Randomized, Double-Blind, Placebo-Controlled, Phase 1 Study in Healthy Volunteers to Evaluate the Safety and Immunogenicity of AGS-v PLUS, a Universal Mosquito-Borne Disease Vaccine

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Vaccine Manufactured by
SEEK

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TEAM ROSTER

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ACT	artemisinin-based combination therapy
AE	adverse event
AR	adverse reaction
BMI	body mass index
BTRIS	Biomedical Translational Research Information System
CFR	Code of Federal Regulations
CRF	case report form
CSO	Clinical Safety Office
CSU	Clinical Studies Unit
CVD	Center for Vaccine Development and Global Health (University of Maryland School of Medicine)
DCR	Division of Clinical Research
DSMB	Data and Safety Monitoring Board
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
EP	European Pharmacopeia
FACS	fluorescence-activated cell sorting
FDA	U.S. Food and Drug Administration
FLU-v	influenza vaccine
FMOC	9-fluorenylmethoxycarbonyl
GCP	Good Clinical Practice
HIV	human immunodeficiency virus
HIV-v	human immunodeficiency virus vaccine
HLA	human leukocyte antigen
HRPP	Human Research Protection Program
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IFA	Incomplete Freund's adjuvant
IFN-γ	interferon gamma
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
IND	Investigational New Drug application
IP	investigational product
IRB	institutional review board
IV	intravenous
kDa	kilodalton
LID	Laboratory of Infectious Diseases
MHC	major histocompatibility complex
MTT	Dimethylthiazol
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
PBMC	peripheral blood mononuclear cell
PI	principal investigator
SAE	serious adverse event
SAR	suspected adverse reaction
SERF	Safety Expedited Report Form
SRCP	Safety Review and Communications Plan

SUSAR	serious and unexpected suspected adverse reaction
TEAE	Treatment Emergent Adverse Event
Th1	Type 1 T helper cells
Th2	Type 2 T helper cells
UMB	University of Maryland, Baltimore
UP	Unanticipated Problem
UPnonAE	Unexpected Problem that is not an AE
USP	United States Pharmacopeia
WBC	white blood cells
WFI	water for injection

PROTOCOL SUMMARY

Full Title:	Randomized, Double-Blind, Placebo-Controlled, Single-center, Phase 1 Study in Healthy Volunteers to Evaluate the Safety and Immunogenicity of AGS-v PLUS, a Universal Mosquito-Borne Disease and Mosquito Control Vaccine
Short Title:	AGS-v PLUS Mosquito Vax
IND Sponsor:	Office of Clinical Research Policy and Regulatory Operations Division of Clinical Research National Institute of Allergy and Infectious Diseases National Institutes of Health Laboratory of Infectious Diseases Clinical Studies Unit
Conducted by:	Center for Vaccine Development and Global Health
Principal Investigator:	Matthew B. Laurens, MD, MPH
Sample Size for Data Analysis:	50 participants
Accrual Ceiling for Vaccinees / Placebo Recipients:	60 participants
Study Population:	Healthy volunteers 18 to 50 years of age
Accrual Period:	2 months
Study Design:	This is a randomized, double-blind, placebo-controlled, single-center, Phase 1 study of AGS-v PLUS administered as 2 vaccinations before a non-infected *Aedes aegypti* and *Aedes albopictus* mosquito feeding.
Study Duration:	The study will begin approximately in April 2019 and will require approximately 14 months to complete. The length of individual subject participation is about 12 months from enrollment. The study will be unblinded once the data from procedures up to day 50 related to the primary and secondary endpoints have been collected for all participants. After unblinding, two follow-up safety calls will be carried out on all participants on days 181 and 366.
Study Agents:	1012 µg of AGS-v PLUS (50 nmol of each of the five peptides in the formulation) non-adjuvanted, adjuvanted in Montanide ISA-51 or in Alhydrogel®.
Study groups:	Group 1. saline placebo (day 1 and day 22). Group 2. AGS-v PLUS non-adjuvanted (day 1 and day 22). Group 3. AGS-v PLUS + adjuvant Montanide ISA-51 (on day 1) and saline placebo on day 22. Group 4. AGS-v PLUS + adjuvant Montanide ISA-51 (on day 1 and day 22).

	Group 5. AGS-v PLUS + Alhydrogel® adjuvant (on day 1 and day 22).
Intervention Description:	Individuals will be vaccinated subcutaneously with vaccine or placebo 21 days apart.
Primary Objectives:	1. To determine the safety and tolerability of AGS-v PLUS when administered as two doses alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses). 2. To measure specific antibody and cellular responses in participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses) from pre-vaccination to post-vaccination.
Secondary Objectives:	1. To measure changes to the antibody and cellular immune responses after clean *Aedes aegypti* and *Aedes albopictus* mosquito feeding on participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses). 2. To evaluate the effect of the AGS-v PLUS on *Aedes aegypti* and *Aedes albopictus* mosquito survival and fecundity after feeding on participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses).
Exploratory Objectives:	1. To evaluate reactions at the bite sites after mosquito feeding on participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (one or two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses). 2. To evaluate the effect of incubating Zika virus coated in vector saliva with peripheral blood mononuclear cells (PBMCs) and/or serum collected from individuals receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus receiving saline placebo (two doses). 3. To evaluate cellular immune responses to *Aedes aegypti*, *Aedes albopictus* and *Anopheles gambiae* mosquito salivary gland proteins in participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® versus saline placebo (two doses) will be assessed from pre-vaccination (day 1) to post-vaccination (day 43) and post-mosquito feeding (day 50).

Primary Endpoints:	1. Incidence and severity of treatment emergent adverse events (AEs) and serious adverse events (SAEs). 2. Geometric mean titer and fold increase in serum AGS-v PLUS specific immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin E (IgE) titers from day 1 to day 43. 3. Geometric mean concentration and fold increase in Type 1 and Type 2 T-helper cell (Th1 and Th2) cytokine responses (from day 1 to day 43) after *in vitro* exposure of PBMCs with AGS-v PLUS antigens.
Secondary Endpoints:	1. Geometric mean titer and the fold increase in AGS-v PLUS specific IgG, IgM and IgE titers seven days after mosquito feeding (day 50) compared to day 1 and day 43 titers. 2. 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. 3. Survival and fecundity in *Aedes aegypti* and *Aedes albopictus* female mosquitos after feeding on vaccinated participants.
Exploratory Endpoints:	1. Reactions to the mosquito bites by size of wheals/papules, redness of the flare, swelling and itching 30 min post-mosquito feeding (day 43), after 2 days (day 45) and after 7 days (day 50). 2. Early innate immune response to the mosquito bites in AGS-v PLUS vaccinated participants compared to placebo from biopsies taken from the bite site 2 days post-feeding. 3. *In* vitro viability of Zika virus coated in mosquito saliva after incubation with serum and/or PBMCs from vaccinated participants. 4. 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 with mosquito saliva from *Aedes aegypti*, *Aedes albopictus* and *Anopheles gambiae*.

PRÉCIS

Mosquito-borne diseases continue to cause significant morbidity and mortality worldwide despite ongoing control efforts. In 2015, there were over 200 million cases of malaria worldwide, causing nearly half a million deaths, with most of the deaths occurring among children under 5 years of age[1]. Mosquitos also transmit arboviruses, including dengue, yellow fever, West Nile virus, chikungunya, Rift Valley fever, Japanese encephalitis, and Zika virus. The outbreak of Zika virus in 2015 in Central and South America, as well as the Caribbean, serves as a reminder of how quickly these viruses can spread and how difficult they can be to control.

AGS-v, the first mosquito-borne disease vaccine already tested in a Phase 1 trial, contained four peptide antigens. AGS-v PLUS was formulated by adding a fifth peptide to AGS-v. With this formulation, the manufacturer aims to increase the breadth of protection and increase the humoral responses compared to AGS-v. In this protocol, we plan to perform a Phase 1 study of a novel, improved universal mosquito-borne disease vaccine. Through modulation of the immune system after a mosquito feeding, this vaccine targets the vector saliva and may provide prophylaxis against multiple arboviral and protozoal diseases. In addition, the vaccine potentially leads to a reduced mosquito lifespan after feeding therefore also reducing transmission of these diseases.

In this protocol, we aim to demonstrate that the safety of AGS-v PLUS vaccine is similar to SEEK's first mosquito-borne disease vaccine, AGS-v, and other peptide-based vaccines (influenza vaccine [FLU-v] and human immunodeficiency virus vaccine [HIV-v]) that have shown good safety profiles in previous Phase 1 and Phase 2 trials. We also aim to demonstrate that vaccination changes how the immune system reacts to mosquito saliva after a controlled clean *Aedes aegypti* and *Aedes albopictus* mosquito feeding. In 2015 and 2016, large outbreaks of Zika virus occurred in the Americas, resulting in an increase in travel-associated cases in US states, widespread transmission in Puerto Rico and the US Virgin Islands, and limited local transmission in Florida and Texas. In 2017, the number of reported Zika virus disease cases in the United States started to decline. Outbreaks of chikungunya have occurred in countries in Africa, Asia, Europe, and the Indian and Pacific Oceans. In late 2013, Chikungunya virus was found for the first time in the Americas on islands in the Caribbean. There is a risk that the virus will be imported to new areas by infected travelers and will be transmitted person-to-person by local *Aedes* mosquitoes.

A successful, universal mosquito-borne disease vaccine offers the benefit of targeting emerging mosquito-borne diseases as well as the many established infections such as dengue, yellow fever and malaria that make dealing with this newly emerging epidemic a challenge.

1 Background Information and Scientific Rationale

Mosquito-borne diseases continue to cause significant morbidity and mortality worldwide despite ongoing vector control efforts. In 2015, there were over 200 million cases of malaria worldwide, causing nearly half a million deaths, with most of the deaths occurring among children under 5 years of age[1]. Mosquitos also transmit arboviruses, including dengue, yellow fever, West Nile virus, chikungunya, Rift Valley fever, Japanese encephalitis, and Zika virus. It is difficult to calculate the true burden of these infections. The symptomology can include asymptomatic infection or acute febrile illness, but can also include acute and severe complications including hemorrhagic fever and death as well as long-term sequelae that can last years. Billions of people are at risk of some of these infections globally with millions affected every year. The recent outbreak of Zika virus that first emerged in Brazil and now includes several countries in Central and South America, the Caribbean, and the U.S. serves as a reminder of how quickly these vector-borne viruses can spread and how difficult they can be to control. Chikungunya outbreaks have been reported in Africa, Asia and Europe and recently in 2013; the first outbreak in America was reported in the Caribbean islands.

In large parts of Africa, South America and Asia, an estimated 3.2 billion people are at risk of contracting malaria[1], and arboviral infections occur worldwide without discrimination between developed and developing nations. Current prevention programs follow a two-pronged approach simultaneously controlling mosquito populations by spraying with insecticides and minimization of suitable habitats and reducing transmission by the provision of insecticide-treated mosquito nets to the population. For malaria, this approach, along with artemisinin-based combination therapy (ACT), has proven highly successful in locations such as Zanzibar[2], but the effective implementation requires continuous investment by and collaboration from international organizations, local governments, healthcare systems, and the people living in those communities. Unfortunately, this approach has not always been possible to sustain, as exemplified by failed eradication efforts in the 1950s-1960s[3].

Efforts in malaria and arboviral elimination continue to include the development of an effective vaccine that can both prevent disease in a healthy individual (prophylactic vaccine) and block disease transmission from an infected individual to the insect vector (transmission blocking vaccine). Most attempts at developing a malaria vaccine have centered on the prophylactic application, but unfortunately they have shown little efficacy. Even the most successful recent candidate vaccine, RTS, S/AS01 (RTS,S) by GlaxoSmithKline, had vaccine efficacy of 27-46% in children and infants, respectively[4]. Even if the RTS,S vaccine is licensed, there will be a recognized need for the continued implementation of current malaria prevention programs[5].

In this protocol, we propose to perform a Phase 1 study of an improved, universal mosquito-borne disease vaccine manufactured by SEEK based on the salivary proteins from the vector and not in the pathogen it carries. SEEK sought to create an ideal malaria and arboviral disease vaccine that addresses both prophylactic and transmission-blocking components. SEEK's approach in developing such a vaccine is defined by the following three principles:

- Because of complex and variable pathogen life cycle and antigenic make up, the vaccine prophylactic effect is not aimed at destroying the parasite itself, but rather at suppressing development of the essential physiologic environment that leads to successful human infection.
- Because several mosquito species are capable of pathogen transmission, the vaccine transmission-blocking effect is not aimed at killing the vector on contact, but rather at preventing the very process of blood feeding which is essential for successful vector procreation.
- Because most nations where malaria and arboviral diseases are endemic have limited healthcare delivery systems and budgets, the vaccine must be economically viable as a large-scale product entirely manufactured synthetically from widely available raw components, and must not require complex storage and delivery systems.

Female mosquitoes have an absolute physiological need for blood-feeding to produce viable eggs and a healthy offspring. As they probe the skin for blood with their proboscis they salivate into the bite site inoculating parasites (i.e. Plasmodium) or arbovirus (i.e., dengue virus, Zika virus) into their human host.

Mosquito saliva contains a range of molecules including vasodilators, anticoagulants and immunomodulators whose purpose is to prevent the disruption of feeding by the host[6]. Mosquito saliva has been shown to inhibit T and B cell proliferation[7] and downregulate the expression of interferon gamma (IFN-γ)[8], a soluble Type 1 T helper cell (Th1) pro-inflammatory immune mediator known to have *in vivo* therapeutic and prophylactic effects against other arthropod-transmitted diseases such as leishmaniasis[9]. Interestingly, mosquito saliva also triggers a non-inflammatory Type 2 T helper cell (Th2) host immune response that causes the characteristic irritation and allergic reaction associated with mosquito bites[10]. With time and continuous exposure, individuals become desensitized to mosquito bites due to a reduction in Th2 cytokine mediators, thus allowing for a more effective development of pro-inflammatory Th1 responses[11]. Moreover, exposure to bites from uninfected mosquitoes reduces the development of *Plasmodium yoelii* in its murine host and that this protection is associated with a shift to a Th1 response at the bite site after repeated exposure to mosquito saliva[12].

Research has shown that mosquito-injected sporozoites can remain at the bite site for at least 5 minutes, enter general circulation by 15 minutes with most gone from the initial bite site by 60 minutes[13,14,15]. During this time, it has been suggested that *Plasmodium* can evade the immune system and travel away from the bite site to the liver via the lymphatic system by infecting activated and non-activated macrophages[16-18]. Activated macrophages can and do kill parasites that infect them (e.g., *Leishmania* and *Plasmodium*), but non-activated macrophages are unable to do so. Macrophage activation is characteristic of a Th1 response and is inhibited by the Th2 response triggered by mosquito saliva. This same process of downregulation of Th1 cytokines by modulation of the host immunity towards a Th2 response at the bite by the mosquito saliva has been reported to facilitate the transmission of chikungunya virus[19], and arboviruses in general[20].

Beyond its direct effect on the parasite in the human host, an immune response to saliva has been shown to reduce malaria transmission. A monoclonal antibody raised against a 100 kilodalton (kDa) mosquito salivary gland protein significantly reduced the invasion by *Plasmodium yoelii* sporozoites of salivary glands in mosquitoes that fed on blood containing that antibody[21]. There is also evidence that antibodies against mosquito midgut and haemolymph antigens reduce the survival and fecundity of mosquitoes that fed on blood containing those antibodies[22,23]. Therefore, the antibodies against salivary antigens may

block transmission of these infections not only by reducing mosquito salivary gland invasion, but also by reducing the population of the mosquito vector.

