, 15 study participants were unable to attend in-person visits beyond day 22 and were administratively censored, resulting in three analysis populations. The safety cohort included all participants who received at least one injection. The intention-to-treat administratively censored (ITT-AC) cohort included all participants with samples available from day 43, analysed in the group to which they were randomized. The per protocol cohort consisted of participants who received two injections corresponding to their randomized treatment group, underwent mosquito challenge, had valid immunological data on days 1, 43, and 50, and were not subject to any major protocol deviation of eligibility criteria or study procedures.

T-tests were used to compare continuous endpoints between each treatment group and the placebo group. Fisher's exact test was used for binary endpoints. All comparisons were at the type I error rate of 0.1 and confidence intervals were calculated at the 90% level. No multiple comparisons adjustments were made as this was a Phase 1 study and all comparisons were considered exploratory. Adverse events were tabulated by treatment arm. For visual assessment of mosquito bite sites, mean redness measured across the widest part (mm) and mean swelling (mm²) were calculated for each group and active groups were compared to placebo at day 43 (30 min post-feeding), day 45, and day 50. For cellular and humoral responses to AGS-v PLUS, fold increases from day 1 to days 43 and 50, and from day 43 to day 50 were calculated for each participant. Mean log₁₀ fold increases were compared for each vaccine group with the placebo group. Cytokine measurements were normalized as described in the [Supplementary Materials: Statistical Analysis Plan](#). The geometric mean antibody titre and geometric mean cytokine concentration of IFN- γ and IL-4 to AGS-v PLUS on days 1, 43, and 50 were calculated and log₁₀ titres and concentrations were compared between each vaccine group and placebo. The decrease in cell death from pre-to post-vaccination representing the viability/infectivity of Zika virus was calculated for each active group and compared to placebo. All analyses were performed using R (version 3.6.3).

Additional details for statistical analyses are in the [Supplementary Materials: Statistical Analysis Plan](#).

Role of the funders

The funding bodies had no role in study design; data collection, analysis, or interpretation; writing of the report; or decision to submit the manuscript.

Results

Sixty-nine participants were screened between 22 July 2019 and 21 February 2020. Fifty-one were enrolled and randomized between 26 August 2019 and 25 February 2020 into five groups: sterile saline placebo (n = 10), two doses non-adjuvanted AGS-v PLUS (n = 10), one dose ISA-51-adjuvanted AGS-v PLUS and one dose saline placebo (n = 11), two doses ISA-51-adjuvanted AGS-v PLUS (n = 10), and two doses Alhydrogel-adjuvanted AGS-v PLUS (n = 10) (Fig. 1). Participant demographics are provided in Table 1.

All randomized participants received at least one injection and were included in safety analyses. All in-person trial activities were cancelled as of 23 March

2020 due to the COVID-19 pandemic. After this date, participants continued telephone safety follow-up, but Day 43 and 50 blood samples were not collected from the last 15 participants, and thus administratively censored (Fig. 1). After randomization, two participants did not receive a second vaccination: one due to Grade 2 anaemia and one withdrew due to the COVID-19 pandemic. Eighteen participants did not undergo mosquito feeding: 15 did not attend due to the COVID-19 pandemic, one subject refused, one was temporarily out of the area, and one did not receive the second vaccine due to unrelated anaemia. One participant was lost to follow up at the Day 181 safety phone follow-up (no participant response to multiple calls and a certified letter).

No participant experienced any treatment-emergent or serious adverse event. Participants reported 134 solicited symptoms: 20 local and 35 systemic in 25 (49%) participants after dose 1 (Fig. 2a and Supplementary Table S1), and 23 local and 56 systemic in 23 (45%) participants after dose 2 (Fig. 2b and Supplementary Table S2). All were considered mild/moderate except for severe fever in a placebo recipient. The most common solicited local symptom was pain,

