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

NIAID Protocol Number
17-I-0059

Sponsored by
Office of Clinical Research Policy and Regulatory Operations (OCRPRO), DCR, NIAID, NIH

IND # 17213

Vaccine Manufactured by
SEEK

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Version Number and Date
Version 5.0
February 09, 2018

TEAM ROSTER

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AE	Adverse Event
AR	Adverse Reaction
BMI	Body mass index
CC	Clinical Center
CFR	Code of Federal Regulations
CRF	Case Report Form
CSO	Clinical Safety Office
CSU	Clinical Studies Unit
DCR	Division of Clinical Research
DLM	Department of Laboratory Medicine
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ELISA	Enzyme-linked Immunosorbent Assay
EP	European Pharmacopoeia
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protection Program
ICH	International Council on Harmonization
IFA	Incomplete Freund's adjuvant
Ig	Immunoglobulin
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
IV	Intravenous
LID	Laboratory of Infectious Disease
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
PCR	Polymerase chain reaction
PI	Principal Investigator
RT	Room Temperature
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SCSU	Special Clinical Studies Unit
SERF	Safety Expedited Report Form
SRCP	Safety Review and Communications Plan
SUSAR	Serious, Unexpected, Suspected Adverse Events
Th1	CD4+ T helper (Th1) cells
UP	Unanticipated Problem
UPnonAE	Unexpected Problem that is not an AE
USP	United States Pharmacopeia
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, a Universal Mosquito-Borne Disease and Mosquito Control Vaccine

Short Title:	Universal Mosquito Vaccine
IND Sponsor:	OCRPRO, DCR, NIAID, NIH/ NIAID LID CSU
Conducted by:	LID
Principal Investigator:	Matthew J. Memoli, MD, MS
Sample Size:	45 participants
Accrual Ceiling:	60 participants
Study Population:	Healthy volunteers 18 to 50 years of age
Accrual Period:	1 year
Study Design:	This is a randomized, double-blind, placebo-controlled, single-center, Phase 1 study of AGS-v administered as 2 vaccinations prior to a non-infected Aedes aegypti mosquito feeding.
Study Duration:	The study will begin approximately in December 2016 and will require approximately 3 years to complete. The length of individual subject participation is about 1 year.
Study Agents	1. AGS-v 2. AGS-v + adjuvant 3. Placebo
Intervention Description:	Individuals will be vaccinated subcutaneously with vaccine or placebo.
Primary Objective:	The primary objectives of the study are:

The primary objectives of the study are:

1. To determine the safety of AGS-v as measured by incidence and type of adverse events (AEs) observed

2. To identify an immune response produced by AGS-v by an increase in one or both of the following immune markers:

    a. Total AGS-v specific immunoglobulin from participant serum 21 days after the first and/or second vaccination

      b. Th1 and Th2-associated cytokine release after incubation of peripheral blood mononuclear cells (PBMCs) collected 21 days after the first or second vaccination with AGS-v antigens

Secondary Objectives: The secondary objectives of this study are to identify other measures of immunogenicity including:

1. Measurement of immune response after clean Aedes aegypti mosquito feeding on participants by:

      a. measuring AGS-v specific total immunoglobulin from serum

      b. measuring Th1 and Th2-related cytokines after incubation of PBMCs with AGS-v antigens

2. Measure AGS-v specific immunoglobulins, primarily IgG1, IgG3, and IgM both after vaccination and clean Aedes aegypti mosquito feeding

3. Evaluate the effect of the AGS-v immune response on Aedes aegypti mosquito feeding and fecundity after feeding on a vaccinated individual or whole blood collected from those individuals in vitro.

4. Evaluate the effect of incubating Zika virus coated in vector saliva with PBMCs and/or serum collected from vaccinated individuals.

Endpoints:

Primary:

1. Incidence and severity (by grading) AEs in AGS-v vaccinated individuals

2. Total AGS-v specific immunoglobulin measured in serum 21 days after the first and/or second vaccination.

3. Interferon-gamma and other cytokine markers of Th1 and Th2 response measured in vitro from PBMCs incubated with AGS-v antigens as indicators of the Th1 vs. Th2 response

Secondary:

1. Total AGS-v specific immunoglobulin in acute serum collected immediately after a clean Aedes aegypti mosquito feeding and convalescent serum collected on follow-up visits (60 days later +/- 14 days)

2. Interferon-gamma and other cytokine markers of a Th1 or Th1/Th2 mixed response measured in vitro from acute PBMCs and serum collected immediately after a clean Aedes aegypti mosquito feeding incubated with AGS-v antigens and convalescent PBMCs and serum collected on follow-up visits (60 days later +/- 14 days)

3. Measurement of changes to Aedes aegypti mosquito life cycle post feeding on blood from participants after vaccination both in vivo and in vitro, including survival, egg laying, and other measures of reproduction.

4. Infectivity of Zika virus after incubation with serum and/or PBMCs from vaccinated participants

Précis

Mosquito-borne diseases continue to cause significant morbidity and mortality worldwide despite on-going control efforts. In 2015, there were >200 million cases of malaria worldwide, causing nearly half a million deaths, with most of the deaths occurring among children under the age of 5 years [1]. Mosquitos also transmit arboviruses, including dengue, yellow fever, West Nile virus, chikungunya, Rift Valley fever, Japanese encephalitis, and Zika virus. The current new outbreak of Zika virus 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.

In this protocol we plan to perform a Phase I study of a novel 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 hope to demonstrate the safety of this vaccine similar to SEEK's other peptide based vaccines Flu-v and HIV-v that have been found to have very good safety profiles in previous Phase I trials. We also hope to demonstrate immunomodulation after a controlled clean Aedes aegypti mosquito feeding to demonstrate proof of concept efficacy of the vaccine. With the current rise of Zika in the Americas and the threat of local mosquito transmission in 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 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 on-going vector control efforts. In 2015, there were >200 million cases of malaria worldwide, causing nearly half a million deaths, with most of the deaths occurring among children under the age of 5 years [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 current 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.

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-prong 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 the development of the disease in a healthy individual (prophylactic vaccine) and block the transmission of the disease 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 I study of a 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 the prophylactic and the transmission blocking components. SEEK's approach in developing such a vaccine is defined by the following three principles:

- Because the complexity and variability of the pathogens' life cycle and antigenic make up, the prophylactic effect of the vaccine is not aimed at destroying the parasite itself, but rather to decrease the development of the essential physiologic environment that leads to a successful infection in humans.

- Because the varied number of mosquito species capable of transmitting the pathogens, the transmission blocking effect of the vaccine is not aimed at killing the vector on contact, but rather to prevent the very process of blood feeding, which is essential for the successful procreation of the vector.

- Because most nations where malaria and arboviral diseases are endemic do not have well developed healthcare systems and limited healthcare budgets, the vaccine must be economically viable as a large-scale product entirely manufactured synthetically from raw components, which are widely available, and must not require complex storage and delivery systems.

Female mosquitoes have an absolute physiological need for blood-feeding in order 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 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 Th2 immune response in the host 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 the more effective development of pro-inflammatory Th1 responses [11]. This process is thought to be associated with the gradual acquisition of partial immunity to infection observed in adults living in malaria-endemic regions. Studies in patients infected by *Plasmodium falciparum*

presenting with uncomplicated malaria suggest that an increase of Th1 cytokines (e.g. IL-12 and IFN-γ) in the acute phase provides protective immunity and may also be involved in preventing severe malaria and complications from malaria. [12] Recent studies support this conclusion by showing that 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[13].

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 [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 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 the 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 infection by the pathogen and (b) block transmission of the disease by preventing invasion of the arthropod vector's salivary glands and/or reduce the survival and/or fecundity of the vector.

As indicated earlier, SEEK aims at manufacturing 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 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 the proteins of a salivary gland lysate from *Anopheles gambiae* 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 Fig. 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 the disease.