Based on the above scientific argument, SEEK's approach to developing a vaccine against mosquito-borne pathogens based on identification of suitable antigenic targets in mosquito saliva capable of inducing, upon vaccination of the mammalian host, a strong anti-mosquito saliva Th1 response would (a) prevent pathogen infection and (b) block disease transmission by preventing the arthropod vector's salivary gland invasion and/or reducing vector survival and/or fecundity.

As indicated earlier, SEEK manufactures this vaccine by an entirely synthetic procedure. However, mosquito saliva contains a multitude of proteins with molecular weights up to and above 100 kDa. Synthetic manufacturing of large proteins by 9-fluorenylmethoxycarbonyl (FMOC) chemistry is currently unfeasible. Therefore, SEEK first had to identify suitable vaccine targets in saliva for manufacturing. This process of target identification started with the simple separation of *Anopheles gambiae* salivary gland lysate proteins into fractions according to their molecular weight (i.e. <20 kDa, 20-40 kDa, 40-80 kDa and >80 kDa). These fractions were used to immunize mice upon which uninfected *A. gambiae* mosquitoes were allowed to feed and the survival and fecundity of these mosquitoes analyzed. As shown in Figure 1, immunization with several mosquito salivary gland proteins fractions significantly reduced mosquito survival. The fraction containing proteins below 20 kDa proved to be the most effective, killing 80% of the mosquito population after only 6 days post-feeding. Fractions containing proteins between 20 and 40 kDa and over 80 kDa also reduced mosquito longevity, but were less effective than the <20 kDa protein fraction. As *Plasmodium* can complete its mosquito life-cycle stage in between 7 to 10 days, the ability of the <20 kDa protein fraction to kill 80% of mosquitoes within 6 days of feeding is important from the transmission blocking point of view as infected mosquitoes will die before they can transmit disease.

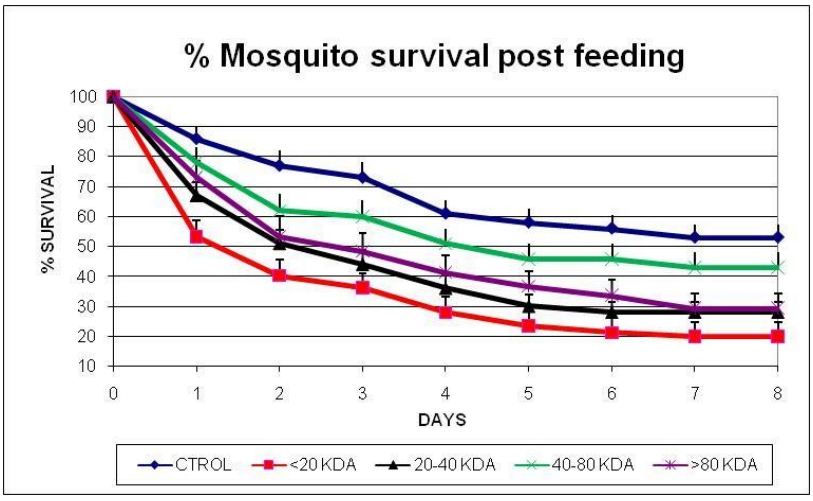

Figure 1. Mosquito survival.
Graph representing the mosquito % survival after feeding on mice immunized with different salivary gland protein fractions (vehicle (control), <20 kDa, 20-40 kDa, 40-80 kDa and >80 kDa). Mosquito salivary glands were dissected and their protein content separated by SDS-PAGE according to their molecular weight (MW). Proteins within the MW ranges of interest were recovered from the gel and used to immunize mice. Fresh *A. gambiae* mosquitoes fed on these mice and were monitored for 8 days post-feed for survival (N=150 mosquitoes per group).

In addition, immunization with the <20 kDa fraction, and the 20-40 kDa fraction significantly ($p<0.05$) reduced the number of eggs produced, eggs laid, larvae arising from eggs and mature adult offspring by the surviving mosquitoes (Figure 2).

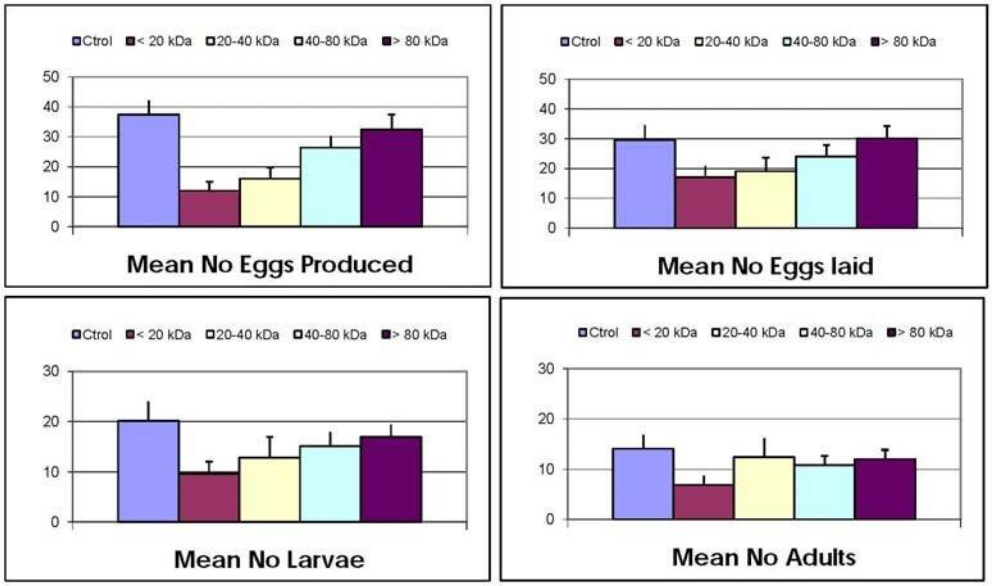

Figure 2. Effect of immunization with salivary gland protein fractions on mosquito fecundity.
Vehicle (control), <20KDa, 20-40KDa, 40-80KDa and >80KDa.

Such reduction in the number of viable offspring would have a significant effect on the number of vectors available for disease transmission at any one time and hence in the overall disease transmission rate.

From this point, SEEK proceeded to identify the proteins present in the fractions containing proteins <20 kDa and between 20 and 40 kDa from data available in the GeneBank sequence repository and by 2D-SDS-PAGE analysis of these fractions. Most of these proteins were still too large to be chemically synthesized and hence further work was required to identify reactive domains in these proteins that could successfully be manufactured and still retain the desired immunogenicity. This stage of the process was carried out using a computer algorithm developed by SEEK. The algorithm identifies and categorizes T-cell epitopes within a protein based on analysis of the structural affinity of a peptide for a given major histocompatibility complex/human leukocyte antigen (MHC/HLA) allele and the reactivity of this complex to T-cells. Any small regions (25+ amino acids) within a protein identified by the algorithm as containing multiple T-cell epitopes were cross-referenced against all available human proteomic data (GeneBank and Swiss-Prot). Any region showing significant sequence similarity was discarded to minimize the risk of inducing an autoimmune challenge following immunization. This algorithm and approach have already been successfully applied to the identification of conserved reactive T-cell regions in both influenza virus[24] and human immunodeficiency virus (HIV). The identified sequences constitute two independent candidate vaccine preparations that have been tested clinical trials (NCT01071031, NCT01181336, NCT03180801, NCT02962908, NCT01226758).

The selection of reactive T-cell regions is based on SEEK's interest in inducing a specific Th1 response to the salivary proteins. As indicated earlier in this document, a switch from a Th2 to a Th1 immune response to mosquito vector saliva is associated with resistance and natural immunity to malaria infection.

Several reactive regions in salivary proteins of <40 kDa each containing multiple T-cell epitopes were identified and synthesized by FMOC chemistry. A cocktail of these small protein-sequences (all less than 8 kDa), identified as AGS-v (or AGS-mix), were used to immunize mice and to establish the type of induced immune response. While multiple peptides may not be needed, the rationale is that the vaccine was developed to be active against a range of hematophagous arthropod vectors (such as *Anopheles* and *Aedes* mosquitos). As shown in Figure 3, immunization with AGS-v induced a strong IFN-γ response from immune cells upon recall with the antigen compared to immunization with a control mix of irrelevant peptides.

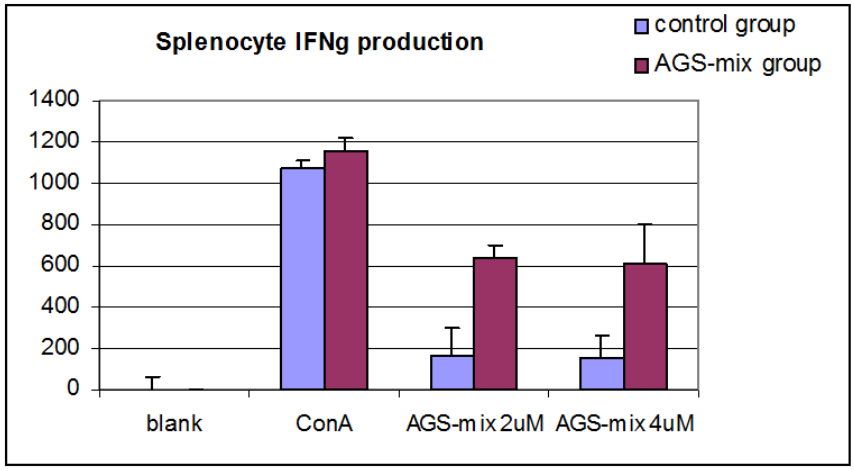

Figure 3. IFNγ response in mice with AGS-mix (AGS-v) or vehicle

Immunization with AGS-v was also found to induce a strong antibody response to the vaccine candidate (Figure 4), characterized by a significant contribution by antibodies of an IgG2a isotype. Both IFN-γ and IgG2a are considered primary mediators of a Th1 response and hence immunization with AGS-v can and does induce the desired Th1 response in mice.

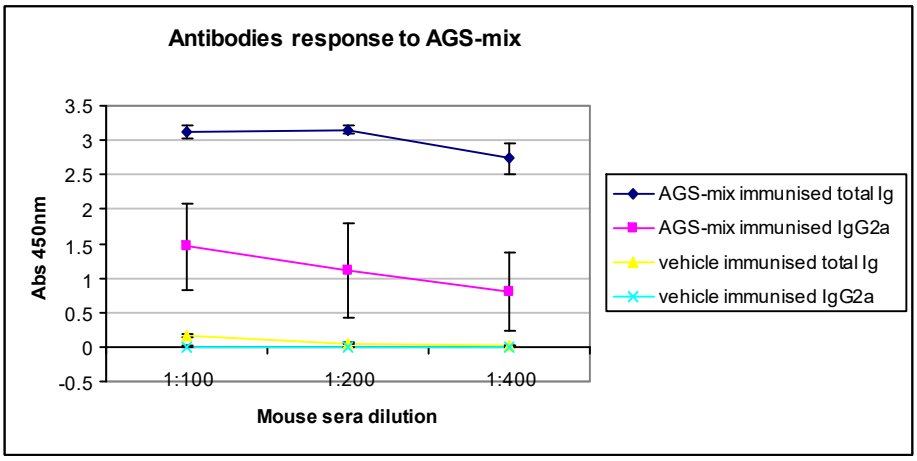

Figure 4. Total Ig and IgG2a AGS-mix (AGS-v)-specific antibody responses in mice measured by ELISA.

Having established that immunization with AGS-v induces a Th1 response in mice, SEEK wanted to establish whether this response was able to reduce the rate of malaria infection in a mouse model.

Traditional experimental models of malaria infection rely on intravenous (IV) injection of large numbers of parasites to successfully establish infection in the mouse. It has been reported that these models do not reflect the natural process of infection by mosquito bite in which parasite numbers several orders of magnitude lower than those used in the experimental models are capable of successfully inducing infection[25]. Interestingly, it has been suggested by the same authors that the systematic use of these models could account for the continued failure of experimental vaccines, which having been shown to be very effective in such models, did not induce any significant protection when tested in real-life infection conditions. It is for this very reason and the nature of the vaccine candidate that SEEK chose to test the product in a natural model of infection (i.e. via the bite of infected mosquitoes).

Preliminary studies using a natural model of infection involving *A. gambiae* infected with *P. yoelii nigeriensis* showed that mice vaccinated with AGS-v were more resilient to infection than mice vaccinated with an irrelevant peptide control mix (Figure 5). AGS-v was found to reduce infection rates 4-fold compared to the control group.

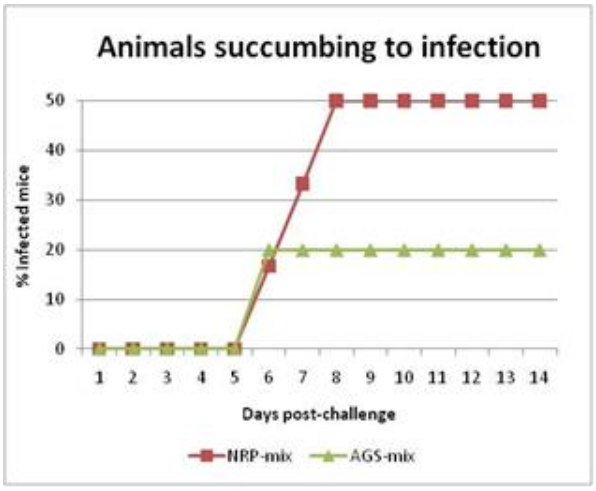

Figure 5. Percentage of animals succumbing to Plasmodium infection transmitted by mosquito bite 21 days post-vaccination with two doses of a Non Relevant Peptide (NRP) mix or AGS-v 15 days apart.

Further experiments in C57BL6, BALBc, and CD1 were performed to measure immunogenicity. All had good responses to the vaccine after two weeks, and antibodies to AGS-v were raised to the vaccine antigens. T-cell responses were seen two weeks after vaccination.

SEEK carried out clinical trials with two additional vaccines developed using the same technology as with AGS-v and AGS-v PLUS. Based on their experience with influenza vaccine (FLU-v) and human immunodeficiency virus vaccine (HIV-v) in clinical trials it is clear that a combination of four to five peptides allows the immune system to mount a response without peptides competing with each other. These studies have also assisted to determine an appropriate dose. For these vaccines, 50 nmol of each peptide proved to be safest and most immunogenic dose and replicated the ratio nmol/Kg in mouse studies. In phase 1 studies, a single dose of 25 nmol or 50nmol of FLU-v or HIV-v with and without adjuvant were given to approximately 10 participants in each arm. In the HIV-v phase 1 trial, both doses induced a significant and sustained antibody response in 60% of volunteers but only the higher 50nmol dose produced a significant vaccine-specific cellular response in approximately 50% of volunteers. In FLU-v phase 1 trial, both adjuvanted doses produced a significant IFN-γ response in more than 80% of volunteers in a dose-dependent manner. Three FLU-v phase 2 studies have confirmed that a single dose of 50nmol FLU-v adjuvanted in Montanide ISA-51 is the safest and most immunogenic dose after over a hundred participants have received this dosing regimen.

In preclinical toxicology studies, a single 50nmol of each peptide dose of AGS-v administered subcutaneously showed no tolerability concerns in New Zealand White Rabbits. In this test, a single dose of subcutaneous AGS-v emulsified in Montanide ISA-51 adjuvant or in solution in water was compared to the vehicle placebos. All animals survived the test and appeared active and healthy during the study. A single subcutaneous injection of AGS-v in suspension caused minimal irritation comparable to vehicle control with full clinical resolution. Adjuvanted AGS-v caused minimal to mild irritation with a slightly lower irritation incidence when compared to vehicle control.