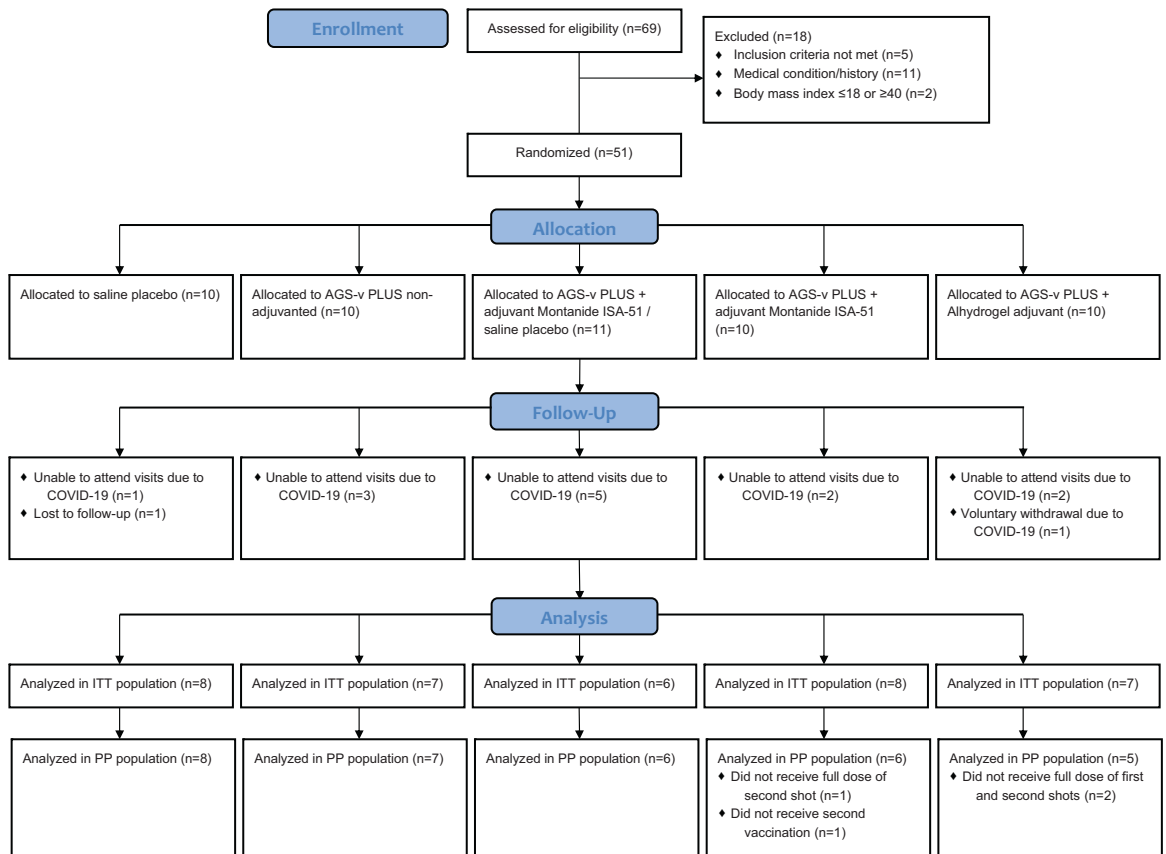


Fig. 1: Screening, enrolment, vaccination, randomization, follow-up, and analysis cohorts of study participants.

	Overall (n = 51)	Placebo (n = 10)	AGS-v PLUS non-adjuvanted (n = 10)	AGS-v PLUS + ISA-51/placebo (n = 11)	AGS-v PLUS + ISA-51 (two doses) (n = 10)	AGS-v PLUS + Alhydrogel (n = 10)
Sex						
Female	21 (41.2%)	2 (20.0%)	3 (30.0%)	6 (54.5%)	5 (50.0%)	5 (50.0%)
Male	30 (58.8%)	8 (80.0%)	7 (70.0%)	5 (45.5%)	5 (50.0%)	5 (50.0%)
Ethnicity						
Hispanic or Latino	2 (3.9%)	0 (0.0%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (10.0%)
Not Hispanic or Latino	49 (96.1%)	10 (100.0%)	10 (100.0%)	10 (90.9%)	10 (100.0%)	9 (90.0%)
Race						
American Indian/Alaskan Native	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Asian	4 (7.8%)	0 (0.0%)	2 (20.0%)	1 (9.1%)	1 (10.0%)	0 (0.0%)
Black/African American	21 (41.2%)	7 (70.0%)	4 (40.0%)	5 (45.5%)	4 (40.0%)	1 (10.0%)
Hawaiian/Pacific Islander	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
White	23 (45.1%)	3 (30.0%)	4 (40.0%)	4 (36.4%)	5 (50.0%)	7 (70.0%)
Mixed Race	2 (3.9%)	0 (0.0%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (10.0%)
Unknown	1 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
Not Reported	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Age, years	36.63 (8.59)	34.40 (7.32)	36.90 (8.32)	41.36 (9.70)	34.50 (9.97)	35.50 (6.64)

Note: Continuous variables are presented as mean (SD) and categorical variables are presented as N (%).

Table 1: Demographic information for all randomized AGS-v PLUS participants.

occurring in 15 (29%) and 11 (22%) participants after dose 1 and 2, respectively. The most common solicited systemic symptoms were headache, malaise, and fatigue, occurring in 9 (18%), 5 (10%) and 7 (14%) participants after dose 1 (Fig. 2a and Supplementary Table S1), and 9 (18%), 11 (22%) and 11 (22%) participants after dose 2, respectively (Fig. 2b and Supplementary Table S2). Of 123 total unsolicited adverse events reported, 19 were deemed related to vaccination in 11 participants (21.6%). These included four lab abnormalities, one bradycardia, and 14 injection/vaccination site reactions that extended beyond the

seventh day after vaccination. Adverse event details are in Supplementary Table S3.