Figure 1. Graph representing the mosquito % survival post feeding
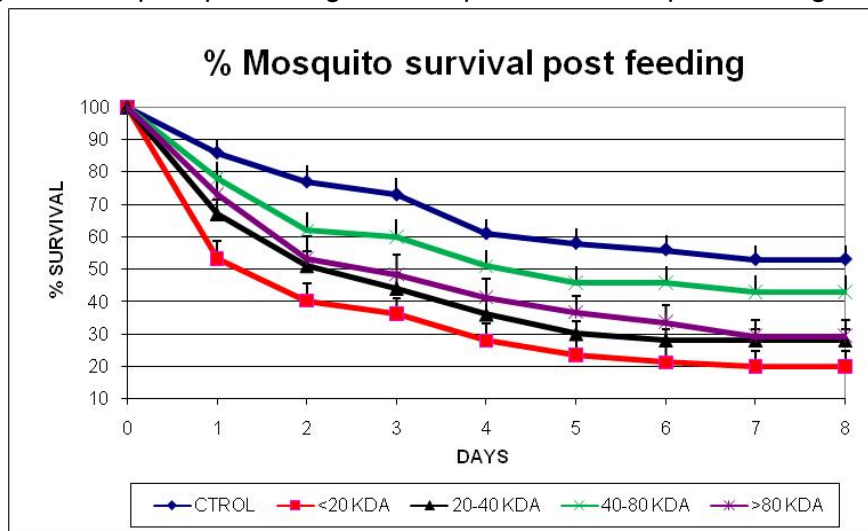
Graph representing the mosquito % survival after feeding on mice immunized with different salivary gland protein fractions (vehicle (control), <20 kDa, 20-40 kDa, 40-80 kDa and >80 kDa). Mosquito salivary glands were dissected and their protein content separated by SDS-PAGE according to their molecular weight. 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 (Fig. 2). 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 transmission rate of the disease.

Figure 2. Effect of immunization with salivary gland protein fractions on mosquito fecundity. (vehicle (control), <20 kDa, 20-40 kDa, 40-80 kDa and >80 kDa) fecundity.
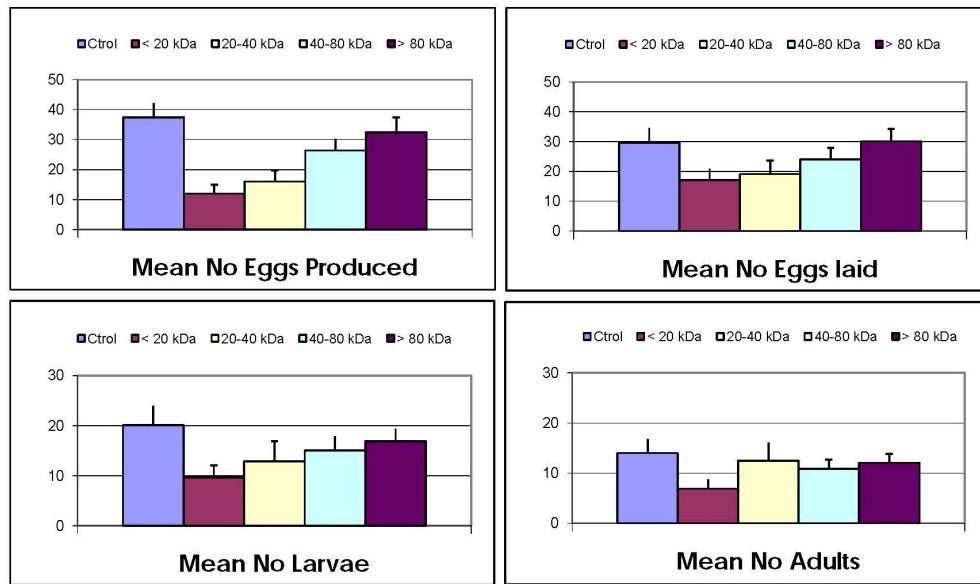
From this point, SEEK proceeded to identify the proteins present in the fractions containing proteins <20 kDa and between 20 and 40 kDa from data available in the GeneBank sequence repository and by 2D-SDS-PAGE analysis of these fractions. Most of these proteins were still too large to be chemically 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 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 SwissProt). Any region showing significant sequence similarity was discarded in order 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 [24] and HIV viruses. The identified sequences constitute two independent candidate vaccine preparations currently undergoing clinical trials in the UK.

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 the saliva of the mosquito vector is associated with an increased 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) and identified as AGS-v (or AGS-mix) were used to immunize mice and to establish the type of induced immune response. While there is no specific reason why four peptides are 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. 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.

Figure 3. IFN-γ response in mice immunized with AGS-mix (AGS-v) or vehicle.
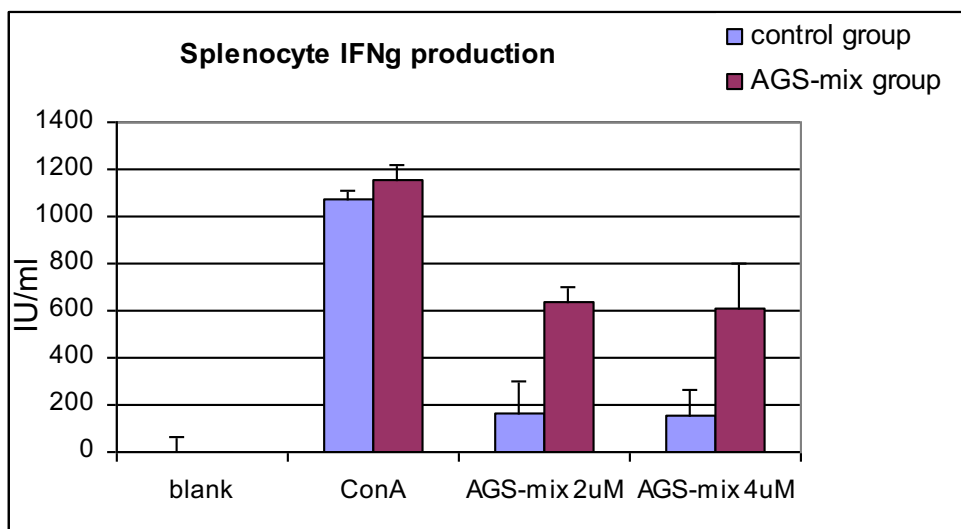
Splenocytes were isolated from mice spleens and incubated with media (blank), a non-specific positive control (Concanavalin A), or different concentrations of the vaccine preparation (2-4uM AGS-mix). After 72h incubation, the levels of IFN-γ (Th1 cytokine) were measured by ELISA.

Figure 4. Total Ig and IgG2a specific-AGS-mix (AGS-v)
response in mouse sera as measured by ELISA.
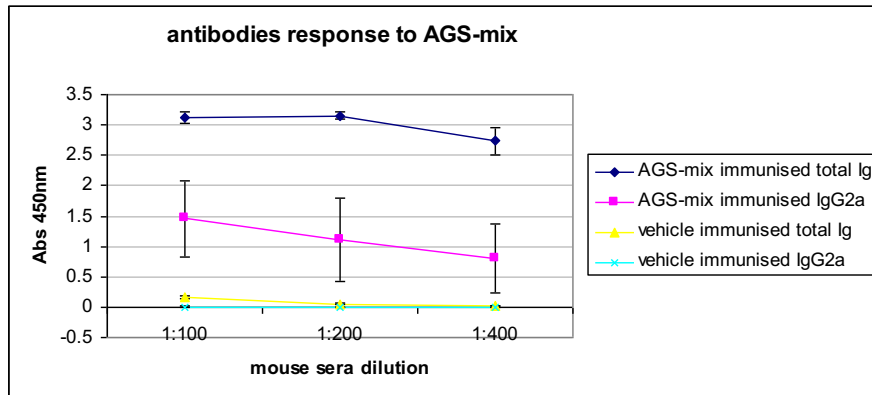
Having established that immunization with AGS-v induces a Th1 response in mice, SEEK wanted to establish whether this response was able to reduce the rate of malaria infection in a mouse model of the disease.