The National Institute of Allergy and Infectious Diseases (NIAID) – National Institutes of Health (NIH), in collaboration with SEEK, has performed a phase 1 clinical study testing the safety, immunogenicity and *in vitro* efficacy of AGS-v administered as two doses non-adjuvanted or adjuvanted in Montanide ISA-51 compared to placebo (NCT 03055000). Investigators identified no safety concerns to date.

SEEK has manufactured an improved AGS-v vaccine named AGS-v PLUS. This new formulation contains the exact four peptides included in AGS-v with the addition of a fifth peptide that provides higher antibody titer and increases the breadth of protection as the protein of origin is found in the saliva of many mosquitoes such as *Anopheles gambiae, Anopheles darling, Culex quinquefasciatus, Aedes aegypti and Aedes albopictus*.

Preclinical immunogenicity data studies show that AGS-v PLUS is capable of inducing strong Th1 responses measured as IFN-γ secretion after *in vitro* exposure of splenocytes from vaccinated mice to the vaccine antigens (Figure 6). Even when AGS-v PLUS is formulated with an adjuvant biased towards inducing Th2 responses such as Alhydrogel®, a strong IFN-γ secretion is detected.

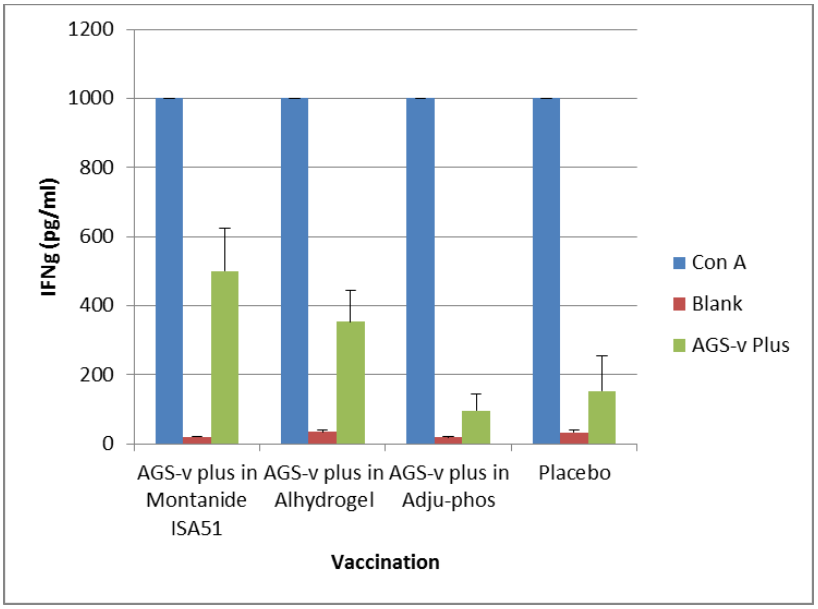

Figure 6. IFN-γ secretion by splenocytes from mice vaccinated twice with AGS-v PLUS formulated in different adjuvants (Montanide ISA-51, Alhydrogel® and Adju-phos).

AGS-v PLUS is also capable of inducing high titers of antigen-specific IgG antibodies (Figure 7) when administered as two subcutaneous doses 21 days apart formulated in different adjuvants; an oil in water emulsion (Montanide ISA-51) or as a suspension with alum based adjuvants (Alhydrogel® and Adju-phos).

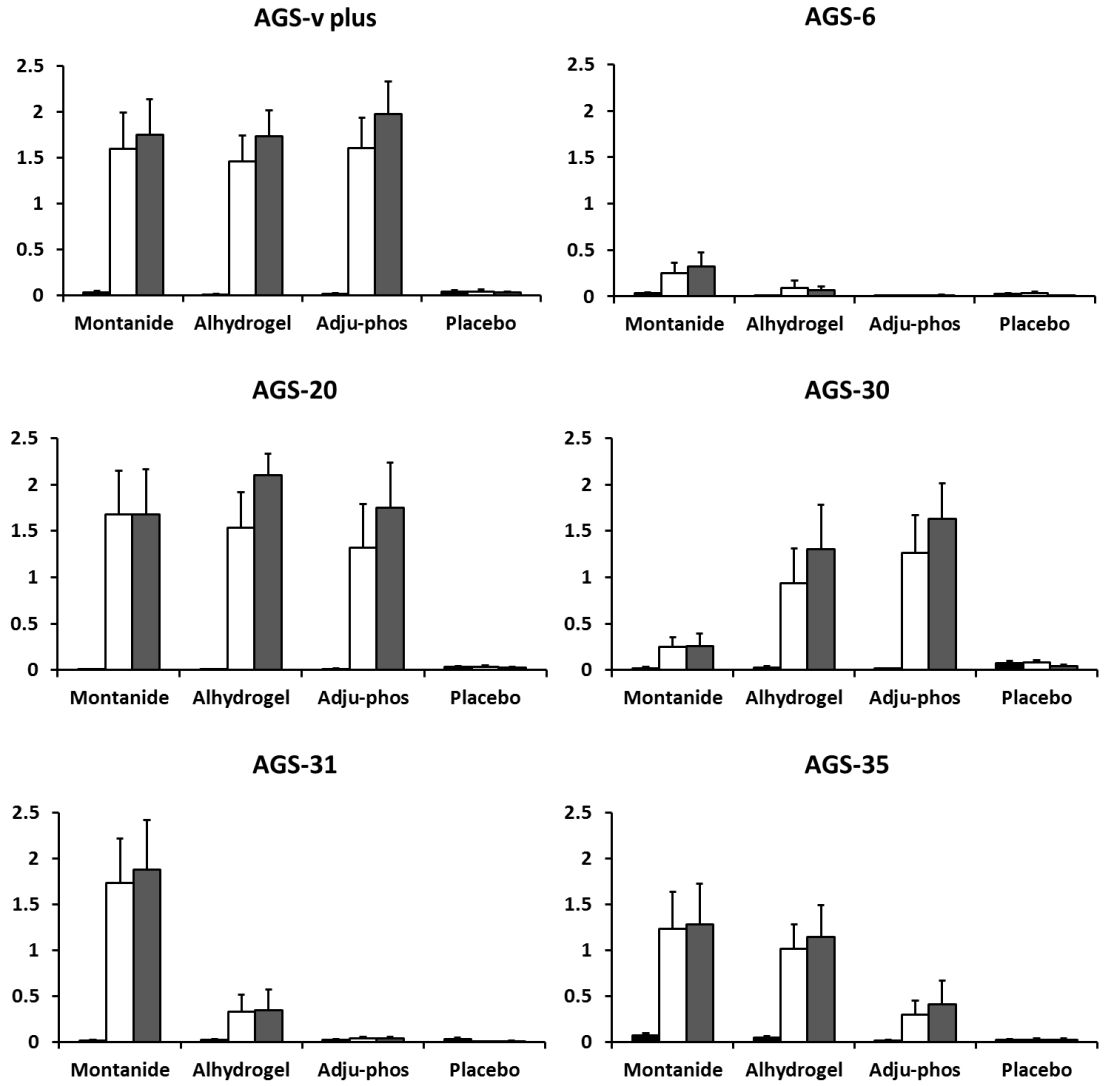

Figure 7. IgG antibody titers against AGS-v PLUS or the single peptides in the serum (1:400) of mice vaccinated with Placebo or AGS-v PLUS formulated in Montanide ISA-51, Alhydrogel® or Adju-phos adjuvants at pre-vaccination (day 0) and post-vaccination (day 42). Y axis=corrected absorbance, X-axis= vaccination received.

As seen in Figure 7, the fifth peptide in the AGS-v PLUS formulation, AGS-20, induces strong antibody responses with all adjuvants tested confirming that addition of this peptide will increase the overall antibody responses to AGS-v PLUS.

In addition to testing two doses of AGS-v PLUS in the different formulations, replicating the groups from the SPITT study (Phase 1 study with AGS-v), the vaccine will also be tested as a single dose in Montanide ISA-51. Animal data showed that ISA-51 was the only adjuvant tested that induced an increase in the antibody response after a single dose (Figure 8). A single-dose vaccine will always have better compliance than one that requires two doses.

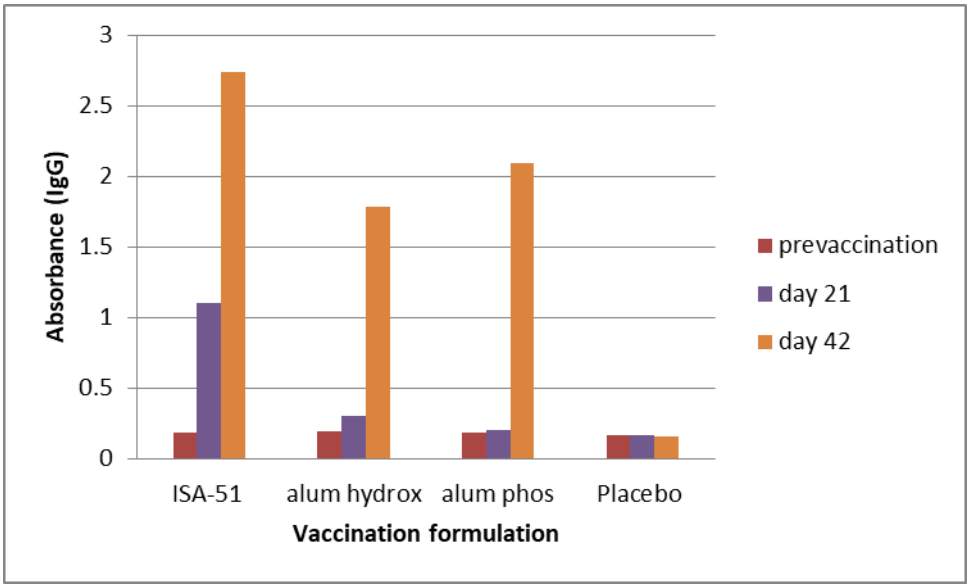

Figure 8. IgG antibody titers against AGS-v of mice vaccinated with Placebo or AGS-v PLUS formulated in Montanide ISA-51, Alhydrogel® or Adju-phos adjuvants at pre-vaccination (day 0), 21 days after first dose (day 21) and 21 days after the second dose (day 42). Y axis=corrected absorbance, X-axis= vaccination received.

In summary, the data presented here supports the validity of SEEK's scientific hypothesis that immunization with mosquito salivary antigens can induce a Th1 specific response to provide both prophylactic and transmission blocking immunity. Other groups have shown the ability of vaccination with salivary proteins from sand flies to reduce infection in diseases such as Leishmaniasis[26,27]. SEEK's vaccine is composed entirely of synthetic products that do not require complex heterologous expression systems for manufacture and hence can be readily scaled up to accommodate for large scale demand. The composition and application of these synthetic products are the subject of a patent by SEEK.

The AGS-v PLUS preparation is a vaccine candidate capable of acting on both the prophylactic and transmission blocking areas of malaria and arbovirus infection control. Moreover, it is a universal vaccine as it does not target a single pathogen but the vectors that transmit multiple pathogens, both viral and protozoal. These vectors are hematophagous arthropods and are dependent on the immunomodulatory activities of arthropod saliva to achieve successful infection of the host[20]. This is particularly relevant since many hematophagous arthropods such as *Aedes aegypti* transmit more than one pathogen (e.g. dengue, Zika and West Nile virus).

In this protocol we plan to demonstrate that the safety of this vaccine is similar to SEEK's other peptide based vaccines (AGS-v, FLU-v and HIV-v) that have been found to have very good safety profiles in Phase 1 and Phase 2 trials. We also aim to demonstrate immunomodulation after a controlled feed with clean *Aedes aegypti* and *Aedes albopictus* mosquitoes. With the current status of Zika and chikungunya in the Americas and the threat of it spreading to the U.S. and the rest of the world, a successful universal mosquito-borne disease vaccine offers the benefit of targeting this emerging disease as well as the many established infections such as dengue, yellow fever and malaria.

2 Study Objectives

2.1 Primary Objective

The primary objectives of the study are:

1. To determine the safety of and tolerability of AGS-v PLUS when administered as two doses alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses).
2. To measure specific antibody and cellular responses in participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses) from pre-vaccination to post-vaccination.

   A previous study tested 2 doses of AGS-v, 21 days apart, as non-adjuvanted or in Montanide. For continuation, AGS-v plus will be tested with the same formulation as AGS-v but also with a different adjuvant, Alhydrogel®. In addition, a single dose of AGS-v plus in Montanide will be tested as animal data showed that only Montanide can trigger an antibody response to AGS-v after a single dose. In order to keep the blinding, participants in this group will receive saline on day 22.

2.2 Secondary Objectives

The secondary objectives of this study are:

1. To measure changes to the antibody and cellular immune responses after clean *Aedes aegypti* and *Aedes albopictus* mosquito feeding on participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses).
2. To evaluate the effect of the AGS-v PLUS on *Aedes aegypti* and *Aedes albopictus* mosquito survival and fecundity after feeding on participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses).

2.3 Exploratory Objectives

1. To evaluate reactions at the bite sites after mosquito feeding on participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (one or two doses), or with Alhydrogel® (two doses) versus saline placebo (two doses).
2. To evaluate the effect of incubating Zika virus coated in vector saliva with peripheral blood mononuclear cells (PBMCs) and/or serum collected from individuals receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® (two doses) versus receiving saline placebo (two doses).
3. To evaluate cellular immune responses to *Aedes aegypti*, *Aedes albopictus* and *Anopheles gambiae* mosquito salivary gland proteins in participants receiving two doses of AGS-v PLUS alone, or with Montanide ISA-51 (single and two doses), or with Alhydrogel® versus saline

placebo (two doses) will be assessed from pre-vaccination (day 1) to post-vaccination (day 43) and post-mosquito feeding (day 50).

3 Study Design

3.1 Description of the Study Design

This is a randomized, double-blind, placebo-controlled, single-center, Phase 1 study of AGS-v PLUS administered at Days 0 and 21 before *Aedes aegypti* and *Aedes albopictus* mosquito challenge at Day 43. Participants will be randomized 1:1:1:1:1 to:

- Group 1: Saline placebo (N=10) on day 1 and day 22.
- Group 2: 1012 µg (50 nmol each peptide) unadjuvanted AGS-v PLUS vaccine (N=10) on day 1 and day 22.
- Group 3: 1012 µg (50 nmol each peptide) Montanide ISA-51 adjuvanted AGS-v PLUS vaccine (N=10) on day 1 and saline placebo on day 22.
- Group 4: 1012 µg (50 nmol each peptide) Montanide ISA-51 adjuvanted AGS-v PLUS vaccine (N=10) on day 1 and day 22.
- Group 5: 1012 µg (50 nmol each peptide) Alhydrogel® adjuvanted AGS-v PLUS vaccine (N=10) on day 1 and day 22.

A placebo control will be used to establish the frequency and magnitude of changes in endpoints that may occur in the absence of active vaccination. Randomization will be used to minimize bias in the assignment of participants to vaccination groups, to increase the likelihood that known and unknown participant attributes (e.g., demographic and baseline characteristics) are evenly balanced across vaccination groups, and to enhance the validity of statistical comparisons across vaccination groups. Blinding of participants and the study team will be used to reduce potential bias during data collection and evaluation of endpoints.

The study will be unblinded once all the data for the primary and secondary endpoints and safety data up to day 50 have been collected. Otherwise, the blind should be broken only if specific emergency treatment/course of action would be dictated by knowing the vaccination status of the participant. In such cases, the investigator may in an emergency determine the identity of the vaccine by opening the sealed code. It is recommended that the investigator contact the sponsor or its designee if possible to discuss the particular situation, before breaking the blind. In the event the blind is broken, the sponsor must be informed as soon as possible. Accidental unblinding (e.g. if the investigator sees the investigational product [IP] administration logs) must be reported within 1 working day to the Sponsor, who will advise on the corrective steps to be taken. The date and reason for the unblinding must be documented in the source document.