After *A. aegypti* and *A. albopictus* feeding, bite site swelling diameter was not statistically different for any group (Supplementary Fig. S5b). Participants receiving AGS-v PLUS/Alhydrogel had lower mean *A. aegypti* mosquito bite redness at Day 45 and those receiving 2 doses AGS-v PLUS/ISA-51 had lower mean *A. aegypti* mosquito bite redness at Day 50 compared to placebo: mean difference 3.8 mm (90% CI 0.5, 7.0; p = 0.061 [t-test]) and 6.5 mm (90% confidence interval [CI] 0.3, 12.8; p = 0.086 [t-test]), respectively. Participants receiving

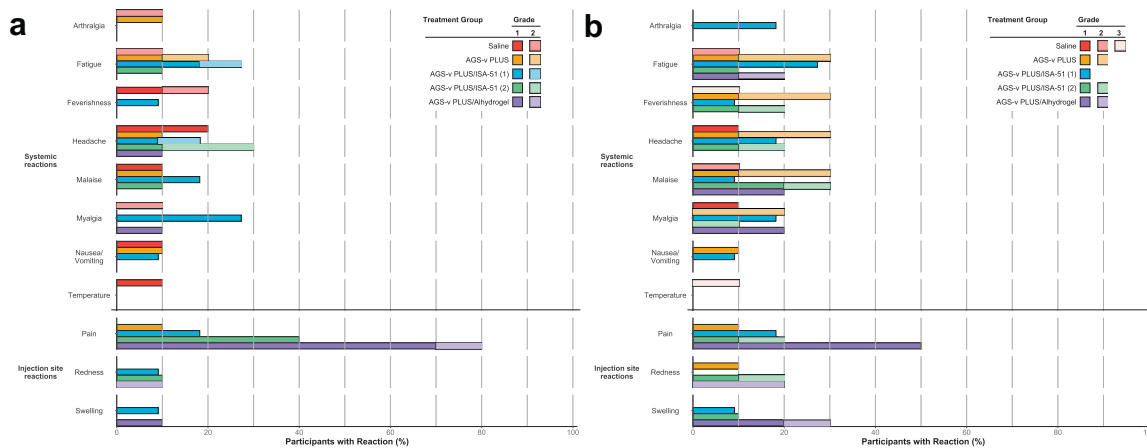


Fig. 2: Solicited injection site and systemic reactions reported after doses one (a) and two (b). All symptoms were mild/moderate except for one severe fever in a placebo recipient after dose two. Participants receiving AGS-v PLUS with or without adjuvant and those receiving placebo had similar solicited symptoms except for injection site pain, which was less common in those receiving placebo.

non-adjuvanted AGS-v PLUS and those receiving AGS-v PLUS/Alhydrogel had lower mean mosquito bite redness after *A. albopictus* feeding than placebo at Day 43: mean difference 22.3 mm (90% CI: 1.2, 43.4; $p = 0.084$ [t-test]) and 23.1 mm (90% CI: 2.4, 43.8; $p = 0.071$ [t-test], respectively) (Supplementary Fig. S5a).

Baseline AGS-v PLUS specific IgM titres were not significantly different when comparing each group to placebo. However, the placebo group did have significantly greater AGS-v PLUS specific IgG titres than the single dose AGS-v PLUS/ISA-51 group at baseline: mean difference: 0.722 (90% CI: 0.205, 1.239, $p = 0.032$ [t-test]) (Supplementary Fig. S6 and Supplementary Table S4). AGS-v PLUS specific IgM fold change was greater in the two-dose AGS-v PLUS/ISA-51 group than the placebo group at Day 43/Day 1: mean difference: 0.565 (90% CI: 0.256, 0.874; $p = 0.010$ [t-test]) (Fig. 3). The group receiving non-adjuvanted AGS-v PLUS and both AGS-v PLUS/ISA-51 groups had greater AGS-v PLUS specific IgM fold change than the placebo group at Day 50/Day 1: mean difference 0.176 to 0.707, $p = 0.025$ to 0.097 [t-test] (Fig. 3). All vaccination groups had greater AGS-v PLUS specific IgG fold changes at Day 43/Day 1: mean difference 0.258 to 1.318 ($p = 0.001$ to 0.029 [t-test]), and at Day 50/Day 1: mean difference 0.256 to 1.430 ($p = 0.009$ to 0.042 [t-test]). All adjuvanted vaccine groups also had greater AGS-v PLUS specific IgG fold changes at Day 50/Day 43: mean difference 0.070 to 0.136 ($p = 0.018$ to 0.058 [t-test]). No significant differences in AGS-v PLUS specific IgE titres or fold

changes existed between placebo and vaccination groups at any time point.