Traditional experimental models of malaria infection rely on the intravenous injection of large numbers of parasites in order 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* show that mice vaccinated with AGS-v are 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.Animals succumbing to infection
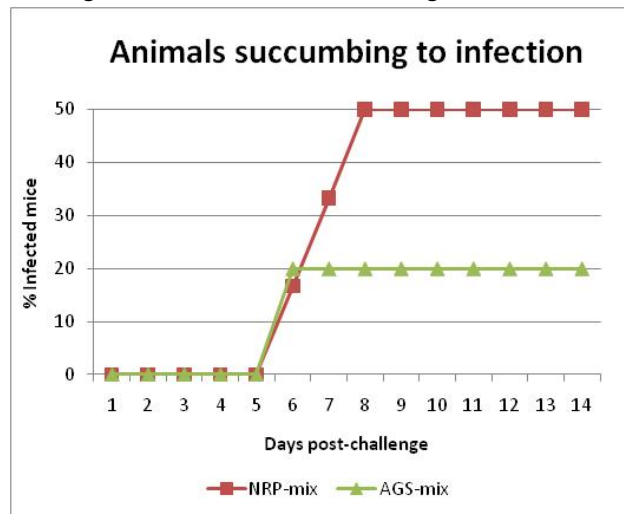
Survival of mice vaccinated with AGS-v vs. mice vaccinated with a control preparation (NRP or non-relevant product) upon challenge via malaria-infected mosquito bite. Groups of 5-6 mice were challenged by bite of infected mosquitoes (low to moderate parasite oocyst burden) on Day 21 post-vaccination (animals received two doses of either AGS-v or NRP-V on Days 1 and 15)

Further experiments in C57BL6, BALBc, and CD1 mice have been performed to measure immunogenicity. All had good responses to the vaccine after two weeks, and antibodies to AGS-v have been raised to the vaccine many months after vaccination and will likely last multiple years. T-cell responses are seen two weeks after vaccination, but are harder to predict long-term.

SEEK has performed clinical studies with two other vaccines using the same technology as AGS-v. Based on their experience with FLU-v and HIV-v in clinical trials it is clear that a combination of four peptides allows the immune system to mount a response without peptides competing with each other. These studies have also assisted in determination of the appropriate dose as 50 nmol of each peptide proved to be the best dose which replicated the ratio nmol/Kg in mouse studies, and therefore was the most immunogenic and safe in humans for these other vaccines. In Phase 1 studies for both HIV-v in HIV-positive males and FLU-v in healthy adults, a dose of 250 ug versus 500 ug with and without adjuvant were given (with approximately 10 people in each arm, for a total of 40 volunteers per Phase 1 study). In the HIV-v Phase 1 trial, either dose induced a significant and sustained antibody response in 60% of volunteers but only the higher 500 ug 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-g response in more than 80% of volunteers in a dose-dependent manner. A FLU-v Phase II study is evaluating the efficacy of a single dose of adjuvanted 500 ug of FLU-v (15 volunteers have received FLU-v vaccine and 12 have received control).

A parenteral tolerance study in New Zealand White Rabbits was conducted to evaluate the local tolerance and irritation potential associated with a single subcutaneous injection of AGS-v.

All animals survived test article or vehicle control formulation administration and appeared active and healthy during the study. Erythema scores were very slight and comparable between Test Formulation 1 (AGS-v in suspension – lyophilized AGS-v reconstituted in 0.5 mL of water for injection) and contralateral Vehicle Control 1 (water for injection). Erythema resolved by the end of the observation period. There was no edema observed at any test site of administration-related findings on macroscopic evaluation.

In this tolerance study with New Zealand white rabbits, the severity of edema, very slight to slight, and erythema, slight to well-defined, scores was comparable between Test Formulation 2 (AGS-v in emulsion – lysophilized AGS-v reconstituted in 0.25 mL of water for injection and emulsified with 0.25 of Montanide ISA-51) and contralateral Vehicle Control 2 (0.25 mL water for injection and adjuvant, 0.25 mL Montanide ISA-51, emulsion); however a slightly lower incidence associated with evidence of resolution at the end of the observation period was observed for Test Formulation 2. These scores correlated with a lower incidence of macroscopic observations for Test Formulation 2.

Under the conditions of this study, a single subcutaneous injection of AGS-v (Test Formulation 1) caused minimal irritation comparable to contralateral vehicle control with full clinical resolution when administered as a suspension in water and minimal to mild irritation when administered as an emulsion using Montanide ISA-51 (Test Formulation 2) with a slightly lower irritation incidence when compared to contralateral vehicle control.

In summary, the data presented here supports the validity of SEEK's scientific hypothesis. This states that immunization with mosquito salivary antigens can induce a Th1 specific response that provides both prophylactic and transmission blocking immunity. Equally importantly, and in contrast to other groups that have shown the ability of vaccination with salivary proteins 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 readily be 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 preparation is a vaccine candidate capable of delivering 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* are responsible for the transmission of more than one pathogen (e.g. dengue, Zika and West Nile virus).

In this protocol we plan to demonstrate the safety of this vaccine similar to SEEK's other peptide based vaccines Flu-v and HIV-v that have been found to have very good safety profiles in Phase I trials. We also hope to demonstrate immunomodulation after a controlled clean Aedes aegypti mosquito feeding as proof of concept efficacy of the vaccine. With the current rise of Zika 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 and malaria that make dealing with this newly emerging epidemic a challenge.

2   Study Objectives

2.1   Primary Objective

The primary objectives of the study are:

1. To determine the safety of AGS-v as measured by incidence and type of AEs observed

2. To identify an immune response produced by AGS-v by an increase in one or both of the following immune markers:

    a. Total AGS-v specific immunoglobulin from participant serum 21 days after the first and/or second vaccination

    b. Th1 and Th2-associated cytokine release after incubation of peripheral blood mononuclear cells (PBMCs) collected 21 days after the first or second vaccination with AGS-v antigens

2.2   Secondary Objectives

The secondary objectives of this study are to identify other measures of immunogenicity including:

1. Measurement of immune response after clean Aedes aegypti mosquito feeding on participants by:

    a. measuring total AGS-v specific immunoglobulin from serum

    b. measuring Th1 and Th2-related cytokines after incubation of PBMCs with AGS-v antigens

2. Measure AGS-v specific immunoglobulins, primarily IgG1, IgG3, and IgM both after vaccination and clean Aedes aegypti mosquito feeding

3. Evaluate the effect of the AGS-v immune response on Aedes aegypti mosquito feeding and fecundity after feeding on a vaccinated individual or whole blood collected from those individuals in vitro.

4. Evaluate the effect of incubating Zika virus coated in vector saliva with PBMCs and/or serum collected from vaccinated individuals.

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 administered at Day 0 and Day 21 prior to Aedes aegypti mosquito challenge at Day 42. Subjects will be randomized 1:1:1 to:

- Group 1: 825 µg (50 nmol each peptide) adjuvanted AGS-v vaccine 2 doses

- Group 2: 825 µg (50 nmol each peptide) non-adjuvanted AGS-v vaccine 2 doses

- Group 3: placebo 2 doses

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.