3.2 Study Endpoints

Primary:

1. Incidence and severity of treatment emergent adverse events (AEs) and serious adverse events (SAEs).
2. Geometric mean titer and fold increase in serum AGS-v PLUS specific immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin E (IgE) titers from day 1 to day 43.
3. Geometric mean concentration and fold increase in Th1 and Th2 cytokine responses (from day 1 to day 43) after *in vitro* exposure of PBMCs with AGS-v PLUS antigens.

Secondary:

1. Geometric mean titer and fold increase in AGS-v PLUS specific IgG, IgM and IgE titers seven days after mosquito feeding (day 50) compared to day 1 and 43.
2. 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.
3. Survival and fecundity in *Aedes aegypti* and *Aedes albopictus* female mosquitoes after feeding on vaccinated participants.

Exploratory:

1. Reactions to mosquito bites by size of wheals/papules, redness of the flare, swelling and itching 30 min post-mosquito feeding (day 43), after two days (day 45) and after 7 days (day 50).
2. Early innate immune response to the mosquito bites in AGS-v PLUS vaccinated participants compared to placebo from biopsies taken from the bite site 2 days post-feeding.
3. *In vitro* viability of Zika virus coated in mosquito saliva after incubation with serum and/or PBMCs from vaccinated participants.
4. 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 with mosquito saliva from *Aedes aegypti*, *Aedes albopictus* and *Anopheles gambiae*.

4 Study Enrollment

Information regarding study materials will be communicated to potential participants who have previously participated in vaccine trials or expressed willingness to participate at the enrollment sites and have signed an authorization form that they are willing to be contacted for future studies. This has been done with institutional review board (IRB) approval. Educational meetings will be offered to the public both within and outside the university system for the purposes of informing individuals of the study. Every effort

will be made to achieve a balanced participant population while avoiding the enrollment of vulnerable populations. Careful screening will be conducted to avoid enrollment of individuals with substance abuse or psychiatric difficulties. Efforts to retain study participants include, but are not limited to: development of professional rapport with potential participants, obtaining valid contact and backup contact information from potential participants, and telephone reminder calls and/or email reminders for upcoming study visits.

The Center for Vaccine Development and Global Health (CVD) will be the site for participant enrollment and inoculation. Mosquito feedings will occur in the Malaria Challenge Suite, where studies of controlled human malaria infection take place. Outpatient follow-up will be conducted at the CVD outpatient clinic.

4.1 Inclusion Criteria

1. Healthy women and men who are ≥18 and ≤50 years of age.
2. Willingness to complete all study visits and comply with all study requirements.
3. A male participant is eligible for the study if he agrees to practicing abstinence or using a condom with spermicide plus an acceptable form of contraception (see inclusion criteria 4) being used by any female partner from 4 weeks before study start to 12 weeks after the second vaccine administration.
4. A female participant is eligible for this study if she is not pregnant or breast feeding and 1 of the following:
    - Of non-child bearing potential (i.e., women who have had a hysterectomy or tubal ligation, or are postmenopausal, as defined by no menses in ≥1 year).
    - Of childbearing potential but agrees to practice effective contraception or abstinence for 4 weeks before study initiation and 12 weeks after the second vaccine administration. Acceptable methods of contraception include a female partner who is the sole sexual partner of the female participant, a male partner who is sterile and is the sole sexual partner of the female participant, or a male partner who uses a condom with spermicide plus 1 or more of the following: 1) implants of levonorgestrel; 2) injectable progestogen; 3) an intrauterine device with a documented failure rate of <1%; 4) oral contraceptives; and 5) double barrier method including diaphragm.
5. Willing to have samples stored for future research.
6. Agrees to abstain from alcohol intake for 24 hours before each study visit.
7. Agrees to not donate blood or blood products throughout the study.
8. Score ≥70% on comprehension quiz at screening

4.4 Exclusion Criteria

1. Participant has any underlying or current medical condition, which, in the opinion of the Investigator, would interfere with the participation in the study.

2. Individual with body mass index (BMI) ≤18 and ≥40.
3. Participants who have a clinically significant (as determined by the PI or designee) baseline Grade 1 or greater toxicity, or any Grade 2 or greater toxicity (regardless of clinical significance) by the toxicity table.
4. Receipt of blood or blood products including immunoglobulin within 3 months before enrollment.
5. Receipt of any unlicensed drug within 3 months or 5.5 half-lives (whichever is greater) before enrollment.
6. Receipt of any unlicensed vaccine within 6 months before enrollment.
7. Participated in study NCT03055000 testing safety and immunogenicity of AGS-v.
8. Self-reported or known history of alcoholism or drug abuse within 6 months before enrollment.
9. Self-reported or known history of psychiatric or psychological issues that require treatment and are deemed by the PI or designee to be a contraindication to protocol participation.
10. History of a previous severe allergic reaction with generalized urticaria, angioedema, anaphylaxis or anaphylactoid reaction.
11. Any condition or event that, in the judgment of the PI or designee, is a contraindication to protocol participation or impairs the volunteer's ability to give informed consent.
12. Known allergy to any vaccine component, including adjuvants.
13. History of severe immunization reaction.
14. Severe allergic reaction to mosquito bites (anaphylaxis)
15. Have taken oral or parenteral (including intra-articular) corticosteroids of any dose within 30 days before study vaccination
16. Have taken high-dose inhaled corticosteroids* within 30 days before each study vaccination
17. Received or plan to receive a licensed, live vaccine within 30 days before or after the study vaccination
18. Received or plan to receive a licensed, inactivated vaccine within 14 days before or after study vaccination
19. Serologic evidence of infection with HIV, hepatitis B virus, or hepatitis C virus
20. Ongoing chronic skin condition, or acute skin condition at the time of vaccination or mosquito feeding, except for mild eczema.
21. History of keloid formation after previous biopsies, lacerations, abrasions, surgeries, or other skin procedures (e.g., cosmetic piercings) that are deemed by the PI or designee to be a contraindication to protocol participation.

22. Pregnancy, breastfeeding, or planning to become pregnant up to one month after mosquito feeding.

* High-dose defined per age as using inhaled high dose per reference chart https://www.nhlbi.nih.gov/sites/default/files/media/docs/asthma_qrg_0_0.pdf

Co-enrollment Guidelines: Co-enrollment in other trials is restricted and discouraged, but may take place with the approval of the PI and after study staff notification. Limitations of total blood volume collection between the two studies must conform with NIH limits on blood collection.

4.5 Justification for Exclusion of Pregnant Women and Children (Special Populations)

In this study, an investigational vaccine will be administered to the participants. Therefore, children, pregnant women, and other individuals at high risk of complications will be excluded as the risk to these individuals may be increased. In addition, the investigational vaccine has not undergone preclinical reproductive safety testing.

Participants younger than 18 years of age will be excluded from the study. Because there are insufficient data regarding dosing or AEs available in adults to judge the potential risk in children, the study is of "greater than minimal risk" and does not meet the criterion of 45 Code of Federal Regulations (CFR) 46, Subpart D, governing the participation of children in research.

4.6 Reasons for Withdrawal

Participants are free to withdraw from the study at any time. Participants will be encouraged to remain in the study to be followed for safety purposes.

A study participant will be discontinued from participation in the study for any one of the following reasons:

- Request by participant to terminate participation;
- Loss to follow-up;
- Request of primary care provider;
- At the request of the IRB/Ethics committee, NIH, NIAID, or the U.S. Food and Drug Administration (FDA);
- The participant's well-being, based on the opinion of the investigator.

Because a true intent-to-treat analysis requires the inclusion of all participants enrolled to the extent possible, this requires an intent-to-treat design in which all participants are followed according to the pre-specified schedule with primary, and perhaps secondary, outcome assessments, regardless of compliance, adverse effects, or other post-enrollment observations— participant refusal excepted.

4.7 Handling of Withdrawals

If voluntary withdrawal occurs, the participant will be asked for permission to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical

supervision until the symptoms of any adverse event resolve or until the participant's condition becomes stable.

It is vital to collect safety data on any participant discontinued because of an AE or SAE, and every effort will be made to undertake protocol-specified safety follow-up procedures. Participants will follow early termination procedures if withdrawn.

4.8 Termination of Study

The study may be discontinued due to the development of laboratory toxicities, at the discretion of the FDA or Division of Microbiology and Infectious Diseases (DMID), or due to natural disaster.

5 Study Agents/Interventions

5.1 Description

5.1.1 AGS-v PLUS

AGS-v PLUS contains five peptides, or drug substances: AGS-6, AGS-20, AGS-30, AGS-31, and AGS-35. Each peptide consists of 32 to 44 amino acids. They are chemically synthesized and available as acetate salts. The peptides contain multiple T-cell reactive regions capable of inducing Th1 responses.

A solution of the five drug substances (50 nmol net peptide/g of each drug substance) is prepared by completely dissolving the drug substances in 50% acetic acid. The five solubilized peptides are mixed to make up AGS-v PLUS bulk solution. This mix is filter-sterilized before filling glass vials automatically with the sterile AGS-v PLUS bulk solution by weight which results in 1012 μg of AGS-v PLUS solution per vial. The vials are then lyophilized to remove the water and acetic acid used in the compounding whereupon a relatively neutral pH, suitable for human injection, will be achieved after reconstitution. The vials are sealed and stored at -20°C.

Bottle labels bear the appropriate label text, including the caution statement: "Caution: New Drug – Limited by Federal (or United States) law to investigational use," as required by governing regulatory agencies. Any addition or modification to the label apart from the variable data must be authorized by the drug supplier.

5.1.2 Adjuvants

The adjuvants used are Montanide ISA-51 and Alhydrogel®.

Montanide ISA-51, manufactured by Seppic (France), is a mixture of oil and water that when combined with an antigen (such as a vaccine) it boosts the response to that antigen. It is comprised of two raw materials Drakeol 6 VR as a mineral oil and mannide monooleate as the surfactant[28]. Drakeol 6 VR is a mineral oil with United States Pharmacopeia/European Pharmacopeia (USP/EP) monograph. It is a mixture of several hydrocarbons with different length obtained from petroleum. Drakeol 6 VR stays at the injection site and is progressively eliminated by competent-cells such as macrophages. It can also be partially metabolized into fatty acids, triglycerides, phospholipids, or sterols. 30% of the mineral oil disappears during the first month and the majority of the oil found outside the injection site is in the liver

and fatty tissues in the form of phospholipids and fatty acids[28]. Mannide monooleate is a non-ionic surfactant based on oleic acid and sugar. Oleic acid is a distribution of various fatty acids with a predominant species C18'. 30-40% of mannide monooleate is removed from the injection site after 24 hours. After 3 months, 30% of the surfactant still remains[28]. Montanide ISA 51 is highly purified and avoids having impurities and high levels of fatty acids that have led to toxicities with other versions of Incomplete Freund's adjuvant (IFA) in the past. It also uses a different ratio of emulsifier to oil resulting in a more consistent and controllable emulsion. It has been used in multiple vaccine trials including those for malaria[28].

Alhydrogel® (Croda Denmark) is a sterilized, aluminium hydroxide wet gel suspension which has been tested free of pyrogenicity. It tolerates re-autoclavation, but is destroyed if frozen. When the pH is maintained at 5-7, which is normal during vaccine production, Alhydrogel® has a positive charge so it readily adsorbs negatively charged antigens (e.g. proteins with acidic isoelectric points kept at neutral pH). Alhydrogel® improves attraction and uptake of antigen by antigen presenting cells (APCs) by generating particles of antigen adsorbed to aluminium salts. This improves Th2 antibody production specific to the antigen. Alhydrogel® adjuvant has been used in multiple commercial vaccine formulations[29] with a 40+ year history of safe and effective use.

5.1.3 Placebo

The placebo contains sterile saline for injection only.

5.2 Study Agents Storage and Stability

The vials of water for injection (WFI), 0.09 normal saline, Montanide ISA-51 and Alhydrogel® should be stored at room temperature (15-30°C).

AGS-v PLUS must be stored at ≤-20°C (±5 °C). AGS-v PLUS is expected to be stable for at least 24 months at this temperature. Stability testing will be performed to cover the duration of the trial. The four peptide formulation (AGS-v) was tested for stability up to 6 months at +25°C 60%RH, 12 months at +5°C and 18 months at -20°C. AGS-v was stable at all conditions tested. Montanide ISA-51, Alhydrogel®, saline ampoules and WFI ampoules should be stored at room temperature (15-30°C).

No study drug can be dispensed beyond the expiration date.

5.3 Preparation

- Please see pharmacy manual for specifics regarding study product preparation.

5.4 Dosing and Administration

Participants will be randomized to one of the following vaccination regimens:

- Group 1: two doses (days 1 and 22) saline (0.5 mL)

- Group 2: two doses (days 1 and 22) non-adjuvanted AGS-v PLUS (50 nmol each peptide) as a suspension in 0.5 mL WFI.
- Group 3: one dose (day 1) of AGS-v PLUS (50 nmol each peptide) in 0.25 mL of adjuvant Montanide ISA-51 emulsified in 0.25 mL of WFI and one dose of saline placebo (0.5 mL) on day 22
- Group 4: two doses (days 1 and 22) of AGS-v PLUS (50 nmol each peptide) in 0.25 mL of adjuvant Montanide ISA-51 emulsified in 0.25 mL of WFI.
- Group 5: two doses (days 1 and 22) of AGS-v PLUS (50 nmol each peptide) as a suspension in 0.375 mL of saline and 0.125 mL of Alhydrogel®.

All doses will be administered by subcutaneous injection in the fatty tissue of the triceps.

The person administering the vaccine will be blinded to the type of formulation by an opaque label placed over the syringe by the pharmacy team. Trained and licensed study staff will administer vaccine.

Vaccine administration will be performed following these instructions as detailed in the CVD SOPs.

Detailed instructions on how to administer a subcutaneous injection can be found in CVD SOPs and in the Pharmacy manual.

5.5 Modification of Study Intervention/Investigational Product for a Participant

The study product will be administered to enrolled participants according to the study schedule. There is no planned modification of study product administration for participants.

5.6 Disposal and Drug Accountability of AGS-v PLUS

5.6.1 Product Accountability

The University of Maryland Medical Center Investigational Drug Service will be responsible for recording the receipt of all vaccine supplies and for ensuring the supervision of the storage and allocation of these supplies. When a shipment is received, an assigned qualified person verifies the quantities received and the accompanying documentation and returns the acknowledgment of receipt to the drug supplier.

Drug administration will be recorded in the source documents, in the electronic case report forms (CRFs) and in the Drug Administration Record form. The latter includes the participant identification, quantity (volume) and date of administration. The containers from which the vaccine was administered to the participants will be retained for dose confirmation.

At the end of the study, delivery records of study vaccine will be reconciled with used / unused stocks and appropriate forms will be completed, to verify that all used, unused or partially used supplies have been returned or destroyed and that no study supplies remain in the Investigator's possession.

All unused vaccine supplies, partially used and empty containers will be returned or destroyed after the study monitor has completed final accountability inspection and in agreement with the drug supplier (SEEK).

5.6.2 Accountability of Study Supplies

All materials supplied are for use only in this clinical study and should not be used for any other purpose.

The PI is responsible for the IP accountability, reconciliation and record maintenance. In accordance with all applicable regulatory requirements, the investigator or designated site staff must maintain IP accountability records throughout the course of the study. The investigator or designated site staff will document the amount of IP received from the drug supplier (SEEK) and the amount administered to participants.