We used IFN- γ levels as a measure of Th1 responses. The group receiving two-dose AGS-v PLUS/ISA-51 and the group receiving AGS-v PLUS/Alhydrogel secreted significantly lower IFN- γ than placebo at baseline after stimulation with AGS-v PLUS antigens: mean difference 0.762 to 0.782 ($p = 0.065$ to 0.086 [t-test]) (Supplementary Fig. S7a and Supplementary Table S5). The AGS-v PLUS/Alhydrogel group also had significantly lower IFN- γ than placebo at baseline in response to *A. gambiae* SGE: mean difference 0.813 (90% CI: 0.048, 1.577, $p = 0.083$ [t-test]). When examining fold changes from baseline after stimulation with AGS-v PLUS antigens, all vaccine groups had a significantly greater fold change in secretion of IFN- γ from baseline compared to placebo at day 43: mean difference 1.854 to 2.221 ($p = 0.006$ to 0.041 [t-test]) (Fig. 4a). All groups receiving adjuvanted AGS-v PLUS also had a significantly greater fold change in IFN- γ secretion in response to AGS-v PLUS antigens compared to placebo at day 50 relative to baseline: mean difference 2.064 to 2.362 ($p = 0.003$ to 0.018 [t-test]). The placebo group had a greater fold change in secretion of IFN- γ from baseline in response to *A. aegypti* SGE compared to the non-adjuvanted AGS-v PLUS group at day 43: mean difference 0.987 (90% CI: 0.099, 1.876, $p = 0.071$ [t-test]), but no other differences existed between placebo and the vaccine groups at any timepoints. No significant differences in fold change in secretion of IFN- γ in response to *A. albopictus* SGE or *A. gambiae* SGE

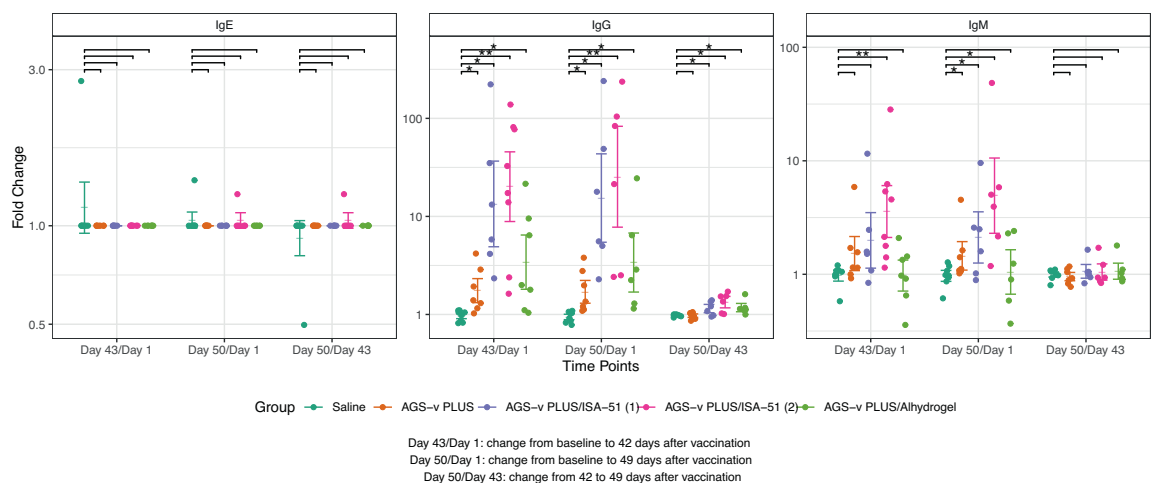


Fig. 3: Mean log₁₀ transformed fold change in AGS-v PLUS specific IgM, IgG and IgE with 90% confidence intervals. The x-axis displays the time points analysed and the y-axis displays the log₁₀ transformed fold change values. Bars at the top of the graphs denote comparisons made with asterisks to denote significance: (* = <0.1, ** = ≤0.01, *** = ≤0.001 [t-test]). Non-adjuvanted AGS-v PLUS and both AGS-v PLUS/ISA-51 groups had greater AGS-v PLUS specific IgM fold change than the placebo group at Day 50 compared to baseline. All vaccination groups had greater AGS-v PLUS specific IgG fold changes at Days 43 and 50 compared to baseline and all adjuvanted vaccine groups had greater AGS-v PLUS specific IgG fold changes at Day 50 compared to Day 43. No significant differences in AGS-v PLUS specific IgE fold changes existed between placebo and vaccination groups at any time point.