3.2 Study Endpoints

Primary:

1. Incidence and severity (by grading) AEs in AGS-v vaccinated individuals

2. Total AGS-v specific immunoglobulin measured in serum 21 days after the first and/or second vaccination.

3. Interferon-gamma and other cytokine markers of Th1 response measured in vitro from PBMCs incubated with AGS-v antigens as indicators of the expected Th1 response

Secondary:

1. Total AGS-v specific immunoglobulin in acute serum collected immediately after a clean Aedes aegypti mosquito feeding and convalescent serum collected on follow-up visits (60 days later +/- 14 days)

2. Interferon-gamma and other cytokine markers of a Th1 or Th1/Th2 mixed response measured in vitro from acute PBMCs and serum collected immediately after a clean Aedes aegypti mosquito feeding incubated with AGS-v antigens and convalescent PBMCs and serum collected on follow-up visits (60 days later +/- 14 days)

3. Measurement of changes to Aedes aegypti mosquito life cycle post feeding on blood from participants after vaccination both in vivo and in vitro, including survival, egg laying, and other measures of reproduction.

4. Infectivity of Zika virus after incubation with serum and/or PBMCs from vaccinated participants

4   Study Population

4.1   Rationale for Participant Selection

Participants will be carefully selected using the inclusion and exclusion criteria described here to select the optimum participants for completing the study objectives and minimize the risk of AEs. NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH information sheet on Employee Research Participation."

For NIH employees:

- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation.

- The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees.

- The employee subject's privacy and confidentiality will be preserved in accordance with NIH Clinical Center and NIAID policies, which define the scope and limitations of the protections.

- For NIH employee subjects, consent will be obtained by an individual independent of the employee's team. Those in supervisory position to any employee and co-workers of the employee will not obtain consent.

- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

4.2   Recruitment Plan

Participants will be recruited through the screening study #11-I-0183 "Screening of Volunteers for Influenza Human Challenge and Vaccine Studies". Participants will be carefully screened and evaluated, and those who meet the study eligibility criteria will be contacted and given the opportunity to be enrolled into the study. A staggered enrollment procedure will be followed with the initial enrollment phase to include 6 patients in the first cohort. These will be block randomized so that 2 receive placebo, 2 vaccine, and 2 vaccine plus adjuvant. If no pausing or halting criteria are met per the safety evaluations described in Section 12 Assessment of Safety for these initial 6 patients one week after the second vaccine administration (~Day 28), the following cohort will include 9 patients. Then, if no pausing or halting criteria or safety concerns arise by one week after the second vaccine administration (~Day 28), the remaining 30 patients will be enrolled in appropriately sized cohorts with 15 patients in each arm to complete the study efficiently.

If a participant has completed the screening study #11-I-0183 more than 60 days prior to Day 0 of this study, they will be asked to come to the NIH Clinical Center for another screening visit under the screening study #11-I-0183 within 60 days prior to the date of vaccination to repeat HIV testing and complete any laboratory or other testing as deemed necessary by the investigator to ensure that it remains safe for the participant to take part in this study. All eligible participants will be consented and enrolled for this study only after completion of all necessary screening studies under protocol #11-I-0183 a maximum of 60 days prior to vaccine administration.

The screening visit will take place prior to Day 0 (Day of vaccine/placebo administration).

4.3   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 subject 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 prior to study initiation through 12 weeks after the second vaccine administration. Acceptable methods of contraception include 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 (including genetic research).

6. Agrees to abstain from alcohol intake for 24 hours prior to each study visit.

7. Agrees to not donate blood or blood products throughout the study.

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) baseline Grade 1 or greater toxicity, or any Grade 3 or greater toxicity (regardless of clinical significance) by the toxicity table.

4. Receipt of blood or blood products (including immunoglobulins) within 3 months prior to enrollment.

5. Receipt of any unlicensed drug within 3 months or 5.5 half-lives (whichever is greater) prior to enrollment.

6. Receipt of any unlicensed vaccine within 6 months prior to enrollment.

7. Self-reported or known history of alcoholism or drug abuse within 6 months prior to enrollment, or positive urine/serum test for drugs of abuse at screening

8. Self-reported or known history of psychiatric or psychological issues that require treatment and are deemed by the PI to be a contraindication to protocol participation.

9. History of a previous severe allergic reaction with generalized urticaria, angioedema, anaphylaxis or anaphylactoid reaction.

10. Any condition or event that, in the judgment of the PI, is a contraindication to protocol participation or impairs the volunteer's ability to give informed consent.

11. Has a known allergy to any of the components of the vaccine.

12. Has a history of severe immunization reaction.

13. Has a severe allergic reaction to mosquito bites

Co-enrollment Guidelines: Co-enrollment in other trials is restricted, but may take place with the approval of the PI and after study staff notification.

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 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 reproductive safety tests.

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.

5 Study Agents/Interventions

5.1 Agent 1: AGS-v, AGS-v + Adjuvant, or Placebo

5.1.1 Formulation, Packaging and Labeling

AGS-v contains four drug substances: AGS-6, AGS-30, AGS-31, and AGS-35. These are peptides consisting of 32 to 44 amino acids. They are obtained by chemical synthesis and are available as acetate salts. The peptides contain multiple T-cell reactive regions capable of inducing Th1 responses.

A solution of the four drug substances (50 nmol net peptide/g of each drug substance) is prepared by completely dissolving the drug substances in water for injection (WFI). The four solubilized peptides are mixed to make up AGS-v solution. This mix is filter-sterilized before filling glass vials automatically with the sterile AGS-v solution. The pharmaceutical formulation solution is filled into glass vials by weight which results in 0.824g of AGS-v solution per vial which is then lyophilized before closing with a rubber stopper and a flip-off cap. The lyophilization removes both the water component; whereupon a relatively neutral pH, suitable for human injection, is achieved by reconstitution.

Bottle labels will bear the appropriate label text 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   Study Agent Storage and Stability

The vials of WFI and Montanide ISA-51 should be stored at room temperature. Stability will be performed to cover the duration of the trial. Stability studies of previous batches of another vaccine Flu-v indicate that it should remain stable for 2 years when stored at -15 to -25°C which should be the case for the AGS-v vaccine also. None of the materials can be dispensed beyond the expiration date.

5.1.3   Adjuvant

The adjuvant is Montanide ISA 51, also known as incomplete Freund's adjuvant (IFA). This 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 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]

This version of IFA , Montanide ISA 51, is highly purified and avoids having impurities and high levels of fatty acids that have lead to toxicities with other versions of 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]

5.1.4   Placebo

The placebo contains water for injection, which is the final product used as placebo.

5.1.5   Preparation

- Please see Pharmacy Manual Section 2.3.
- Two members of the study staff, Dr. Alison Han (associate investigator) and Lindsay Czajkowski (NP), will perform emulsification and blinding of all syringes and accountability for the AGS-v study agent. Therefore, these two

members of the study staff will be unblinded and will not participate in any other aspects of the study or patient care.

- Once prepared and drawn into the syringe, it should be administered within 3 hours.
- Once emulsification of the adjuvant and vaccine occurs, it should be administered within 15 minutes.

5.2   Dosing and Administration

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

- Group 1: AGS-v (unadjuvanted) as a suspension in WFI (0.5 mL) on Day 0 and on Day 21
- Group 2: ISA-51-adjuvanted AGS-v emulsified in WFI (0.5 mL) on Day 0 and on Day 21
- Group 3: WFI (0.5 mL) on Day 0 and Day 21

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

The person administering the vaccine will be blinded to the type of formulation (adjuvanted or not adjuvanted) because an opaque label will be placed over the syringe by the pharmacy. The NIH Special Clinical Studies Unit (SCSU) or other appropriate clinical unit nurses will administer the vaccines. No member of the study team will be administering the blinded study product.

Administration of the vaccine will be performed following these instructions as detailed in the NIH Clinical Center subcutaneous vaccine administration guidelines[29]:

To locate the injection site on the arms, fold one arm across the chest. Place your hand on the shoulder and draw an imaginary line below your hand. Place another hand on the elbow. Draw an imaginary line down the outer side of the arm and down the center front of the arm, starting at the elbow. The area inside these imaginary lines is where injections are given.