A Drug Dispensing Log must be kept current and will contain the following information:

- The identification of the participant to whom the drug was dispensed.
- The date(s) of the drug dispensed to the participant.

The inventory must be available for inspection by the study monitor at any point during the study. Drug supplies, excluding empty containers, will either be collected at the end of the study by the study monitor, returned or destroyed by the investigator as agreed in writing with the drug supplier (SEEK). The PI will keep records indicating identification and quantity of each unit disposed of or returned, the method of destruction (taking into account the requirements of local law), and the person who disposed of the test substance. Such records must be submitted to the drug supplier.

5.6.3 Retention of Samples

It will be the responsibility of the drug supplier (SEEK) to ensure adequate samples of all study drug are retained in accordance with the regulatory guidelines.

5.7 Concomitant Medications and Procedures

At each study visit/contact from screening to study day 366 the study team will question the participant about any medication taken starting from 30 days before screening, including herbals, vitamins and holistic/naturopathic medications. Concomitant medication, including vaccines and any other medication, will be recorded in the CRFs with trade name and/or generic name of the medication, medical indication, start and end dates of treatment.

6 Study Schedule

This study will take place at the CVD, University of Maryland School of Medicine. All aspects of the protocol will be carried out in accordance with CVD University of Maryland guidelines, Good Clinical Practice (GCP) and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) involving human-participant research. Please see Figure 9 below.

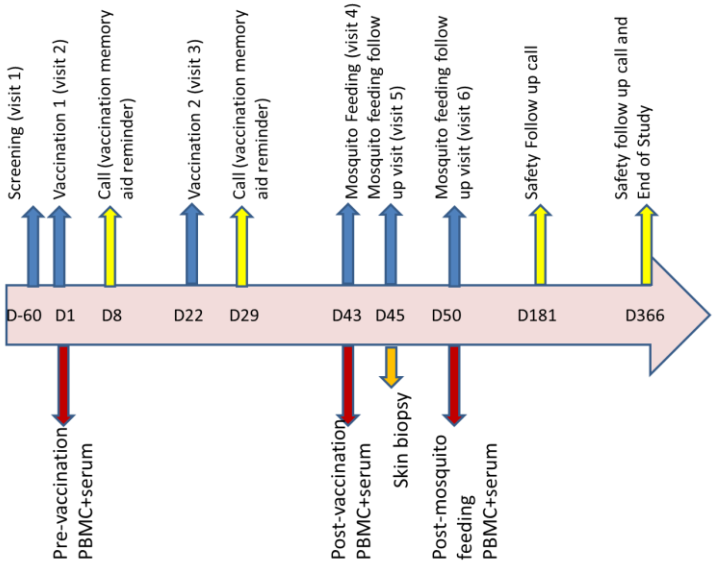

Figure 9. Study schedule

6.1 Screening (Days -60 to 0) (outpatient visit)

- Potential participants will be provided with a verbal description of the study (purpose and study procedures), and will be asked if they have any questions and to read/sign the consent form. The consent form will be signed and potential participants must score ≥70% on a comprehension quiz before the performance of any study procedures. The comprehension quiz may be repeated once.
- The study staff will discuss with the potential participant his/her medical history, study eligibility criteria and concomitant medication use.
- Vital signs (height, weight, oral temperature, blood pressure, heart rate) will be obtained. BMI will be calculated to ensure eligibility.
- Documentation of usual responses to mosquito bites as none (0), mild (1), moderate (2), or severe (3).
- A physical exam will be performed.
- For females who are capable of bearing children, a serum pregnancy test will be performed at the clinical trial study site at the time of screening regardless of age unless written medical demonstration of sterility or amenorrhea (defined as one year with medical evaluation) can be provided.
- A blood sample will be collected from an arm vein to screen for health as follows:
  - Hematology: hemoglobin (Hgb), white blood cell count (WBC), and platelet count;
  - Chemistry: glucose (random), alanine aminotransferase (ALT), and creatinine;
  - Serology: HIV, hepatitis C virus (HCV), and hepatitis B surface antigen (HBsAg).
- Medications taken in the previous 30 days will be recorded.

6.2 Enrollment/Baseline and First Vaccination (Day 1) (outpatient visit)

Randomization will use computer generated randomization codes. These codes will be sent to the pharmacy where the unblinded pharmacist will have the key and prepare the appropriate agent for administration. All vaccination syringes will be labeled to cover contents so that the blinded staff cannot identify the different treatments.

The first vaccine or placebo administration will take place on Day 1. The research team will confirm continuing consent with the volunteer before performing any and all procedures. Once it is determined that the participant still meets inclusion/exclusion criteria for the research study, participants will receive the first of two injections.

The following procedures will be performed at enrollment:

- Review of medical/concomitant medication history
- Targeted physical exam/assessment as needed
- Vital signs pre-vaccination
- Vital signs 30-45 minutes post vaccination
- Blood collection for routine safety labs (Hemoglobin, white blood cell count, platelets, serum creatinine, serum alanine aminotransferase, random glucose)
- serum/whole blood prevaccination for research (as per Appendix B)
- Urine pregnancy test (females of childbearing potential only)
- Review of inclusion/exclusion criteria
- Vaccine or placebo administration
- Vaccine injection site and AE assessment pre-vaccination and
- 30-45 minutes post vaccination
- AE memory aid, thermometer, and ruler will be distributed before discharge home

6.3 Day 8 (+2 days) (phone call)

- Review of medical/medication history
- Review of AEs and AE memory aid from first vaccination

6.4 Day 22 (+/- 3 days) (outpatient visit)

The second study vaccine administration will take place if the participant continues to meet all inclusion and none of the exclusion criteria following these procedures:

- Review of medical/medication history
- Targeted physical exam/assessment as needed

- Vital signs (temperature, heart rate, blood pressure)
- Vital signs at least 30-45 minutes post vaccination
- Blood collection for routine safety labs (Hemoglobin, white blood cell count, platelets, serum creatinine, serum alanine aminotransferase, random glucose)
- Urine pregnancy test (females of childbearing potential only)
- Vaccine or placebo administration
- Vaccine injection site reaction and AE assessment pre-vaccination and at least 30-45 minutes post vaccination
- AE memory aid, thermometer, and ruler will be distributed before discharge home if the previous set is lost

6.5 Day 29 (+2 days) (phone call)

- Review of medical/medication history
- Review of AEs and AE memory aid from second vaccination
- Participants will be asked not to apply cream, perfume or deodorant on the day of mosquito exposure.

6.6 Day 43 Mosquito Feeding (+7 days) (outpatient visit)

The following procedures/assessments and testing will be performed:

- Review of medical/medication history and addition of any new information
- Targeted physician assessment and physical exam if needed
- Vital signs (temperature, heart rate, blood pressure)
- Urine pregnancy test (females of reproductive potential only)
- Blood collection for routine safety labs (hemoglobin, white blood cell count, platelets, serum creatinine, serum alanine aminotransferase, random glucose)
- serum/whole blood for research before mosquito feeding as per Appendix B.
- Mosquito feeding: Starved clean female Aedes aegypti and Aedes albopictus mosquitos will be selected from a mosquito colony approved for human feeding studies in Laboratory of Malaria and Vector Research (LMVR), NIAID. Clean mosquitoes are referred to as mosquitoes colonized in an insectary and that are pathogen free. Clean mosquitoes are raised in an incubator dedicated for clinical trials. This ensures these insects are never exposed to human blood after emergence as adult mosquitoes up to the time they are utilized for feeding on human volunteers enrolled in the clinical trial. The insectary has well-established safety precautions to prevent outside mosquitoes from entering the facility. Mosquitoes will be aspirated into a secured Plexiglas feeding device and brought to the CVD from the LMVR Insectary.
    - There will be 10 participants in each treatment arm. Each participant will be exposed to *Aedes aegypti* on the right arm and *Aedes albopictus* mosquitoes on the left arm.

- The feeding site will be wiped clean with mild unscented soap and water and the device will be placed on the participant's arm for 10-20 minutes. The mosquitos will feed through a disposable mesh on the bottom of the feeding device. This device permits the evaluation of mosquito feeding on the human participant at the end of exposure. In the unlikely event of no feeding or poor feeding (only 0-2 mosquitoes fed or probed as noted by trained staff for a particular strain), the volunteer may undergo a repeat feed with 5-10 fresh mosquitoes ONCE.
- Participants will be asked not to apply cream, perfume or deodorant on the day of mosquito exposure. Once the mosquitos have fed they will be brought back to the lab for further study.
- The PI or study doctor will perform mosquito bite assessments at least 30 min after feeding.

- AE assessment.

6.7 Day 45 (+/- 1 day) (outpatient visit)

Two days after the mosquito feeding the participants will return for a follow-up. The following procedures/assessments and testing will be performed:

- Review of medical/medication history and addition of any new information
- Targeted physician assessment and physical exam if needed
- AE assessment
- Mosquito bite assessment
- Collection of 3 skin biopsies: 2 from sites of mosquito bites previously marked by permanent marker, and a third from an unbitten area of normal skin

6.8 Mosquito Feeding follow-up (Day 50 + 2 days)

Seven days after mosquito feeding, participants will return for a follow-up. The following procedures/assessments and testing will be performed:

- Review of medical/medication history and addition of any new information
- Vital signs (temperature, heart rate, blood pressure)
- Targeted physician assessment and physical exam if needed
- Whole blood and serum collection for research as per Appendix B
- Blood collection for routine safety labs (Hemoglobin, white blood cell count, platelets, serum creatinine, serum alanine aminotransferase, random glucose)
- Mosquito bite assessment by the PI or trained study staff.
- Assessment of biopsy healing
- AE assessment

6.9 Telephone Safety Follow-up (Day 181 ±14 days, and Day 366 ±14 days)

All participants will be followed for a minimum of 12 months from the time of enrollment in the study. However, the study will be unblinded when the safety data up to day 50 and data for primary and secondary endpoints have been collected for all participants. Any participant who experiences complications due to vaccine or placebo administration will be followed until resolution of such complications or appropriate referral to the necessary medical care has been made.

Follow-up visits will take place over a telephone call on days 181 and 366 (+/- 14 days). The study doctor will discuss ongoing, unresolved AEs with the participant and if deemed necessary, schedule a clinic visit. The procedures/evaluations to be conducted at these visits are shown in Appendix A. The follow-up call on day 366 will be the final study intervention unless the participant requires additional follow-up of study-related complications.

7 Study Procedures/ Evaluations

7.1 Clinical and Laboratory Evaluations

See Appendix A for clinical and laboratory procedures performed during the vaccination and mosquito feeding visits as well as follow-up portions of the study. See Appendix B for Blood Volumes for Specimen Collection. Many of these tests are being performed to monitor safety and ensure that participants are healthy enough to undergo vaccination and mosquito feeding. The CVD has established limits for blood draws for research purposes, and the planned blood draws are within these limits.

7.1.1 Laboratory Testing of Collected Samples for Primary and Secondary Endpoints

- AGS-v PLUS specific IgG, IgM and IgE assay will be performed using enzyme-linked immunosorbent assay (ELISA). The time points assayed will be day 1, day 43 and day 50.
- Th1 and Th2 response assays: PBMCs collected from participants will be incubated with AGS-v PLUS and saliva from *Aedes aegypti*, *Aedes albopictus* and *Anopheles gambiae* and measurements of Th1 (Interferon-gamma) and Th2 (Interleukin-4) cytokines will be performed using ELISA. The time points assayed will be day 1, day 43 and day 50.
- Biopsies taken on day 45 will be used to assess expression of inflammatory markers.
- Feeding on human volunteers**:** 24 hour after emergence from pupae, male and female *Aedes aegypti* and *Aedes albopictus* mosquitoes will be allowed to mate for 24-48 hours. After this period, female mosquitoes will be separated from males. These mosquitoes would have only been sugar-fed up until now. Female mosquitoes will be placed in a secured Plexiglass feeding device and allowed to feed on the volunteer's arms on Day 43 (approximately 21 days after they have completed the vaccination schedule). Each participant from each arm will be exposed to the bites of *Aedes aegypti* on the right arm and *Aedes albopictus* on the left arm. Mosquitoes that had a blood meal will then be removed and followed up to assess any changes to their life cycle, i.e., life-span, number of eggs laid, number of fertile eggs, number of surviving larvae etc. The mosquito colony used in this study will not be fed on human blood before exposure to participants, but on non-mammalian blood to minimize the presence of blood borne pathogens.
- Zika virus killing, *in vitro* toxicity assay:
  - Blood samples will be obtained from all volunteers 21 days after vaccination is completed (day 43 pre-feeding). PBMCs+sera containing immune cells and antibodies generated against AGS-v PLUS will be tested for their ability to kill or inactivate Zika virus coated with salivary gland proteins from *Aedes aegypti* and *Aedes albopictus*. Viability of the virus will be tested by infecting Vero cells with the PBMC/virus supernatants. Infectivity of the Zika virus will be assessed by measuring the viability of the Vero cells using MTT assay.
  - Zika virus will be obtained from commercial suppliers and will be propagated *in vitro*.

7.2 Reactogenicity

On a daily basis during the 7-day period after each injection participants will evaluate and record temperature, any local (redness, swelling, and pain) or general symptoms (e.g., fever, chills, headache, myalgia, arthralgia, malaise, sweats, fatigue, nausea, vomiting) they experience on the AE memory aid. At the day 8 and day 29 visits (phone call) the AE memory aids will be reviewed via phone and study staff will record reactogenicity during their discussion with each participant.

The AE memory aid will be completed by the participant in the evening. Participants will be instructed on how to complete the card.

7.3 Mosquito Bite Assessment

Mosquito bites will be assessed by the PI or trained study staff on days 43 (30 minutes after mosquito feeding), 45, and 50. Local reaction will be assessed by grading the size in mm of redness and swelling/induration.

8 Potential Risks and Benefits

8.1 Risks of Placebo administration

Subcutaneous injection of sterile saline poses minimal risk. The primary risks of placebo administration are a local injection-site reaction or infection at the site of injection. Signs of a local injection-site reaction include pain, swelling, and redness at the site of injection. These reactions resolve after short period of time and pose little risk to the participant. Applying cold packs and administering over the counter pain medications if necessary can generally treat these reactions. Signs of infection include pain, redness, swelling, edema, and purulent drainage at the site.

8.2 Risks of AGS-v PLUS ± adjuvant vaccination

Vaccine reactions must be considered a risk of all vaccines. These include minor and severe vaccine reactions. A minor vaccine reaction typically occurs within a few hours of injection and resolve within a short period of time posing little risk to the participant. Symptoms include local pain, swelling, and redness at the site of injection and may include systemic symptoms like fever, malaise, muscle pain, headache, and loss of appetite. Severe reactions usually do not result in long-term problems for the participant, but can be disabling. They are rarely life threatening, but can be in <0.5% of individuals. These reactions can include severe anaphylactic reactions and seizure. Topical symptomatic therapy may be administered if necessary to treat vaccine reactions including cold compresses, and topical anti-pruritic and pain relief. Additional standard of care therapy for pain or anaphylaxis will be administered according to CVD standards if the reaction becomes severe or life threatening.

The Sponsor has carried out a similar study testing the safety and immunogenicity of AGS-v, a vaccine that contains four of the five peptides in AGS-v PLUS (NCT 03055000). No safety concerns were raised by the safety review committee during the trial.