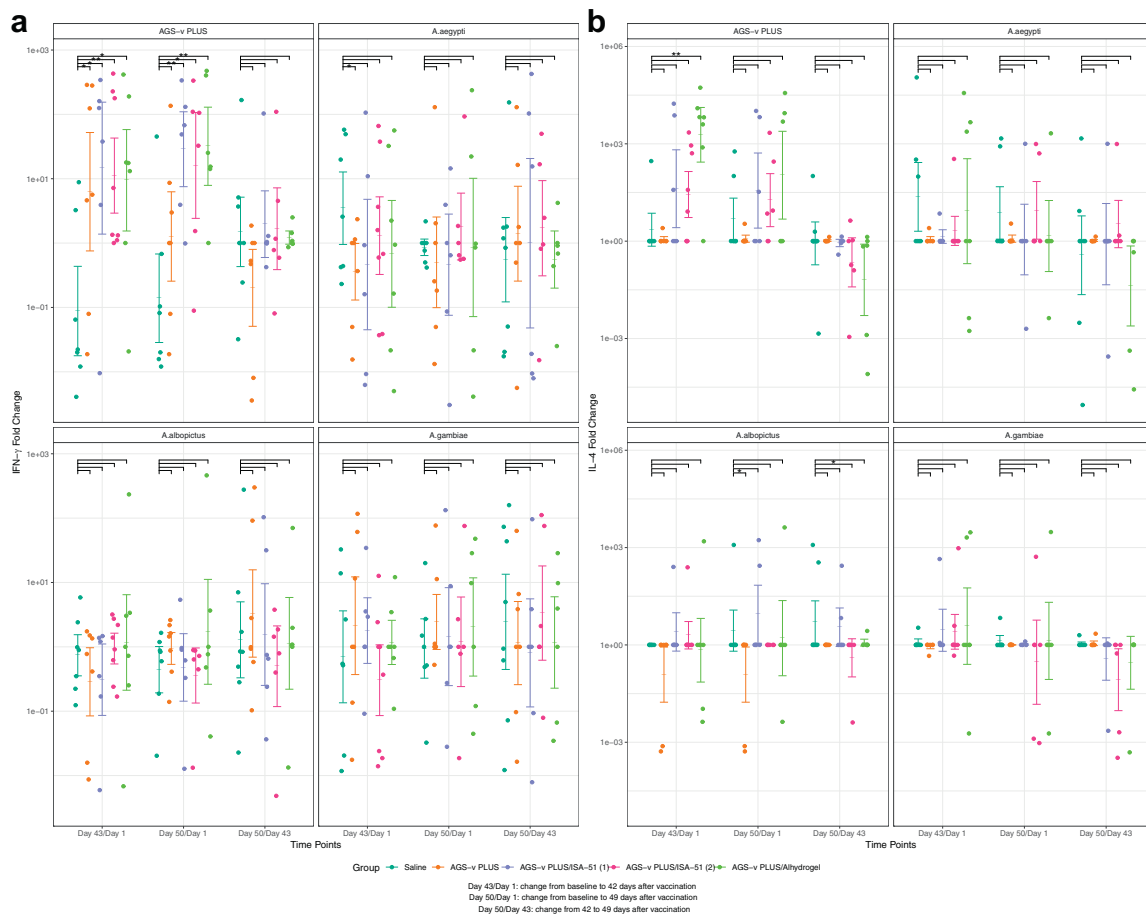


Fig. 4: a). Mean log₁₀ transformed fold change in IFN- γ secretion in response to AGS-v PLUS, *A. aegypti* salivary gland extracts (SGE), *A. albopictus* SGE, and *A. gambiae* SGE with 90% confidence intervals. The x-axis displays the time points analysed and the y-axis displays the log₁₀ transformed fold change values. Bars at the top of the graphs denote comparisons made with asterisks to denote significance: (* = <0.1, ** = ≤ 0.01 , *** = ≤ 0.001 [t-test]). Significant differences existed in fold change in secretion of IFN- γ in response to AGS-v PLUS antigens between placebo and all vaccine groups at day 43 versus baseline and between placebo and all groups receiving adjuvanted AGS-v PLUS at day 50 versus baseline. In response to *A. aegypti* SGE, significant differences in secretion of IFN- γ existed only between the placebo group and the non-adjuvanted AGS-v PLUS group at day 43 versus baseline. No significant differences in fold change in secretion of IFN- γ were observed between placebo and the vaccine groups at any timepoint. b). Mean log₁₀ transformed fold change in IL-4 secretion in response to AGS-v PLUS, *A. aegypti* SGE, *A. albopictus* SGE, and *A. gambiae* SGE with 90% confidence intervals. The x-axis displays the time points analysed and the y-axis displays the log₁₀ transformed fold change values. Significant differences in IL-4 secretion in response to AGS-v PLUS antigens existed only between the AGS-v PLUS/Alhydrogel group and placebo group at day 43 versus baseline. In response to *A. albopictus* SGE, significant differences existed only between the non-adjuvanted AGS-v PLUS group and placebo group at day 50 versus baseline and the two dose AGS-v PLUS/ISA-51 group at Day 50 versus Day 43. No other significant differences existed in fold change in IL-4 secretion in response to AGS-v PLUS antigens, *A. aegypti* SGE, or *A. gambiae* SGE between placebo and the vaccine groups at any time points.

were observed between placebo and the vaccine groups at any timepoint.

IL-4 levels were measured as a marker of Th2 responses. No significant differences were detected at baseline between placebo and any vaccine groups for IL-4 secretion in response to either AGS-v PLUS antigens or to *A. aegypti*, *A. albopictus*, or *A. gambiae* SGE (Supplementary Fig. S7b and Supplementary Table S6). After stimulation with AGS-v PLUS antigens, the AGS-v PLUS/Alhydrogel group had a greater fold change in IL-

4 secretion from baseline to day 43 compared to placebo: mean difference 2.926 (90% CI: 1.686, 4.165; $p = 0.002$ [t-test]) (Fig. 4b). In response to *A. albopictus* SGE, the non-adjuvanted AGS-v PLUS group had a lower fold change in IL-4 secretion from baseline to day 50 compared to placebo: mean difference 1.355 (90% CI: 0.033, 2.677, $p = 0.093$ [t-test]), and the two dose AGS-v PLUS/ISA-51 group had a lower fold change in IL-4 secretion from Day 43 to Day 50: mean difference 1.101 (90% CI: 0.013, 2.190, $p = 0.097$ [t-test]). No other

significant differences existed in fold change in IL-4 secretion in response to AGS-v PLUS antigens, *A. aegypti* SGE, or *A. gambiae* SGE between placebo and the vaccine groups at any time points.