Cleanse the area of the skin thoroughly before a needle is inserted. Cleanse the skin with a back and forth motion using an alcohol swab. This motion moves bacteria away from the injection site. Allow the alcohol to dry completely by air.

- Take the cover off the needle. Be careful not to contaminate the needle. Place the cover on its side. Hold the syringe in one hand like pencil or a dart. Grasp the skin between the thumb and index finger with your other hand and pinch up.
- Quickly thrust the needle all the way into the skin. Do not "push" the needle into the skin slowly or thrust the needle into the skin with great

force. Do not press down on the top of the plunger while piercing the skin.

- Insert the needle at a 90° (right) angle. This angle is important to ensure that the medications will be injected into the fatty tissue. However, for persons with little subcutaneous fat on thin skin, you may be taught to use a 45° angle.
- After the needle is completely inserted into the skin, release the skin that you are grasping. Press down on the plunger to release medication into the subcutaneous layer in a slow, steady pace. Count 10 seconds before removing the needle from the skin.
- As the needle is pulled out of the skin, gently press a gauze pad onto the needle insertion site. Pressure over the site while removing the needle prevents skin from pulling back, which may be uncomfortable. The gauze also helps seal the punctured tissue and prevents leakage.

It is not serious if you notice blood at the site after the needle is removed. You may have nicked a surface blood vessel when you injected, and blood is following the needle track out to the surface. Simply press the site with a 2 x 2 gauze pad. Also, a small amount of clear fluid may appear at the site. This may be medication that is following the needle track to the surface. Again, apply pressure using a 2 x 2 gauze pad.

5.3   Disposal and Drug Accountability of AGS-v

5.3.1 Product Accountability

The Clinical Center pharmacy 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 eCRFs and in the Drug Administration Record form. The latter includes the subject identification, quantity (volume) and date of administration. The containers from which the vaccine was administered to the subjects 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 and that no study supplies remain in the Investigator's possession.

All unused vaccine supplies, partially used and empty containers will be returned to the drug supplier.

5.3.2   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 investigational product (IP) accountability, reconciliation and record maintenance. In accordance with all applicable regulatory requirements, the investigator or designated site staff must maintain investigational product accountability records throughout the course of the study. The investigator or designated site staff will document the amount of investigational product received from SEEK and the amount administered to subjects.

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

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

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 or returned by the investigator or designee to SEEK. When requested in writing by the drug supplier, unused drug supplies may be destroyed by the investigator provided such disposition does not expose humans to risks from the drug. Records will be maintained by the investigator of any such alternate disposition of the test drug. These records must show the identification and quantity of each unit disposed of, 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.

5.3.3   Retention of Samples

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

5.4   Concomitant Medications and Procedures

All concomitant prescription medications, over-the-counter medications, or herbal remedies taken during study participation must be approved by the PI and will be recorded in the participant's source documents. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

6   Study Schedule

This study will take place at the NIH Clinical Center. All aspects of the protocol will be carried out in accordance with NIH guidelines, GCP and ICH involving human-participant research. Please see Figure 6 below.

Figure 6. Study Schedule

6.1 Randomization and Blinding

Randomization will occur using computer generated randomization codes. This code will be sent to the pharmacy where the unblinded pharmacist will have the key and prepare the appropriate agent for administration. All vaccination vials will be labeled and look identical so the blinded staff cannot identify placebo v AGS-v vaccine doses.

Under normal circumstances, the blind should not be broken until all participants have completed the study and the database is finalized. 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 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.

In general, randomization codes will be disclosed fully only if the study is completed and the clinical database is closed. However, for the interim analysis planned for this study unblinding will occur when the interim database is locked. This unblinding is limited to the study statistician and Data Safety Monitoring Board (DSMB).

6.2 Randomization and Vaccine or Placebo Administration (Day 0)

The first vaccine or placebo administration will take place on Day 0. This visit will occur in the SCSU or other appropriate CC unit/clinic. The research team will thoroughly discuss the consent with the volunteer prior to 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 of AGS-v +adjuvant 0.5 mL dose, AGS-v 0.5 mL dose, or placebo. All participants will be educated by study staff on prevention of mosquito bites while participating in study including, but not limited to, minimizing skin exposure with long sleeves/pants and the use of repellent.

The following procedures will be performed after informed consent is obtained:

- Review of medical/medication history
- Documentation of usual responses to mosquito bites as none (0), mild (1), moderate (2), or severe (3)
- Physician exam/assessment
- Vital signs including weight pre vaccination
- Vital signs (+/- 15 minutes) post vaccination at 30 minutes, 60 minutes, and 120 minutes
- Blood collection for routine safety labs (as per Appendix A), total serum IgE, as well as serum/whole blood for research (as per Appendix B).
- Routine urinalysis
- Serum pregnancy test (females only)
- Review of inclusion/exclusion criteria
- Vaccine or placebo administration
- Required 2 (two) hour observation time (post vaccine injection) at the clinical research site
- Vaccine injection site and AE assessment pre-vaccination, 60 minutes and 120 minutes post vaccination (+/- 15 minutes)
- Any other clinical tests that are medically indicated or appropriate to ensure the safety of the individual participant as deemed by the PI
- Diary card, thermometer, and ruler will be distributed prior to discharge home

6.3 Day 7 (+/- 2 days)

- Clinic visit
- Review of medical/medication history
- Physician exam/assessment
- Vital signs including weight
- Blood collection for routine safety labs
- Routine urinalysis
- Serum pregnancy test (females only)
- Vaccine injection site assessment
- Review of diary card

6.4 Day 14 (+/- 2 days)

- Clinic visit
- Review of medical/medication history
- Physician exam/assessment
- Vital signs including weight
- Blood collection for routine safety labs

- Routine urinalysis
- Serum pregnancy test (females only)
- Vaccine injection site assessment

6.5 Day 21 (+/- 2 days)

This visit will take place in the SCSU or other appropriate CC unit/clinic. 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
- Physician exam/assessment
- Vital signs and including weight
- Vital signs (+/- 15 minutes) post vaccination at 30 minutes, 60 minutes, and 120 minutes
- Blood collection for routine safety labs as well as serum/whole blood for research
- Routine urinalysis
- Serum pregnancy test (females only)
- Vaccine or placebo administration
- Required 2 (two) hour observation time (post vaccine injection) at the clinical research site
- Vaccine injection site and AE assessment pre-vaccination, 60 minutes and 120 minutes post vaccination (+/- 15 minutes)
- Any other clinical tests that are medically indicated or appropriate to ensure the safety of safety the individual participant as deemed by the PI
- Diary card, thermometer, and ruler will be distributed prior to discharge home

6.6 Day 28 (+/- 2 days)

- Clinic visit
- Review of medical/medication history
- Physician exam/assessment
- Vital signs including weight
- Blood collection for routine safety labs
- Routine urinalysis
- Serum pregnancy test (females only)
- Vaccine injection site assessment
- Review of diary card

6.7 Day 35 (+/- 2 Days)

- Clinic visit
- Review of medical/medication history
- Physician exam/assessment

- Vital signs including weight
- Blood collection for routine safety labs
- Routine urinalysis
- Serum pregnancy test (females only)
- Vaccine injection site assessment

6.8   Day 42 Mosquito Feeding (+14 days)

This will take place in the SCSU or other appropriate CC unit. The following procedures/assessments and testing will be performed:

- Review of medical/medication history and addition of any new information
- Physician assessment and physical exam
- Vital signs including weight
- Routine urinalysis
- Placement of IV catheter in arm
- Serum pregnancy test (females only)
- Blood collection for routine safety labs and serum/whole blood for research prior to mosquito feeding.
  - Per Appendix B: serum 8 ml tube x 1, whole blood 8.5 ml tube x 10, and Paxgene 2.5 ml x1
- Mosquito feeding:
  - Starved female Aedes aegypti mosquitos will be selected from a mosquito colony approved for human feeding studies in Laboratory of Malaria and Vector Research (LMVR), NIAID. 5 -10 female mosquitoes will be secured in the feeding device prior to feeding and brought to the CC from the LMVR Insectary.
  - 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 20 minutes. The mosquitos will feed through a disposable mesh on the bottom of the feeding device.. In the unlikely event of no feeding or poor feeding (only 0-2 mosquitoes fed or probed as noted by trained staff), the volunteer may undergo a repeat feed with 5-10 fresh mosquitoes ONCE.
  - Participants will be asked not to apply cream, perfume or deodorant the day of mosquito exposure. Once the mosquitos have fed they will remain in the same device and be brought back to the lab for further study.
- The skin will be inspected pre-feeding. Redness, swelling and number of visible bites will be recorded pre-feeding, immediately post feeding and 30 minutes post feeding (+/- 15 minutes)
- Research blood collection (+/- 15 minutes) after mosquito feeding will be collected at:
  - 30 minutes: whole blood (8.5 mL tubes x 5), serum (8 ml tube x 1), and 2.5 mL in Paxgene tube
  - 60 minutes: 2.5 mL for Paxgene tube
  - 120 minutes: 2.5 mL Paxgene tube

- 180 minutes: whole blood (8.5 mL tubes x 5), serum (8 ml tube x 1), and 2.5 mL in Paxgene tube
- Any other clinical tests that are medically indicated or appropriate to assure the safety of the individual participant as deemed by the PI.
- AE assessment

6.9   Day 44 Telephone Call (+/- 1 day)

A brief telephone call will be made on Study Day 44 to elicit general state of health and any adverse events. The volunteer will report the size of redness and swelling on their arm at 48 hours.

6.10   Follow-up

All participants will be followed for a minimum of 10 months after completing Day 42 of the study. 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 in the clinic approximately every 60 days (±14 days) for the next 5 months or more often if deemed medically necessary; the procedures/evaluations to be conducted at these visits are shown in Appendix A. The 10-month post Day 42 visit will be the final study visit 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.

7.1.1   Laboratory Testing of Collected Samples for Primary and Secondary Endpoints

- Primary Endpoints:
  - o AGS-v specific immunoglobulin assay will be performed using enzyme-linked immunosorbent assay (ELISA).
  - o Th1 and Th2 response assays: PBMCs collected from participants will be incubated with AGS-v and measurements of various Th1 and Th2 cytokines will be performed using Luminex and/or ELISA
- Secondary Endpoints:

- o AGS-v specific immunoglobulin and interferon-gamma assay will be performed as stated above on samples collected after *Aedes aegypti* mosquito feeding occurs.

- o Specific IgG subclasses will be measured using ELISA

- o Mosquito Studies:

  - ▪ Feeding on human volunteers**:** 24 hour after emergence from pupae, male and female *Aedes aegypti* mosquitoes will be allowed to mate during 24-48 hour. After this period, female mosquitoes will be kept separated until human exposure. 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 42 (approximately 21 days after they have completed the vaccination schedule). 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 but on non-mammalian blood to minimize the presence of blood borne pathogens in them.

- o Zika virus killing, in vitro toxicity assay:

  - ▪ Blood samples will be obtained from all volunteers 21 days after vaccination is completed. PBMCs+sera containing immune cells and antibodies generated against AGS-v will be tested for their ability to kill Zika virus mixed with salivary gland proteins from *Aedes aegypti*. This test will be carried out in 96 well plates.

  - ▪ Salivary gland proteins will be obtained after dissecting the salivary glands from female *Aedes aegypti*, sonicating them and centrifuging the sonicate to obtain the supernatant containing all the mosquito salivary proteins.

  - ▪ Zika virus will be obtained from a commercial supplier (ATCC® VR-1838). This strain can be propagated in vitro by infecting VERO cells. Cells are plated 18-24 hours prior to infection and infected when cultures are 75-80% confluent. The medium is removed, cell monolayer is washed with PBS or serum free medium prior to inoculation with a small volume of inoculum (e.g. 1 mL per 25 cm^2) diluted to provide an optimal MOI (e.g. 0.1-0.01). The inoculum is left to adsorb for 1-2 hours at 37°C in a humidified 5% CO_2 atmosphere. End adsorption by adding virus growth medium. Cells are incubated for 2-4 days at 37°C in a humidified 5% CO_2 atmosphere, until cytopathic effect is progressed through 80-90% of the monolayer. At this point the propagated virus is collected.

  - ▪ In order to assess that the AGS-v specific immune responses have killed the Zika virus, the PBMC+Zika+salivary protein cultures will be spun to remove any cells. Any viable Zika virus will be found in the

supernatant which will be used to attempt re-infection of VERO cells and/or PCR. If infection is not successful and/or PCR is negative it will be an indication that the virus was destroyed.

7.2   Reactogenicity

On a daily basis during the 7-day period after each injection subjects will evaluate and record temperature, any local (redness, swelling, and pain) or general symptoms (e.g., fever, chills, headache, myalgia, arthralgia, sweats, fatigue, nausea, vomiting) they experience on the diary card. The diary card will be brought to each clinic visit. The diary cards will be reviewed and evaluated for AEs.

The diary card will be completed by the subject in the evening. Subjects will be instructed on the use and bring it to every clinic visit. The diary card will be reviewed by the study team during the post injection follow up visits. Diary cards will be collected from subjects, and retained as source documentation. If diary cards are not completed or returned, the study team will review the AEs that occurred during that timeframe to the best of the subject's recollection.

8   Potential Risks and Benefits

8.1   Risks of Placebo administration

Subcutaneous injection of water for injection (WFI) poses minimal risk. The primary risks of the placebo vaccinations are a local vaccine reaction or infection at the site of injection. Signs of a local vaccine 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 drainage at the site.

8.2   Risks of AGS-v ± 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. These reactions can include severe anaphylactic reactions and seizure. Primarily topical symptomatic therapy will be administered if necessary to treat vaccine reactions including cold compresses, topical antipuriritics and pain relievers. Additional standard of care therapy for pain or anaphylaxis will be administered if the reaction becomes

severe or life threatening. This therapy includes but is not limited to analgesics, epinephrine, diphenhydramine, hydroxyzine, and systemic steroids.

SEEK has completed clinical trials in the EU for the FLU-v and HIV-v vaccines. The technology utilized for peptide identification and vaccine development for the Flu-v and HIV-v vaccines is the same technology utilized for peptide identification and vaccine development for the AGS-v vaccine. FLU-v, HIV-v and AGS-v all utilize the same adjuvant, Montanide ISA-51. Therefore, we expect similar safety findings for AGS-v as was observed with FLU-v and HIV-v. Minor vaccine reactions have been observed, but no severe vaccine reactions have been observed in any participants receiving either of these vaccines. Safety results from the FLU-v and HIV-v studies are presented below and no safety concerns were identified during those Phase I trials.

In the Phase 1a Study Flu-v-001, no deaths occurred and only 1 SAE was reported with this being considered unrelated to FLU-v (appendicitis). There was no apparent increase in AEs with a change in dose from the 250 µg or 500 µg (500 µg of FLU-v is the equivalent of 825 µg of AGS-v as both contain 50 nmol of each peptide), but an approximate 2-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; however, the forearm site and 1 mL volume is suspected to be the cause rather than the adjuvant itself. Montanide ISA-51 has been used in cancer vaccines and local reactogenicity has not been documented to be greater than moderate or Grade 2. Case reports of erythema nodosum and sterile abscesses have also been documented in vaccines adjuvanted with Montanide ISA-51[30-34], but these were not observed in the FLU-v trials of healthy volunteers.

In the Phase 1b Study Flu-v-002, all 32 subjects 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 Montanide ISA-51 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, ECG).