The manufacturer, SEEK, has completed clinical trials in the European Union for the FLU-v and HIV-v vaccines. The technology utilized for peptide identification, vaccine development and manufacturing for the FLU-v and HIV-v vaccines is the same technology utilized for AGS-v PLUS vaccine. FLU-v, HIV-v and AGS-v all utilize Montanide ISA-51 as the adjuvant too. Therefore, we expect similar safety findings for AGS-v PLUS as were observed with FLU-v, HIV-v and AGS-v. Below is a brief safety summary for all trials above mentioned:

- Phase 1 AGS-v 001: Safety issues had been mild in severity. The vaccinations were well tolerated. In the unadjuvanted AGS-v group the most common AEs were Grade 1 or 2 injection site pain, fatigue, and headache (37.5%, 31.3%, 31.3% of participants respectively). Some participants also experienced Grade 1 or 2 systemic reactions of myalgia/feverishness/chills/diaphoresis (6.1-18.8% of participants). The adjuvanted AGS-v group experienced a higher level of related AEs, with the most common being injection site pain, swelling, erythema, and pruritis (52.9%, 47.1%, 41.2%, and 41.2% respectively). They also experienced some systemic grade1 and 2 AEs such as fatigue (41.2%) and feverishness (35.3%), as well as myalgia, chills and diaphoresis (5.9-29.4%). The placebo group experienced fewer AEs with only injection site pain and erythema in 25% and 6.3% respectively. They experienced a smaller amount of systemic AEs as well ranging from 6.3% to 12.5% for headache, fever, chills, fatigue, and myalgia. The most common AEs on Mosquito Feeding Day were Grade 1 erythema (93.8%) and Grade 1 local swelling (93.8%).

- Phase 1 FLU-v-001: no deaths occurred and only one SAE was reported with this being considered unrelated to FLU-v (appendicitis). There was no apparent increase in adverse events (AEs) with a change in dose from the 250 µg or 500 µg (500 ug of FLU-v is the equivalent of 825 ug of AGS-v as both contain 50 nmol of each peptide and a total of four peptides), but an approximate two-fold increase in AEs was seen in the adjuvant versus non-adjuvant groups. This latter point is likely responsible for the largely local administration site reaction AEs, and the forearm site and 1 ml volume is suspected to be the cause rather than the adjuvant itself. In conclusion, no safety or tolerability concerns were identified.

- Phase 1b FLU-v-002: all 32 participants reported AEs: 32 mild, 10 moderate and one severe (a pre-syncopal episode in the placebo group). There were no deaths or SAEs. The most frequently reported AEs were related to the injection site reactogenicity and are consistent with those already documented with use of the adjuvant. There were no other clinically significant changes in the other safety parameters (complete physical examination, clinical chemistry, hematology and urinalysis, vital signs including oral temperature, electrocardiogram [ECG]).

- Phase 2b FLU-v003: A total of 175 participants were enrolled to take part in a study carried in the Netherlands as part of a large European Consortium (UNISEC). Participants were allocated (2:2:1:1) to receive: 2 doses non-adjuvanted FLU-v, 1 dose of FLU-v adjuvanted in Montanide ISA-51, 2 doses of non-adjuvanted placebo or 1 dose adjuvanted placebo. The FLU-v vaccine formulations were safe and tolerated. A small number of participants mainly in the adjuvanted FLU-v group experienced severe reactions at the injection site that resolved with the use of over the counter anti-inflammatory drugs and required no additional follow-up by the medical team. The majority of solicited AEs that were related to the vaccination were observed at the site of injection and those were mild to moderate in intensity. An increase in the number of adverse

events was observed with the addition of adjuvant Montanide ISA-51 to the formulation. It is well known that emulsions prepared in this adjuvant have a tendency to create indurations at the site of injection which slowly disappear as the antigen is processed and the water in oil emulsion is degraded. Adjuvanted placebo had considerable more treatment emergent adverse events (TEAEs) definitely related to treatment than non-adjuvanted placebo (74.1% vs 18.8% participants). Addition of FLU-v to the placebo formulations increased the number of participants experiencing TEAEs (74.1 % to 93% of participants in adjuvanted placebo to adjuvanted FLU-v, and 18.8% to 34.5% participants in non-adjuvanted placebo to non-adjuvanted FLU-v). This indicates that the antigen itself is immunogenic and induces a small inflammatory reaction that translates into AEs at the injection site. These AEs were mostly mild to moderate with the participants in adjuvanted FLU-v group showing the highest number of these AEs. A total of 20 participants experienced adverse events classified as severe in intensity with only 7 of them being definitely related to the study drug and defined as reactions at the site of the injection (1/58 in non-adjuvanted FLU-v, 5/57 in adjuvanted FLU-v, 0/32 in non-adjuvanted placebo and 1/27 in adjuvanted placebo).

- Phase 2b FLU-v004: 121 participants were randomized (1:1:1) into one of three arms: 2 doses adjuvanted placebo (Montanide ISA-51+WFI), two doses adjuvanted FLU-v or one dose adjuvanted FLU-v +adjuvanted placebo. Participants were then challenged with an H1N1 influenza virus. Overall the data showed a good safety profile for the IMP. The single dose FLU-v showed a better profile than the 2 doses. However, there were no significant issues with the single dose arm and no concerns with the 2-dose arm. Injection site reactions were as expected and of an 'acceptable' level. A greater incidence of local reactions was seen in active arms, which may suggest a local reaction to the protein in addition to the adjuvant. The number of definitely related AEs in both FLU-v treatment groups was considerably higher than the placebo but of similar severity. The main AE was induration at the site of the injection and was considerably higher in the vaccine groups than placebo (46.8% and 60.8% compared to 18%).

- Phase 1b HIV-v-001: all 59 participants reported an AE. Fatigue and injection site pain were the most commonly reported AEs. The majority of AEs were of mild severity (69.7%). There were 5 SAEs in 3 participants with 3 SAEs occurring in the one participant and only 2 SAEs occurring within 30 days of vaccination. None of the SAEs were considered to be related to the study drug. No participants died and no participant discontinued the study due to an AE.

8.3 Risks of adjuvant

Montanide ISA-51

Clinical experience with Montanide ISA 51 dates back to the 1990s and most trials were related to cancer and Acquired Immune Deficiency Syndrome (AIDS). Currently, cancer trials in melanoma, colorectal, prostate, cervical, brain cancer and leukemia are ongoing. Frequency of vaccination is often between 2 and 4 weeks, and the number of injections can reach up to 40. Route of immunization is most often subcutaneous and volume of injection can reach up to 3 mL. Most common local reactions are local pain, tenderness, erythema and granuloma at the injection site. Less frequently, mild to moderate transient indurations and swelling are described. General reactions are mainly 'flu-like symptoms such as chills,

fever and headaches. Lethargy and nausea are also observed. The intensity is usually mild or moderate. No biological changes are generally observed.

Aluminium based adjuvants

Alhydrogel® has been used in many commercial vaccines[29]. After almost a century, aluminum salts maintain their dominance as adjuvants in human vaccines. This reflects the fact that aluminum adjuvants are extremely effective at enhancing antibody responses, are well tolerated, do not cause pyrexia and have the strongest safety record of any human adjuvants. Hence, aluminum adjuvants remain the gold standard against which all new adjuvants need to be compared and any new adjuvant must prove it provides better protection, tolerability or safety, or preferably all, when compared to aluminum adjuvant. Aluminum adjuvants suffer from a number of minor toxicities potentially explained by its mechanisms of action. For example, aluminum induces injection site pain and tenderness that may reflect cell necrosis and induction of inflammasome activation and IL1 production which explains why some participants develop persistent lumps and granulomas at the injection site. Aluminum adjuvants may also induce contact dermatitis to aluminum in a fraction of immunized participants. Aluminum adjuvant-containing vaccines can cause post-immunization headache, arthralgia and myalgia, which could reflect alum's propensity to induce IL-1, with IL1 administration to human participants reproducing these symptoms. On the positive side, aluminum adjuvants rarely cause severe local reactions and are not normally associated with systemic inflammatory problems such as pyrexia[29].

8.4 Risks of blood draw

Risks of blood draw include pain, bruising, bleeding, and rarely fainting or infection. To minimize this risk, the skin is cleaned with alcohol before puncture; sterile, unused needles and lancets will always be used; and pressure will be held at the puncture site after removal of the needle or lancet. Although the quantity of blood drawn would not lead to any ill effects on the participants' health, some adults feel faint with phlebotomy. This risk will be minimized by having trained technicians perform the procedure, and by placing participants in a recumbent position if they feel light headed or appear as if they are about to faint. Clinicians will be available for evaluation if there is any untoward effect.

8.5 Risks of Aedes aegypti and Aedes albopictus Mosquito Feeding

The risk of mosquito feeding is minimal, but participants may suffer pruritis, mild rash, or irritation at the site of the bites. This may require topical symptomatic therapy. In rare cases a more severe irritation could occur, anaphylactic reaction, or secondary infection at the site of the bite where antibiotics or systemic anti-inflammatory medication may be required.

8.6 Risk of Skin Biopsies

From each participant, three 3 mm skin biopsies will be taken, one from the A. aegypti bite (right arm), one from the A. albopictus bite (left arm) and the last one from normal unbitten skin (control). Risks of biopsy include local pain, bleeding, redness, infection, a scar and possible keloid formation. Oral or topical antibiotics and oral analgesics will be used to manage pain and infection as needed. Any non-routine complications resulting from the procedure will be addressed in consultation with the NIH

Clinical Center (CC) Dermatology service. Injection of local anesthetic may cause a minimal burning discomfort or bruising at the site of the needle puncture.

8.6 Potential Benefits

The vaccine is still under development. There is no direct benefit to the participant. The information collected from this study will allow a better understanding of the safety and immunogenicity of AGS-v PLUS and may inform further development of this or other universal mosquito-borne disease vaccines.

9 Research Use of Stored Human Samples, Specimens, or Data

Intended Use: Samples and data collected under this protocol may be used to study aspects of Zika virus infection and other mosquito-borne diseases. Genetic testing will not be performed. A separate signed informed consent document will be obtained for any other research not described in this protocol.

Storage: Access to stored samples will be limited using a locked freezer in a locked laboratory. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers or in locked cabinets in locked rooms accessed only by study staff. Only investigators and designated study staff will have access to the samples and data.

Tracking: Samples will initially be stored in laboratories of primary and associate investigators. Samples will be tracked using a database located on password-protected computers, which will be maintained by the investigators and their designees. Only investigators and their designees will have access to this database.

Disposition at the Completion of the Protocol:

- In the future, other investigators may wish to study these samples and/or data. In that case, IRB approval must be sought before any sharing of samples and/or data. Any clinical information shared about the samples would similarly require prior IRB approval.

- At the completion of the protocol (termination), samples will be stored at suitable conditions for a minimum of 1 year. After this time and in agreement between the Sponsor and vaccine manufacturer, SEEK, the samples will either be destroyed, or after IRB approval, transferred to another existing protocol.

- At the completion of the protocol (termination), data will be stored for a minimum of 3 years. After this time and in agreement between the Sponsor and vaccine manufacturer, SEEK, the data will either be destroyed, or after IRB approval, transferred to another existing protocol.

Reporting Loss or Destruction of Samples/Specimens/Data to the IRB:

- Any loss or unanticipated destruction of samples or data (for example, due to freezer malfunction) that meets the definition of protocol deviation and/or compromises the scientific integrity of the data

collected for the study will be reported to the IRB in accordance with local regulations. The PI will also notify the IRB if the decision is made to destroy the samples.

- Additionally, participants may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the participant and to the IRB. This decision will not affect the subject's participation in this protocol or any other protocols at CVD.

10 Data Sharing Plan

10.1 Sharing of Human Data

Human data generated in this study will be shared for future research as follows:

- De-identified data in an NIH-funded or approved public repository.
- Identified data in Biomedical Translational Research Information System (BTRIS).
- De-identified or identified data with approved outside collaborators under appropriate agreements.

Data will be shared through:

- ClinicalTrials.gov
- BTRIS.
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations at scientific meetings.

11 Remuneration Plan

Participants will be compensated for time undergoing study procedures according to standard compensation policy at CVD.

Study visits will be compensated according to the number of visits the participant completes. Participants will only be reimbursed for the protocol visits and interim visits requested by the investigators if medically necessary. Remuneration will be provided to the participants as the study visits are completed.

12 Assessment of Safety

12.1 Documenting, Recording, and Reporting AEs

At each contact with the participant, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

- Immediately documented in the participant's medical record/source document,
- Recorded in the study database
- Reported as outlined below (e.g., Investigational New Drug (IND) Sponsor, IRB, FDA).

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All abnormal laboratory findings will be reviewed on a routine basis by the PI or designee to identify potential safety signals. An abnormal lab not included on the toxicity table should be assessed in a similar fashion to the criteria above.

12.2 Definitions

Adverse Event (AE)
An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam, or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Treatment Emergent Adverse Event (TEAE)
A TEAE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam, or laboratory finding), symptom, or disease, that manifested after administration of the study treatment, whether or not considered related to the treatment.

Adverse Reaction (AR)
An AE that is caused by an investigational agent (drug or biologic).

Protocol Deviation
A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the stie and implemented promptly.
These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sec tions 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, Section 5.1.1
5.2.0 Noncompliance, Sections 5.20.1, and 5.20.2.

Suspected AR (SAR)
An AE for which there is a reasonable possibility that the investigational agent caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. A SAR implies a lesser degree of certainty about causality than AR, which implies a high degree of certainty.

Serious Adverse Event (SAE)
An SAE is an AE that results in 1 or more of the following outcomes:

- Death

- A life-threatening event (places the subject at immediate risk of death from the event as it occurred)
- An inpatient hospitalization or prolongation of an existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- A medically important event*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event (UAE)

An AE is unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND sponsor to make this determination.

Serious and Unexpected SAR (SUSAR)

A SUSAR is a SAR that is both serious and unexpected.

Unanticipated Problem (UP)

A UP is any event, incident, experience, or outcome that is

1. Unexpected in terms of nature, severity, or frequency in relation to
   a. The research risks that are described in the IRB-approved research protocol and informed consent document, Investigator's Brochure, or other study documents; and
   b. The characteristics of the participant population being studied; and
2. Possibly, probably, or definitely related to participation in the research; and
3. Places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk).

Serious Unanticipated Problem

A UP that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

UP that is not an AE (UPnonAE)

An UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, breaches of confidentiality, accidental destruction of study records, or unaccounted-for study agent will be reported.

12.3 Investigator Assessment of AEs

The investigator will assess all AEs with respect to seriousness (criteria listed above), severity (intensity or grade), and causality (relationship to study agent and relationship to research) according to the following guidelines.

12.3.1 Severity

The investigator will grade the severity of each AE according to Appendix C. For items not found in Appendix C, the investigator will use the FDA "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" September 2007, which can be found at: . https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical

All lab and vital sign abnormalities found before administration of the vaccine or placebo will be documented as a baseline AE and will be assessed for clinical significance and the participant will be reassessed for inclusion or exclusion to receive vaccination. After administration of vaccine or placebo, all new gradable abnormalities not found at baseline will be reported as AEs.

Severity grading for clinical events that are not found in the FDA Healthy Volunteer Toxicity Table will be graded according to the following grading scale:

- Grade 1 (Mild)
  Events causing no or minimal interference with daily activity and not requiring medical intervention
- Grade 2 (Moderate)
  Events causing greater than minimal interference with daily activity but not requiring medical intervention
- Grade 3 (Severe)
  Events causing inability to perform daily activity and/or requiring medical intervention
- Grade 4 (Potentially Life-Threatening)*
  Events causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
- Grade 5 (Death)
  Events causing death

***Note:** A severity assessment of "potentially life-threatening" is not necessarily the same as life-threatening as an "SAE" criterion. The latter means that the event is an immediate threat to life as opposed to a potential threat to life.