Stimulated PBMCs and serum from days 1 and 43 from participants were used to determine if immune responses to AGS-v PLUS were able to reduce the infectivity of Zika virus mixed with mosquito saliva by measuring survival of Vero cells after infection. Compared to placebo, the single dose AGS-v PLUS/ISA-51 group had significantly greater percent difference in survival (post-versus pre-vaccination) of Vero cells inoculated with viral cultures that had been exposed to immune cells and serum from day 43 versus day 1: mean difference 6.461 (90% CI: 0.343, 12.580; $p = 0.085$ [t-test]) (Fig. 5).

Five participants were excluded from the per-protocol analysis: three for lower-than-intended volume vaccines for one or both doses, one for missing second vaccination and one for receiving second vaccination despite a Grade 2 lab abnormality. Although several differences were seen between the per protocol analysis and the ITT-AC analysis, the study team deemed these differences were due to the small sample size. Full results are in the [Supplementary Materials: Intention to Treat-Administratively Censored Analysis Report](#) and [Per Protocol Analysis Report](#).

Discussion

In this randomized, double-blind, placebo-controlled Phase 1 study of AGS-v PLUS with or without adjuvant, all formulations had a favourable safety and tolerability profile. Participants receiving placebo and those receiving

AGS-v PLUS with or without adjuvant had similar solicited symptoms, except that injection site pain was less commonly reported in placebo recipients. Participants who received AGS-v PLUS, with or without adjuvant, also had no significant increase in redness or swelling after *A. aegypti* or *A. albopictus* mosquito feeding. In fact, some groups had smaller mean areas of redness compared to placebo. Differences seen may be due to personal history of mosquito bites, individual differences in responses to mosquito bites, or vaccine-induced modification of the local response. Planned analyses of gene expression profiling using RNA expression profiles from skin biopsy specimens at *A. aegypti* and *A. albopictus* skin bite sites compared to unbiten skin sites will further investigate vaccine-induced changes at the molecular level. Maryland is known to have *A. albopictus* mosquitoes, so most participants have likely been exposed to at least one of these species.²³ We saw no significant boosting of systemic IFN- γ responses after mosquito feeding, which may be related to the AGS-v PLUS vaccine peptides, as only one was derived from *A. aegypti*/*A. albopictus* salivary proteins or that the effect is more local rather than systemic. In addition, mosquito SGE are highly immunogenic *in vitro* due to the presence of mosquito salivary gland cells and lack of sterility, so IFN- γ levels measured when PBMCs were stimulated with mosquito SGE were often at the upper limit of detection. This made small differences difficult to detect.

No reliable immunological correlations of protection exist for mosquito saliva targeted vaccines. Mice vaccinated with the first-generation vaccine, AGS-v, mounted robust antibody and IFN- γ responses to AGS-v antigens and survival was more than doubled after challenge with

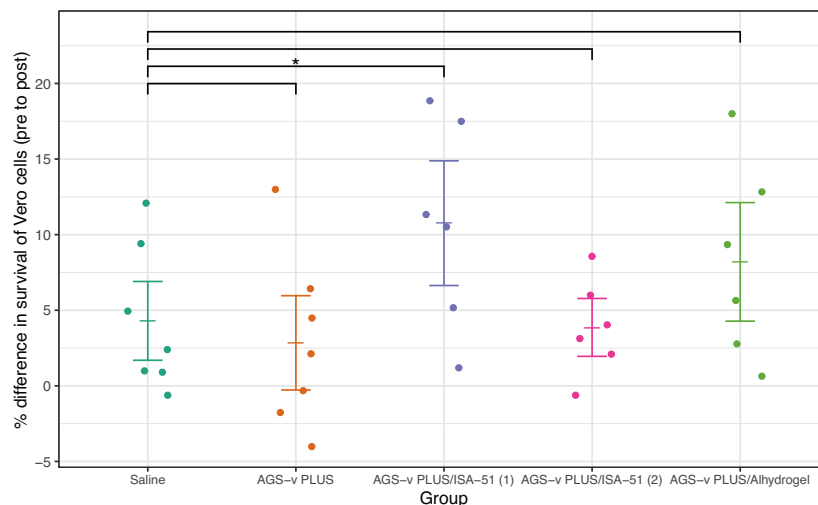


Fig. 5: Percent difference in Vero cell killing by Zika virus pre-vaccination to post-vaccination. The x-axis displays the groups, and the y-axis displays percent difference in killing pre-to post-vaccination. Bars at the top of the graphs denote comparisons made with asterisks to denote significance: (* = <0.1 , ** = ≤ 0.01 , *** = ≤ 0.001 , [t-test]). Compared to placebo, the single dose AGS-v PLUS/ISA-51 group had significantly greater percent difference in survival of Vero cells inoculated with Zika exposed to immune cells and serum from participants from day 43 compared to immune cells and serum from participants from day 1.