In the Phase 1b Study HIV-v-001 performed in HIV positive patients not healthy volunteers, all 59 subjects 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 subjects with 3 SAEs occurring in the 1 subject (lower abdominal pain, shigella infection, and gastroenteritis) 37 days prior to vaccination, 31 and 128 days after vaccination respectively. The other 2 SAEs (perianal abscess with streptoccacl infection and Kaposi's sarcoma) occurred at 101 and 56 days after vaccination. None of the SAEs were considered to be related to the study drug. No subjects died and no subject discontinued the study due to an AE.

8.3   Risks of adjuvant

Given the use of Montanide ISA-51 in our trial and its theoretical potential for induction of autoimmune disease, we will monitor for the following list of potentially immune-mediated medical conditions for 12 months after vaccination.

We will monitor for the occurence of an unexpected event of a vaccine-related immune-mediated condition by performing interim history at each interval follow-up visit, thorough review of systems of new symptoms, physical exams, and blood work to include comprehensive metabolic panel and complete blood count with differential. If necessary as based on history or exam, we may refer subjects for evaluation or send additional tests such as but not limited to c-reactive protein, thyroid stimulating hormone, hemoglobin A1c, cryoglobulins, ANCA, and rheumatoid factor. Below is the provided list of immune-mediated conditions that will be monitored.

Gastrointestinal disorders
- Celiac disease
- Crohn's idseas
- Ulcerative colitis and proctitis

Liver disorders
- Autoimmune cholangitis
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

Metabolic diseases
- Addison's disease
- Autoimmune thyroiditis
- Diabetes mellitus type 1
- Grave's disease

Musculoskeletal disorders
- Antisynthetase syndrome
- Dermatomyositis
- Juvenile chronic arthritis
- MCTD
- Polymyalgia rheumatica
- Polymyositis
- Psoriatic arthropathy
- Relapsing polychondritis
- Rheumatoid arthritis
- Sclerderma (including CREST And systemic diffuse form)
- Spondyloarthritis
- Systemic lupus erthematosus
- Systemic sclerosis

Neuroinflammatory disorders

- Acute disseminated encephalomyelitis
- Cranial nerve disorders
- Guillain Barré syndrome
- Immune-mediated peripheral neuropathies and plexopathies
- Multiple sclerosis
- Narcolepsy
- Optic neuritis
- Transverse myelitis
- Myasthenia gravis

Skin disorders
- Alopecia areata
- Autoimmune bullous skin disease such as pemphigus, pemphigoid, and dermatitis herpetiformis
- Cutaneous lupus erythematosus
- Erythema nodosum
- Morphoea
- Lichen planus
- Psoriasis
- Sweet's syndrome
- Vitiligo

Vasculitides
- Large vessel vasculitides such as giant cell arteritis and Takayasu's arteritis and temporal arteritis
- Medium and small sized vessels: polyarteritis nodosa, Kawasaki's, miscrosocpic polyangiitis, Wegener's granulomatosis, Churg-Staruss, Buerger's, Henoch Schonlein purpeura and Behcet's

Others
- Antiphospholipid syndrome
- Autoimmune hemolytic anemia
- Autoimmune glomerular nephritis
- Autoimmune myocarditis/cardiomyopathy
- Autoimmune thrombocytopenia
- Goodpasture syndrome
- Idiopathic pulmonary fibrosis
- Pernicious anemia
- Raynaud's phenomenon
- Sarcoidosis
- Sjogrens' syndrome
- Steven-Johnson syndrome
- Uveitis

8.4   Risks of blood draw

Risks of blood draw include pain, bruising, bleeding, and rarely fainting or infection. The amount of blood drawn will be within the limits allowed for adult subjects by the NIH

Clinical Center (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf). Exceptions to this limit will be submitted to the Institutional Review Board (IRB) for prior approval.

8.5   Risks of Aedes aegypti 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   Potential Benefits

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 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. Only investigators 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 (both at NIH and outside) may wish to study these samples and/or data. In that case, IRB approval must be sought prior to 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 and data will either be destroyed, or after IRB approval, transferred to another existing protocol.

Reporting the 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. The PI will also notify the IRB if the decision is made to destroy the samples.

- Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator 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 NIH.

10   Data Sharing Plan

10.1   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 BTRIS.
- De-identified or identified data with approved outside collaborators under appropriate agreements.

10.2   Data will be shared through:

- An NIH-funded or approved public repository. Insert name(s): ClinicalTrials.gov____
- BTRIS.
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

10.3   The data will be shared:

- At the time of publication or shortly thereafter.

11   Remuneration Plan

Participants will be compensated according to Table 1. Participant remuneration

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Table 1. Participant remuneration

Day 0	$200
Day 7 (± 2 days)	$70
Day 14 (± 2 days)	$70
Day 21 (± 2 days)	$200
Day 28 (± 2 days)	$80
Day 35 (± 2 days)	$80
Day 42 (+ 14 days)	$400
Follow up visits ~60 days (± 14 days) X5	$60 x 5=$300

Expected total for completion of ALL study visits	*$1,400*
Study-requested interim visits:	$75

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 CRIMSON

- Reported as outlined below (e.g., 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 principal investigator 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
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.

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

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. An 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: 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 an 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.)

UP that is not an AE (UPnonAE)

A 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 the FDA "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" September 2007, which can be found at: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/ Guidances/Vaccines/ucm074775.htm

Microscopic hematuria will be graded using the above toxicity table except for the Grade 1 values, which are being adjusted to be consistent with the CC Department of Laboratory Medicine normal laboratory values. Grade 1 will be >3-10 RBC/HPF instead of 1-10 RBC/HPF listed in the FDA toxicity table.

All lab and vital sign abnormalities found prior to 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
- 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

*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) and sent to the Sponsor Clinical Safety Office (CSO) by fax or e-mail attachment. 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. 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.
- Unblind the subject.

- Report to the DSMB and the IRB.
- Advise the research subject to notify the obstetrician of study participation and study agent exposure.

12.5 Investigator Reporting Responsibilities to the NIAID IRB

12.5.1 Special Reporting Situations

Safety events of interest that may require expedited reporting 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 subject/patient exposure to the study drug, e.g., name confusion)

Special reporting situations should be recorded. Any special reporting situation that meets the criteria of a serious adverse event should be recorded as a serious adverse event and reported as described in Section 12.4.2.

12.5.2 Expedited Reporting to the NIAID IRB

Serious and non-serious UPs, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. SAEs that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 days of site awareness, regardless of expectedness.

12.5.3 Waiver of Reporting Anticipated Protocol Deviations, Expected UPnonAEs and Deaths to the NIAID 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.

12.5.4 Annual Reporting to the NIAID IRB

The following items will be reported to the NIAID IRB in summary at the time of Continuing Review:

- Serious and non-serious UPs
- Expected SAEs that are possibly, probably, or definitely related to the research
- SAEs that are not related to the research
- All AEs, except expected AEs granted a waiver of reporting
- Serious and non-serious protocol deviations
- Serious, continuing, and minor non-compliance

- Any trends or events which in the opinion of the investigator should be reported
- A summary of accumulated safety data

12.6   Follow-Up of AEs and SAEs

AEs that occur following enrollment of the participant (by signing the informed consent) will be followed until the final outcome is known or until the end of the 10-month 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 CRIMSON and on the SERF.

SAEs that occur after the 10-month study follow-up period 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).

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 Subject

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

The pausing criteria for a single subject in this study include any of the following:
- A subject experiences an SAE that is possibly, probably, or definitely related to a study agent;
- A subject 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 subject.

The CSO, in collaboration with the principal investigator, 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 principal investigator within 1 business day 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 principal investigator and/or the relevant DSMB, will determine whether or not it is safe to resume administration of the study agent to the subject. The principal investigator will notify the IRB of the decision on resumption of the study agent.

A subject 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 subjects 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 subjects 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 subjects that are grade 3 or above and are possibly, probably, or definitely related to the study agent;

OR
- any safety issue that the principal investigator and/or the CSO determines should halt the study.