12.3.2 Causality

Causality (likelihood that the event is caused by the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related

- Reasonable temporal relationship
- Follows a known response pattern
- Clear evidence to suggest a causal relationship
- There is no alternative etiology

Probably Related

- Reasonable temporal relationship
- Follows a suspected response pattern (based on similar products)
- No evidence of a more likely alternative etiology

Possibly Related

- Reasonable temporal relationship
- Little evidence for a more likely alternative etiology

Unlikely Related

- Does not have a reasonable temporal relationship

OR

- Good evidence for a more likely alternative etiology

Not Related

- Does not have a temporal relationship

OR

- Definitely due to an alternative etiology

Note: Other factors should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

12.4 Investigator Reporting Responsibilities to the Sponsor

12.4.1 AEs

AE data will be submitted to the IND sponsor when requested for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

12.4.2 SAEs

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) or Red Cap system and sent to the Sponsor Clinical Safety Office (CSO) by fax or e-mail attachment, or directly online via secure server. Deaths and immediately life threatening SAEs will be reported within 1 business day after the site becomes aware of the event. All other SAEs will be reported within 3 business days of site awareness.

CSO CONTACT INFORMATION:
Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
E-mail: rchspsafety@mail.nih.gov

12.4.3 Unanticipated Problems

UPs that are also AEs must be reported to the CSO and sent by fax or e-mail attachment using the NIH Problem Report Form no later than 7 calendar days of site awareness of the event. Report Ups that are also AEs to the CSO on a SERF or a local IRB UP form. UPs that are not AEs are not reported to the Sponsor CSO.

12.4.4 Pregnancy

All pregnancies will be reported on the Pregnancy Notification/Outcome Form to the CSO within 1 business day from site awareness.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness.

Although pregnancy itself is not an AE, events that meet SAE criteria during pregnancy, delivery, or in the neonate (e.g., congenital anomaly/birth defect) are reportable on the SERF.

In the event of pregnancy, the following steps will be taken:

- Discontinue the study agent and procedures but continue to follow-up for safety.
- Report to the Data and Safety Monitoring Board (DSMB) and the IRB.
- Advise the research participant to notify the obstetrician of study participation and study agent exposure.

12.5 Investigator Reporting Responsibilities to the University of Maryland, Baltimore (UMB) IRB

12.5.1 Reporting Protocol Deviations

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations as per UMB IRB reporting guidelines in Appendix D. All deviations must be promptly reported to the sponsor per the sponsor's protocol deviation reporting procedures.

All protocol deviations must be addressed in study subject data collection forms. A completed copy of the protocol deviation form must be maintained in the Regulatory File, as well as in the subject's chart for subject-specific protocol deviations. Protocol deviations must be sent to the UMB IRB per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

12.5.2 Special Reporting Situations

Protocol deviations of interest that may require expedited reporting to the UMB IRB (as per UMB IRB reporting guidelines in Appendix D) and/or safety evaluation include, but are not limited to:

- Overdose of a study drug
- Suspected abuse/misuse of a study drug
- Inadvertent or accidental exposure to a study drug
- Medication error involving a product (with or without participant/patient exposure to the study drug, e.g., name confusion)

All protocol deviations should be recorded in the study database.

12.5.3 Expedited Reporting to the UMB IRB

SAEs, serious and non-serious UPs, deaths, serious deviations, and serious or continuing non-compliance will be reported as per UMB IRB reporting guidelines in Appendix D.

12.5.4 Waiver of Reporting Anticipated Protocol Deviations, Expected UPnonAEs and Deaths to the UMB IRB

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected AEs will not be reported to the IRB unless they occur at a rate greater than that known to occur in vaccine recipients. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are UPs.

12.5.5 Annual Reporting to the UMB IRB

Annual reporting to the UMB IRB will be in compliance with UMB IRB guidelines.

12.6 Follow-Up of AEs and SAEs

AEs that occur following enrollment of the participant after first vaccination will be followed until the final outcome is known or until stable, if chronic, or until the end of the study follow-up period. AEs that have not resolved by the end of the study follow-up period will be recorded as “ongoing.” Any participant who experiences complications due to vaccine/placebo administration will be followed until resolution of such complications or appropriate referral to the necessary medical care has been made.

SAEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the participant is lost to follow-up),

the reason a final outcome could not be obtained will be recorded by the investigator in the study database and on the SERF.

SAEs that occur after the Day 366 phone call that are reported to and are assessed by the investigator to be possibly, probably, or definitely related will be reported to the CSO, as described above (section 12.4.2 SAEs).

12.7 Sponsor's Reporting Responsibilities

SUSARs as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA and all participating investigators as IND Safety Reports.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

12.8 Pausing Rules for an Individual Participant

Pausing is the suspension of administration of study agent to a single participant until a decision is made whether or not to resume administration of the study agent.

The pausing criteria for a single participant in this study include any of the following:

- A participant experiences an SAE that is possibly, probably, or definitely related to a study agent;
- A participant experiences two Grade 3 or greater AEs that are possibly, probably, or definitely related to a study agent;
- Any safety issue that the site investigator determines should pause administration of a study agent to a single participant.

The CSO, in collaboration with the PI, may also pause for an entire group if a safety concern is identified.

12.8.1 Reporting a Pause

If a pausing criterion is met, a description of the AE(s) or safety issue must be reported by the PI or designee within 1 business day of recognition of the event to the CSO, the IRB, and the DSMB by fax or email.

12.8.2 Resumption of a Paused Study

The CSO, in collaboration with the PI and the DSMB, will determine whether or not it is safe to resume administration of the study agent to the participant. The PI will notify the IRB of the decision on resumption of the study agent.

A participant who does not resume study agent will continue to be followed for safety.

12.9 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all participants and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

The halting rules are:

- 1 or more participants experience the same or similar SAEs that are possibly, probably, or definitely related to the study agent;

OR

- 2 or more of the same or similar AE in different participants that are grade 3 or above and are possibly, probably, or definitely related to the study agent;

OR

- Any safety issue that the PI and/or the CSO determines should halt the study.

The PI and/or CSO will determine if the study should be halted. In addition, the FDA may halt the study at any time following review of any safety concerns.

12.9.1 Reporting a Study Halt

If a halting rule is met, a description of the AE(s) or safety issue must be reported by the PI or designee within 1 business day to the Co-Investigator, IND Sponsor, CSO, the IRB, and the DSMB by fax or email.

12.9.2 Resumption of a Halted Study

The IND sponsor, in collaboration with the PI and the DSMB will determine if it is safe to resume the study. The PI will notify the IRB of the decision on resumption of the study.

Participants who do not resume study agent will continue to be followed for safety.

12.10 Study Discontinuation

Office of Clinical Research Policy and Regulatory Operations (OCRPRO), the study sponsor, the IRB, and the FDA have the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

1. The incidence or severity of AEs in this study indicates a potential health hazard to participants
2. Participant enrollment is unsatisfactory
3. Data recording is inaccurate or incomplete
4. Investigators do not adhere to the protocol, or applicable regulatory guidelines in conducting the study

The IRB, the NIAID, the FDA, or other government agencies, as part of their duties to ensure that research participants are protected may discontinue the study at any time. Subsequent review of serious, unexpected and related AEs by the IRB, the DSMB, the sponsor, the FDA, and other regulatory

authorities may also result in suspension of further trial interventions/administration of study agent at a site. The FDA, other regulatory authorities, and the study sponsor retain the authority to suspend additional enrollment and study agent administration for the entire study as applicable.

12.11 Premature Withdrawal of a Participant

Participants may withdraw before vaccine administration and no further testing or follow-up will be performed. The PI or designee will discuss with the participant why she/he wants to prematurely withdraw from the study to determine the best course of action for the participant. If the participant would like to withdraw after vaccination, clinical laboratory tests and procedures for safety purposes will continue as obtainable and at a frequency determined by the PI. If the participant does not return for scheduled follow-up visits, the study staff will make every reasonable effort to contact the participant by phone, mail, or email, or a combination of the latter and reiterate that follow-up visits are strongly encouraged for safety reasons.

An individual participant will be withdrawn for any of the following:

- An individual participant's decision. (The investigator should attempt to determine the reason for the participant's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the participant or to the integrity of the study data.
- A change in the participant's baseline condition after enrollment so that the participant no longer meets one or more of the inclusion/exclusion criteria.
- The investigator determines that continued participation in the study would not be in the best interest of the participant.

12.12 Replacement of a Participant

If a participant withdraws or appears ineligible to continue the study before vaccine/placebo administration, he/she will be removed and no data will be used in analysis or publication of the study. The participant may be replaced in the accrual with a new volunteer who qualifies and consents to the study, and who can complete both vaccinations 14-28 days before the planned date of mosquito challenge. Safety data from all participants that have withdrawn will be used and included in the safety analysis.

12.13 Safety Oversight

12.13.1 Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the CSO, which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

12.13.2 Sponsor Medical Monitor

A Medical Monitor, representing the IND Sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The Sponsor Medical Monitor will be responsible for performing routine safety assessments in an SRCP.

12.13.3 Data and Safety Monitoring Board

The NIAID intramural data and safety monitoring board (DSMB) includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interest as defined by NIAID policy. The DSMB will review the study protocol, consent document(s), and investigator brochure prior to initiation and twice a year thereafter, or as may be determined by the DSMB.

The DSMB may convene additional reviews as necessary. The DSMB will review the study data as needed to evaluate the safety, efficacy, study progress, and conduct of the study.

All deaths, SAEs, UPs, pregnancies, and IND safety reports will be reported to the DSMB at the same time they are submitted to the IRB and CSO unless otherwise specified herein.

All cases of intentional or unintentional unblinding will be reported to the DSMB not later than one business day from the time of study personnel awareness.

The principal investigator will notify the DSMB at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The principal investigator will submit the written DSMB summary reports with recommendations to the IRB(s).

13 Site Monitoring Plan

According to the ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the sponsor's clinical monitoring guidelines. Clinical monitors will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be:

1) To verify the existence of signed informed consent documents and documentation of the informed consent process for each monitored participant;

2) To verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs;

3) To compare clinical data abstracts with individual participants' records and source documents (participants' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original participant information); and

4) To help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (FDA; University of Maryland, Baltimore; Office for Human Research Protections [OHRP]) and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, clinical data abstracts, and pertinent hospital or clinical records) readily available for inspection by the FDA, IRB, site monitors, and NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI and study staff before enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

14 Statistical Considerations

14.1 Study Hypothesis

The study hypothesis is that:

- AGS-v PLUS specific immunoglobulin will increase after vaccination with AGS-v PLUS compared to placebo

-PBMCs collected after vaccination with AGS-v PLUS incubated with AGS-v PLUS antigens will show a greater Th1 response as compared to placebo vaccination. Interferon–gamma is the main cytokine marker for Th1 responses.

14.2 Sample Size Justification and Analysis Plan

The primary objective of this study is to evaluate safety.

For the safety endpoint we are interested in identifying the number of participants with at least one SAE that is possibly, probably or definitely related to vaccine (halting rule 1). With 10 participants in each group we would have an 80% chance of observing at least one participant with at least one SAE if the true underlying rate for that SAE in a vaccinated arm is 0.15. We will have a 10% chance of observing at least one SAE if the true underlying rate is .01. If we collapse across the vaccinated arms we would have 40 participants. With 40 participants we would have a 95% chance of observing at least one participant with AEs if the true rate is 0.07. There is a 4% chance of observing at least one SAE out of 40 if the true rate is .001. With this sample size we will have a high probability of beginning to see a severe adverse event safety signal if one exists. Table 1 shows the probability of observing at least one participant with an SAE for various true SAE rates with samples sizes of 10 and 40.

In halting rule 2 we will halt the study if at least 2 or more of the same or similar AE's (possibly, probably or definitely related to vaccine) that are grade 3 or above are observed. With 10 participants in each group we would have an 80% chance of observing 2 or more participant with at least one AE if the true underlying rate in a vaccinated arm is 0.27. We will have a less than a 1% chance of observing at least 2 AE if the true underlying rate is .01. If we collapse across the vaccinated arms we would have 40 participants. With 40 participants we would have an 84% chance of observing at least two participants with AEs if the true rate is 0.08. There is a 6% chance of observing at least two AE out of 40 if the true rate is .01. With this sample size we will have a high probability of beginning to see an adverse event safety signal if one exists. Table 2 shows the probability of observing at least one participant with an SAE for various true SAE rates with samples sizes of 10 and 40.

Since this is a Phase 1b study we are looking for activity of the vaccine that would indicate the vaccines should be studied further. Since we do not wish to expose more participants than necessary to an experimental vaccine but we also don't wish to miss an active vaccine we will design the study to have 85% power with a one sided type I error rate of 0.1. We will also not adjust for the multiple comparisons (4 active arms being compared to placebo).

With 10 participants per arm, there will be 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(R version 3.4.1).

Table 1. Probability of observing at least 1 SAE for samples sizes of 10 and 40 with different true SAE rates

True rate of severe adverse events	Probability of observing at least one participant with SAE (Halting rule 1)	
	N=10	N=40
.001	**0.01**	**0.04**
0.01	0.10	0.33
0.02	0.18	0.55
0.03	0.26	0.70
0.04	0.34	0.80
0.05	0.40	0.87
0.06	0.46	0.92
0.07	0.52	0.95
0.08	0.57	0.96
0.09	0.61	0.98
0.1	0.65	0.99
0.11	0.69	0.99
0.12	.72	0.99
0.13	0.75	0.99
.14	0.78	0.99
0.15	0.80	0.99

Table 2. Probability of observing at least 2 SAE's for samples sizes of 10 and 40 with different true SAE rates

True rate of severe adverse events	Probability of observing at least 2AE's (halting rule 2)	
	N=10	N=40
.01	<.001	.06
.08	.19	.84
.27	.80	>.99

14.3 Analysis Plan

T-tests will be used to compare immunogenicity endpoint measurements for the primary endpoint and other continuous endpoints between each treatment group and the placebo group. For binary endpoints Fishers exact test will be used. AEs will be tabulated by treatment arm.

The fold increase in cellular and humoral responses to AGS-v PLUS from day 1 to days 43 and 50, and from day 43 to day 50 will be calculated for each participant. The mean fold increases on each active group will be compared to the mean fold increases in the placebo group. The geometric mean antibody titers and geometric mean cytokine concentrations to AGS-v PLUS on days 1, 43, and 50 will be calculated and compared between the different treatment groups.

The fold increase in cellular responses to mosquito saliva from day 1 to days 43 and 50, and from day 43 to day 50 will be calculated for each participant. The mean fold increases on each active group will be compared to the mean fold increases in the placebo group. The geometric mean cytokine concentrations to mosquito saliva on days 1, 43 and 50 will be calculated for each treatment group and compared to placebo.

For the mosquito analysis, the following parameters will be compared on the active groups versus placebo for each mosquito (*Aedes aegypti* and *Aedes albopictus*):
-mean number of days mosquitoes survive after feeding on vaccinated participants
-mean number of eggs laid
-mean number of mosquitoes emerging from the laid eggs

For the visual assessment of the mosquito bite the following parameters will be calculated for each group and active groups will be compared to placebo at three different time points: day 43 (30 min post feeding), day 45 and day 50:
-mean number of visible bites
-mean redness measured across the widest part (mm)
-mean swelling measured using the ball point pen method (mm^2). Ball point pen measurement of induration uses the pen to slide on top of the skin so it halts at the edge of true induration (versus redness)

From the biopsies taken on day 45, the following comparisons will be made:

-gene expression: the level of expression of inflammatory cytokines at the bite site compared to normal skin (control) will be calculated for each participant for each mosquito bite. The mean will be calculated in each group and compared to placebo.