a rodent malaria, *P. yoelii nigeriensis*, delivered via *A. gambiae* mosquito bites. All second-generation vaccine formulations induced robust anti-AGS-v PLUS IgG and IFN- γ fold change responses in our participants. The similarity in anti-AGS-v PLUS IgG fold change responses between single dose AGS-v PLUS/ISA-51 and two dose AGS-v PLUS/ISA-51 deserves further investigation and may indicate that a single dose regimen could suffice. AGS-v PLUS with ISA-51 recipients showed *in vitro* decreased Vero cell death due to Zika infection suggesting that vaccine-induced immune responses may limit Zika virus infectivity. Follow-up studies using challenge models or in endemic areas will confirm if these immunological and *in vitro* results translate into human protection from infection.

Our study was limited by the COVID-19 pandemic that began mid-trial and associated smaller-than-expected samples available for immunological assays. Despite this limitation, we did document statistically significant immunological differences between vaccine recipients and placebo. Our study was done in an area known to have *Aedes* species endemic to the region, but no *Anopheles* species, which may limit the generalizability to areas where people are most at risk for mosquito borne illnesses.

To our knowledge, this is the first clinical trial to evaluate *in vitro* the effect of the vaccine against the infectivity of ZIKV, an arbovirus and only the second study evaluating a vector saliva vaccine, following the Phase 1 study of the first generation vaccine, AGS-v.¹⁹ Our results show that AGS-v PLUS demonstrates a promising safety, tolerability, and immunogenicity profile, especially when combined with ISA-51. Given the high rates of morbidity and mortality of mosquito borne diseases worldwide and the lack of effective prophylactic treatment for most arboviral infections, these first results of human testing hold promise for clinical efficacy, potentially saving many lives. Next steps in the development of AGS-v PLUS include assessing clinical efficacy in areas endemic for mosquito-borne diseases. Controlled human malaria infection challenge studies where infection is transmitted by the bite of infected mosquitoes could also be used to evaluate efficacy of the vaccine and investigate correlates of protection. Combination of AGS-v PLUS with other approved or in development pathogen-specific vaccines for diseases transmitted by mosquitoes could result in a synergistic effect on efficacy.

Contributors

DJFK, JGV, SH, OP, MJM, and MBL participated in the conceptualisation of the vaccine trial. DJFK, MB, MTS, JRL, MJM, and MBL executed the vaccine trial, mosquito feedings, skin biopsies, participant follow up and trial oversight. MTS, TRW, CRM, JRL, FO, SK, and JGV reared mosquitoes, developed and processed mosquito related assays, and analysed mosquito related laboratory data. OP developed and processed

laboratory assays related to immunogenicity of the vaccine. AM and SH provided statistical support and analysed the data. DJFK and MBL wrote the original draft manuscript and verified the underlying data. All authors contributed to review and editing of the manuscript. GS and OP developed and provided the study product and acquired funding for the clinical trial. All authors read and approved the final version of the manuscript.

Data sharing statement

The clinical trial protocol, statistical analysis plan, and de-identified participant data in aggregate are included in the [Supplementary Materials](#).

Declaration of interests

The views expressed in this publication are those of the author(s) and not necessarily those of Innovate UK or the UK's Department of Health and Social Care.

Imutex Limited is a joint venture with PepTcell Limited (trading as SEEK) in which Open Orphan has a 49% shareholding, and PepTcell has 51%. Dr. Olga Pleguezuelos is an employee of ConserV Bioscience, a subsidiary of PepTcell Limited (trading as SEEK) and a shareholder in PepTcell Limited (trading as SEEK). Gregory Stoloff is an employee and shareholder in PepTcell. Imutex Ltd owns the intellectual property rights around AGS-v and AGS-v PLUS. All remaining authors declare no conflicts of interest. OP and GS are also named inventors in the following issued patents: AU 2008297213, AU 2013200258, BE 2783694, CH 2783694, CN 20101815526, DE 602008054283.1, DK 2783694, EP 2783694, ES 2783694, FI 2783694, FR 2783694, GB 2783694, IE 2783694, IN 296636, IT 2783694, JP 5981883, JP 6227018, MC 2783694, MT 2783694, MX 310667, NO 2783694, NZ 602090, OA 14958, PL 2783694, PT 2783694, RU 2466737, SE 2783694, SG 187401, TR 2783694, TW 548646, US 8,986,703, ZA 2010/00914.