The viability/infectivity of Zika virus after culture with PBMCs and serum from vaccinated participants will be assessed by infecting Vero cells with the cell supernatant followed by measuring viability of Vero cells using MTT assay. The higher the infectivity of the Zika virus the lower the MTT value will be for Vero cells. The fold changes from day 0 to day 43 will be measured for each participant and the mean calculated for each group and compared to placebo.

For randomization, the first 10 participants will be randomized into 5 arms with 2 in each arm. An interim safety analysis after the first group of 10 participants have received the first immunization and completed the Day 8 phone call will be done by the DSMB by teleconference. Recruitment activities will continue but vaccination of new participants will be halted until the DSMB review has been completed. Once the interim safety analysis is done on the initial enrolment, an additional set of 40 participants will be enrolled and randomized using a permuted block randomization with a block size of 10.

14.3.1 Interim Analysis

Data for all groups collected up to day 50 will undergo interim analysis for safety and immunogenicity, and an interim study report will be generated. Due to the importance of this data, these results may be shared with the greater scientific community via meeting abstracts, professional presentations, and manuscript submissions to scientific journals. The data to be presented and the authorship will be approved by the study investigators, vaccine developer, and sponsor before any official communication.

15 Ethics/Protection of Human Participants

15.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research participant. It is an ongoing conversation between the human research participant and the researchers, which begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks, and benefits. Participants will be given the opportunity to ask questions and have them answered.

The participants will sign the informed consent document before undergoing any procedures. The participant may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the participant's medical record. The rights and welfare of the participant will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.2 Participant Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to medical records. Electronic records will be kept in secure electronic systems. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, or the sponsor's designee.

16 Data Handling and Record Keeping

16.1 Data Capture and Management

Study data will be collected and maintained in the study database and collected directly from participants during study visits and telephone calls. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into these systems will be performed by authorized individuals. The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

Data that may potentially unblind the vaccine assignment (e.g., vaccine preparation/accountability data, and vaccine allocation) will be handled with special care to ensure that the integrity of the blind is maintained and the potential for bias is minimized. This can include making special provisions, such as segregating the data in question from view by the investigators, sponsor clinical team, or others as appropriate until the time of database lock and unblinding.

16.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP Guidelines and 21 CFR 312.62. Study records will be maintained at a minimum by the PI for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.Records will be maintained in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from NIAID/OCRPRO.

SCIENTIFIC REFERENCES

1. *World Malaria Report 2015.* World Health Organization;2015.

2. Bhattarai A, Ali AS, Kachur SP, et al. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLoS medicine.* 2007;4(11):e309.

3. Najera JA, Gonzalez-Silva M, Alonso PL. Some lessons for the future from the Global Malaria Eradication Programme (1955-1969). *PLoS medicine.*

   2011;8(1):e1000412.

4. Rts SCTP. 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 medicine.*

   2014;11(7):e1001685.

5. Greenwood B, Targett G. Do we still need a malaria vaccine? *Parasite immunology.* 2009;31(9):582-586.

6. Ribeiro JM. Role of saliva in blood-feeding by arthropods. *Annual review of entomology.* 1987;32:463-478.

7. Wasserman HA, Singh S, Champagne DE. Saliva of the Yellow Fever mosquito, Aedes aegypti, modulates murine lymphocyte function. *Parasite immunology.* 2004;26(6-7):295-306.

8. Cross ML, Cupp EW, Enriquez FJ. Differential modulation of murine cellular immune responses by salivary gland extract of Aedes aegypti. *The American journal of tropical medicine and hygiene.* 1994;51(5):690-696.

9. Silvestre R, Cordeiro-Da-Silva A, Santarem N, Vergnes B, Sereno D, Ouaissi A. SIR2-deficient Leishmania infantum induces a defined IFNgamma/IL-10 pattern that correlates with protection. *Journal of immunology.* 2007;179(5):3161-3170.

10. 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. *Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology.* 1996;77(3):238-244.

11. Peng Z, Simons FE. Mosquito allergy: immune mechanisms and recombinant salivary allergens. *International archives of allergy and immunology.* 2004;133(2):198-209.

12. Donovan MJ, Messmore AS, Scrafford DA, Sacks DL, Kamhawi S, McDowell MA. Uninfected mosquito bites confer protection against infection with malaria parasites. *Infection and immunity.* 2007;75(5):2523-2530.

13. Doolan DL, Dobaño C, Baird JK. Acquired immunity to malaria. *Clin Microbiol Rev.* 2009; 22(1): 13-36.

14. Sidjanski S, Vanderberg JP. Delayed migration of Plasmodium sporozoites from the mosquito bite site to the blood. *The American journal of tropical medicine and hygiene.* 1997;57(4):426-429.

15. Ponnudurai T, Lensen AH, van Gemert GJ, Bolmer MG, Meuwissen JH. Feeding behaviour and sporozoite ejection by infected Anopheles stephensi. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 1991;85(2):175-180.

16. Vanderberg JP, Stewart MJ. Plasmodium sporozoite-host cell interactions during sporozoite invasion. *Bulletin of the World Health Organization.*

   1990;68 Suppl:74-79.

17. Vanderberg JP, Chew S, Stewart MJ. Plasmodium sporozoite interactions with macrophages in vitro: a videomicroscopic analysis. *The Journal of protozoology.* 1990;37(6):528-536.

18. Krettli AU, Dantas LA. Which routes do Plasmodium sporozoites use for successful infections of vertebrates? *Infection and immunity.*

2000;68(5):3064-3065.

19. 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.

20. Schneider BS, Higgs S. The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 2008;102(5):400-408.

21. Brennan JD, Kent M, Dhar R, Fujioka H, Kumar N. Anopheles gambiae salivary gland proteins as putative targets for blocking transmission of malaria parasites. *Proceedings of the National Academy of Sciences of the United States of America.* 2000;97(25):13859-13864.

22. Suneja A, Gulia M, Gakhar SK. Blocking of malaria parasite development in mosquito and fecundity reduction by midgut antibodies in Anopheles stephensi (Diptera: Culicidae). *Archives of insect biochemistry and physiology.* 2003;52(2):63-70.

23. Gulia M, Suneja A, Gakhar SK. Effect of anti-mosquito hemolymph antibodies on fecundity and on the infectivity of malarial parasite Plasmodium vivax to Anopheles stephensi (Diptera:Insecta). *Japanese journal of infectious diseases.* 2002;55(3):78-82.

24. Stoloff GA, Caparros-Wanderley W. Synthetic multi-epitope peptides identified in silico induce protective immunity against multiple influenza serotypes. *European journal of immunology.* 2007;37(9):2441-2449.

25. Vaughan JA, Scheller LF, Wirtz RA, Azad AF. Infectivity of Plasmodium berghei sporozoites delivered by intravenous inoculation versus mosquito bite: implications for sporozoite vaccine trials. *Infection and immunity.*

1999;67(8):4285-4289.

26. Valenzuela JG, Belkaid Y, Garfield MK, et al. Toward a defined anti-Leishmania vaccine targeting vector antigens: characterization of a protective salivary protein. *The Journal of experimental medicine.*

2001;194(3):331-342.

27. Belkaid Y, Kamhawi S, Modi G, et al. Development of a natural model of cutaneous leishmaniasis: powerful effects of vector saliva and saliva pre-exposure on the long-term outcome of Leishmania major infection in the mouse ear dermis. *The Journal of experimental medicine.*

1998;188(10):1941-1953.

28. Wu Y, Ellis RD, Shaffer D, et al. Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with Montanide ISA 51. *PloS one.* 2008;3(7):e2636.

29. Petrovsky N. Comparative safety of vaccine adjuvants: a summary of current evidence and future needs. *Drug. Saf.* 2015, 38(11): 1059-1074.

30. National Institutes of Health: Giving a subcutaneous injection 2015; http://www.cc.nih.gov/ccc/patient_education/pepubs/subq.pdf, 2016.

Appendix A: Schedule of Procedures/Evaluations

Study Phase >	Screen	Injection 1	Follow-up call	Injection 2	Follow-up call	Feeding	Follow-up	Follow-up	Follow-up	Follow-up call
Study Day >	**DAYS -60 to 0**	**D1**	**D8**	**D22**	**D29**	**D43**	**D45**	**D50**	**D181**	**D366**
Outpatient Visit	X	X		X		X	X	X		
Comprehension quiz	X									
Written Consent	X									
Medical/Medication History	X	X	X	X	X	X	X	X	X	X
Physician Assessment and PE (targeted and as needed after screening)	X	X		X		X	X	X		
Review of inclusion/exclusion criteria and review of consent	X	X		X						
Vital signs (temperature, heart rate, blood pressure)	X	X		X		X		X		
Vital signs, Vaccine injection site and AE assessment pre-vaccination and 30-45 min post-vaccination		X		X						
Provision of memory aid, ruler and thermometer		X		X						
Review of AEs			X	X	X	X	X	X		
Review of Memory Aid			X		X					
Telephone Call to Assess Symptoms and AEs			X		X				X	X
Pregnancy test (serum/urine)‡	X	X		X		X				
Vaccine administration		X		X						
Randomization		X								
Mosquito Feeding						X				
Mosquito Bite Assessment						X	X	X		
Safety Labs: hemoglobin, white blood cells, platelets, serum creatinine, serum alanine aminotransferase, random glucose	X	X		X		X		X		
Screening Labs: HIV, hepatitis B and C	X									
Serum and Whole Blood Collection		X				X		X		
Skin Biopsy							X			
Skin Biopsy inspection								X		

† Vital Signs: participants must be sitting for a minimum of 5 minutes before these procedures being performed; vital signs include blood pressure, heart rate, temperature. Weight and height will be taken at the screening visit only.

‡ Serum pregnancy testing at the screening and urine pregnancy testing for all other visits. On Days 1, 22, 43, study vaccination or mosquito feeding will not be initiated until negative results are available. Females of childbearing potential only.

Appendix B: Blood Volumes for Specimen Collection

Study Schedule/Procedures	Volume	Screening	Day 1 prevaccination	Day 22 prevaccination	Day 43 (pre-mosquito feeding)	Follow-up Day 50
Serum creatinine, serum alanine aminotransferase, hepatitis BsAg, hepatitis C Ab & b-hCG, Pregnancy, if needed	8.5 mL per blood draw	8.5 mL	x	x	x	x
HIV	3.5 mL	3.5 mL	X	X	X	X
Complete blood count	4 mL per blood draw	4 mL	4 mL	4 mL	4 mL	4 mL
Serum creatinine, serum alanine aminotransferase	5 mL per blood draw	x	5 mL	5 mL	5 mL	5 mL
Serum for Immunology studies	4mL	x	4mL	x	4ml	4mL
Whole blood for Immunology studies	50 mL	x	50 mL	x	50 ml	50 mL
Daily Volume (mL)		16 mL	63 mL	9 mL	63 mL	63 mL
Cumulative Volume			79 mL	88 mL	151 mL	214 mL

Appendix C: Toxicity Tables

Vital Signs				
Vital Signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C)*	38.0-38.4 100.4-101.2 (**°F**)	38.5-38.9 101.3-102.1(**°F**)	39.0-40.0 102.2 – 104.0 (**°F**)	>40.0 >104.0(**°F**)
Hypertension (systolic) mm Hg **	141-150	151-160	>160	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) mm Hg	91-95	96-100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) mm Hg	85-89	80-84	<80	ER visit or hospitalization for hypotensive shock
Bradycardia – beats per minute	50-54 or 45-50 if baseline <60	45-49 or 40-44 if baseline <60	<45 or <40 if baseline <60	ER visit or hospitalization for arrhythmia
Tachycardia – beats per minute	101-115	116-130	>130 or ventricular dysrhythmia	ER visit or hospitalization for arrhythmia

* Oral temperature; no recent hot or cold beverages or smoking

** Assuming seated position, 5 minutes at rest conditions, not sleeping subjects, measurements on the same arm and several concordant results

Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
WBC Decreased –cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	<1,000
WBC Increased –cell/mm^3	11,001 – 15,000	15,001 – 20,000	20,001 – 25,000	>25,000
Hgb g/dL (Female)	11.0 – 11.5	9.5 – 10.9	8.0 – 9.4	<8.0
Hgb g/dL (Male)	12.0 – 12.5	10.0 – 11.9	8.5 – 9.9	<8.5
Platelets Decreased –cell/mm^3	120,000 – 130,000	100,000 – 119,999	25,000 – 99,000	< 25,000

Chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
ALT Increased IU/L	46 – 105	106 – 175	176 - 450	>450
Creatinine mg/dL (Increased) (Female)	>ULN – 1.7	1.8 – 2.0	2.1 – 2.5	>2.5 or requires dialysis
Creatinine mg/dL (Increased) (Male)	>ULN – 1.7	1.8 – 2.0	2.1 – 2.5	>2.5 or requires dialysis
Glucose-hypoglycemia mg/dL	65-67	55-64	45 - 54	<45
Glucose-hyperglycemia random- mg/dL	140-159	160-200	>200	Insulin requirements or hyperosmolar coma

Appendix D: UMB IRB reporting guidelines

REPORTABLE NEW INFORMATION

Please post this prominently in your research or office space.

Report the information items that fall into one or more of the following categories to the IRB within 5 business days using this form:

Information that does not fall under any of the categories does not require reporting to the IRB.

1) Information that indicates a new or increased risk. For example:
   a. New information (e.g., an interim analysis, safety monitoring report, publication in the literature, sponsor report, or investigator finding) indicates an increase in the frequency or magnitude of a previously known risk, or uncovers a new risk.
   b. An investigator brochure, package insert, or device labeling is revised to indicate an increase in the frequency or magnitude of a previously known risk, or describe a new risk.
   c. Withdrawal, restriction, or modification of a marketed approval of a drug, device, or biologic used in a research protocol.
   d. Protocol violation that harmed subjects or others or that indicates subjects or others might be at increased risk of harm.
   e. Complaint of a subject that indicates subjects or others might be at increased risk of harm or at risk of a new harm.
   f. Any changes significantly affecting the conduct of the research.
2) Any harm experienced by a subject or other individual which in the opinion of the local investigator is **unexpected** and at least **probably related** to the Human Research procedures and suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.
   a. A harm is "unexpected" when its specificity or severity are inconsistent with risk information previously reviewed and approved by the IRB in terms of nature, severity, frequency, and characteristics of the study population.
   b. A harm is "a least probably related to the Human Research procedures" if in the opinion of the local investigator, the research procedures more likely than not caused the harm (greater than 50% probability).
3) Non-compliance with the federal regulations governing human research or with the requirements or determinations of the IRB, or an allegation of such non-compliance.
4) Failure to follow the protocol due to the action or inaction of the investigator or research staff.
5) Breach of confidentiality.
6) Change to the protocol taken without prior IRB review to eliminate an apparent immediate hazard to a subject.
7) Incarceration of a subject in a study not approved by the IRB to involve prisoners.
8) Complaint of a subject that cannot be resolved by the research team.
9) Suspension or termination of the research by the sponsor or the investigator.
10) Unanticipated adverse device effect (Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects).
11) Audit, inspection, or inquiry by a federal agency.
12) Written reports of study monitors.
13) For Veterans Administration (VA) research only: any local or internal serious adverse event or serious problem that is both unanticipated and related to the research.
14) External IRB determination of serious non-compliance, continuing non-compliance, serious and continuing non-compliance, an unanticipated problem involving risk to research subjects or others, suspension or termination of (external) IRB approval. (This RNI category is to be used only when another IRB (external IRB) has made these determinations for this protocol).

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