Acknowledgements

We would like to thank the participants from the Baltimore, MD, area for their time and commitment to this study. We would also like to thank the study coordinators, lab and pharmacy staff, regulatory oversight and quality assurance staff, and data entry staff from the Center for Vaccine Development and Global Health, University of Maryland School of Medicine, for their assistance with the study. We would like to thank Peter Armbruster, Department of Biology, Georgetown University, for providing *Aedes albopictus* eggs to start a colony for this study. The project was funded by the UK Department of Health and Social Care (DHSC) through an SBRI program managed by Innovate UK (Project number 971556). This report is work commissioned by Innovate UK and DHSC. This project was also funded in part by the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases (M.T.S., T.R.W., C.R.M., J.R.L., F.O., S.K., J.G.V., S.H., M.J.M.). This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024 (A.M.). M.J.M was supported by SEEK, Inc., by a collaborative research agreement which included payment for mosquito breeding and personnel. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. DJF-K was supported by the National Institute for Allergy and Infectious Diseases award 1K23AI155838-01A1.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.ebiom.2022.104375>.

References

- 1 Institute for Health Metrics and Evaluation (IHME). *Global Burden of Disease 2019 Cause and Risk Summary: Malaria, Dengue, Yellow Fever, Zika*; 2020. Accessed April 6, 2021.
- 2 Moore SM. The current burden of Japanese encephalitis and the estimated impacts of vaccination: combining estimates of the spatial distribution and transmission intensity of a zoonotic pathogen. *PLoS Negl Trop Dis*. 2021;15(10):e0009385.
- 3 Gaythorpe KA, Hamlet A, Jean K, et al. The global burden of yellow fever. *Elife*. 2021;10:e64670.
- 4 Wong JM, Adams LE, Durbin AP, et al. Dengue: a growing problem with new interventions. *Pediatrics*. 2022;149(6):e202105522.
- 5 RTSS Clinical Trials Partnership. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. *PLoS Med*. 2014;11(7):e1001685.
- 6 RTSS Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet*. 2015;376:31–45.
- 7 Fauci AS, Morens DM. Zika virus in the Americas—yet another arbovirus threat. *N Engl J Med*. 2016;374(7):601–604.
- 8 World Health Organization. *World Malaria Report 2016*. Geneva: World Health Organization; 2016.
- 9 World Health Organization. *World Malaria Report 2020: 20 years of global progress and challenges*. Geneva: World Health Organization; 2020.
- 10 McCracken MK, Gromowski GD, Garver LS, et al. Route of inoculation and mosquito vector exposure modulate dengue virus replication kinetics and immune responses in rhesus macaques. *PLoS Negl Trop Dis*. 2020;14(4):e0008191.
- 11 Pinggen M, Schmid MA, Harris E, McKimmie CS. Mosquito biting modulates skin response to virus infection. *Trends Parasitol*. 2017;33(8):645–657.
- 12 Peng Z, Yang M, Simons FE. Immunologic mechanisms in mosquito allergy: correlation of skin reactions with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. *Ann Allergy Asthma Immunol*. 1996;77(3):238–244.
- 13 Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. *Trans R Soc Trop Med Hyg*. 2008;102(5):400–408.
- 14 Thangamani S, Higgs S, Ziegler S, Vanlandingham D, Tesh R, Wikel S. Host immune response to mosquito-transmitted chikungunya virus differs from that elicited by needle inoculated virus. *PLoS One*. 2010;5(8):e12137.
- 15 Cross ML, Cupp EW, Enriquez FJ. Differential modulation of murine cellular immune responses by salivary gland extract of *Aedes aegypti*. *Am J Trop Med Hyg*. 1994;51(5):690–696.
- 16 Peng Z, Simons FE. Mosquito allergy: immune mechanisms and recombinant salivary allergens. *Int Arch Allergy Immunol*. 2004;133(2):198–209.
- 17 Donovan MJ, Messmore AS, Scrafford DA, Sacks DL, Kamhawi S, McDowell MA. Uninfected mosquito bites confer protection against infection with malaria parasites. *Infect Immun*. 2007;75(5):2523–2530.
- 18 Marin-Lopez A, Wang Y, Jiang J, Ledizet M, Fikrig E, AgBR1 and NeSt1 antisera protect mice from *Aedes aegypti*-borne Zika infection. *Vaccine*. 2021;39(12):1675–1679.
- 19 Manning JE, Oliveira F, Coutinho-Abreu IV, et al. Safety and immunogenicity of a mosquito saliva peptide-based vaccine: a randomised, placebo-controlled, double-blind, phase 1 trial. *Lancet*. 2020;395(10242):1998–2007.
- 20 Neafsey DE, Waterhouse RM, Abai MR, et al. Mosquito genomics. Highly evolvable malaria vectors: the genomes of 16 *Anopheles* mosquitoes. *Science*. 2015;347(6217):1258522.
- 21 Arensburger P, Megy K, Waterhouse RM, et al. Sequencing of *Culex quinquefasciatus* establishes a platform for mosquito comparative genomics. *Science*. 2010;330(6000):86–88.
- 22 Leta S, Beyene TJ, De Clercq EM, Amenu K, Kraemer MUG, Revie CW. Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *Int J Infect Dis*. 2018;67:25–35.
- 23 Hahn MB, Eisen L, McAllister J, Savage HM, Mutebi JP, Eisen RJ. Updated reported distribution of *Aedes (stegomyia) aegypti* and *Aedes (stegomyia) albopictus* (Diptera: Culicidae) in the United States, 1995–2016. *J Med Entomol*. 2017;54(5):1420–1424.