The principal investigator 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 principal investigator within 1 business day to the 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 principal investigator and the DSMB will determine if it is safe to resume the study. The principal investigator will notify the IRB of the decision on resumption of the study.

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

12.10 Study Discontinuation

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 prior to 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 subject will be withdrawn for any of the following:
- An individual subject's decision. (The investigator should attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- A change in the subject's baseline condition after enrollment so that the subject 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 subject.

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. If a participant does not receive 2 vaccinations, but does not withdraw from the study, that participant's data may be used in the analysis, but an additional participant may be enrolled who qualifies and consents to the study to receive 2 vaccinations. This may require the overall sample size to be larger than 45 participants, but the number to have received two vaccinations will be 45 participants. 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 DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The DSMB will review the study prior to initiation, after the first group of participants has been vaccinated, and at least twice a year thereafter. The Board may convene additional reviews as necessary. The board will review the study data to evaluate the safety, study progress, and conduct of the study. All UPs and all IND Safety Reports will be reported by the PI to the DSMB at the same time they are submitted to the IRB or IND Sponsor. All SAEs that are possibly, probably or definitely related to the study agent will be reported to the DSMB within 1 business day after the site becomes aware of the event. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB.

13   Site Monitoring Plan

According to the International Council on Harmonization (ICH) Good Clinical Practice (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 "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO 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 and 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 principal investigator and study staff prior to 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 specific total immunoglobulin will increase after vaccination with AGS-v or adjuvanted AGS-v and/or PBMCs collected after vaccination incubated with AGS-v antigens will show a greater Th1 response as measured by interferon-gamma as compared to placebo vaccination.

14.2   Sample Size Justification and Analysis Plan

The sample size for this study is based on the primary comparison of total immunoglobulin and interferon-gamma release with each of the experimental vaccine arms to placebo vaccine. 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 subjects than necessary to an experimental vaccine but we also don't

wish to miss an active vaccine we will design the study to have 90% power with a 1-sided type I error rate of .1. We also will not adjust for the 3 multiple comparisons (all 3 arms being compared).

With 15 subjects per arm, there will be 90% power to detect a true difference in the primary efficacy endpoint of .95 standard deviations using t-test with significance level of .1 (one sided).

For the safety endpoint we are interested in identifying AEs that may occur with the vaccines. With 15 patients in each group we would have a probability of .9 of observing at least one AE if the true underlying rate for that AE in a vaccinated arm is 0.14. If we collapse across the vaccinated arms we would have 30 patients. With 30 patients we would have a probability of .9 of observing at least one AE if the true rate is 0.075. With this sample size we will have a high probability of beginning to see an adverse safety signal if one exists. Table 2 shows the probability of observing at least 1 AE for various true AE rates with samples sizes of 15 and 30.

Table 2. Probability of observing at least one AE.

True rate of adverse events	Probability of observing at least one AE	
	N=15	N=30
0.01	0.14	0.26
0.02	0.261	0.455
0.03	0.367	0.599
0.04	0.458	0.706
0.05	0.537	0.785
0.06	0.605	0.844
0.07	0.663	0.887
0.08	0.714	0.918
0.09	0.757	0.941
0.1	0.794	0.958
.14	0.900	0.989

With 15 patients per arm, there is low probability of being able to detect differences in adverse events rates between groups unless the differences are very large. But these comparisons will be made between the 3 arms of the study. In order to gain some power, the 2 experimental arms will also be collapsed and the AE event rates will be compared to the placebo arm. In order to be conservative and not miss a difference in significant safety issues, we will perform tests at the 1-sided .05 level without controlling for multiple comparisons. A Fishers exact test will be used to compare proportions of specific adverse events between groups. The following table shows the probability of observing at least one event for various true underlying rates for 15 (single arm) or 30

patients (collapse across experimental arms). Table 3 gives the detectable differences with 80% power.

Table 3. Difference in probabilities that can be detected with 80% power for sample sizes of 15 in each group or 15 in one group and 30 in the other

15 per arm		15 in arm 1 combined arms so n=30	
Arm 1 rate	Arm 2 rate	Arm 1	combined
0.01	0.36	0.01	0.31
0.025	0.40	.025	0.34
0.05	0.47	0.05	0.38

14.3   Analysis Plan

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

For randomization, the first 6 patients will be block randomized. Once an interim safety analysis is done on the initial enrollment, the remaining 39 patients will be randomized and enrolled in appropriately sized cohorts until there are 15 patients per arm.

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 prior to 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   Non–English-Speaking Participants

If a non–English-speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the CC Short Written Consent Form for Non–English-Speaking Research Participants in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12, and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreters will translate the IRB-approved English consent form verbatim and facilitate discussion between the participant and investigator.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record (CRIMSON), including the name of the interpreter. Further, all instances of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used three times or more for the same language within an IRB approval period, this will be reported to the IRB immediately.

15.3   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. 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 CRIMSON and CRIS 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 Guideline. Study records will be maintained by the PI for a minimum of 3 years and 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.

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Appendix A: Schedule of Procedures/Evaluations

Study Phase >	Screen	Injection 1			Injection 2			Feeding	Telephone Call	Follow Up
Study Day >	~ DAY -60 Under Protocol 11-I-0183	D0	D7 ± 2 days	D14 ± 2 days	D21	D28 ± 2 days	D35 ± 2 days	D42 +14 days	Day 44	1 visit every 60 days (± 14 days)
Outpatient Visit		X	X	X	X	X	X	X		X
Written Consent		X								
Medical/Medication History		X	X	X	X	X	X	X		X
Assessment and PE		X	X	X	X	X	X	X		X
Review of I/E criteria and review of consent		X								
Vital signs †		X	X	X	X	X	X	X		X
Review of Diary Card			X			X				
Telephone Call to Assess Symptoms									X	
Pregnancy test‡		X	X	X	X	X	X	X		X
Urinalysis		X	X	X	X	X	X	X		X
Vaccine administration		X			X					
Randomization		X								
Mosquito Feeding								X		
CBC + diff		X	X	X	X	X	X	X		X
Acute Care, Mineral, and Hepatic Panels		X	X	X	X	X	X	X		X
LDH, Uric Acid, Creatine Kinase, and Total Protein		X	X	X	X	X	X	X		X
Serum/Whole Blood Collection		X			X			X		X
IgE		X								

†Vital Signs: participants must be sitting for a minimum of 5 minutes prior to these procedures being performed; vital signs include blood pressure, mean arterial pressure, heart rate, respiratory rate, temperature, weight, pulse oximetry; height will be taken at the screening visit only.
‡Serum pregnancy testing at the screening and all other visits. On Days 0, 21, 42, study vaccination or mosquito feeding will not be initiated until results are available. Females only.

Appendix B: Blood Volumes for Specimen Collection

Study Schedule/Procedures		Day 0	Day 7 (+/- 2 days)	Day 14 (+/- 2 days)	Day 21 (+/- 2 days)	Day 28 (+/- 2 days)	Day 35 (+/- 2 days)	Day 42 (+ 14 days)	1 visit every 60 days (+/- 14 days)
b-hCG, Pregnancy	4mL per blood draw	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL
CBC + Diff	3mL per blood draw	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL
Acute Care, Mineral, and Hepatic Panels,	4mL per blood draw	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL	4 mL
LDH, Uric Acid, Creatine Kinase, and Total Protein	*Included with the above*	X	X	X	X	X	X	X	X
IgE, Total	4mL per blood draw	4 mL							
Serum/Whole Blood Collection	8 mL X 1 for serum, 8.5 mL X 5 for whole blood, and 2.5 mL for RNA per blood draw	53 mL + 8.5 mL x 5 more for whole blood			53 mL			95.5 mL prior to feeding, 111 ml post feeding	53 mL
Daily Volume (mL)		110.5 mL	11 mL	11 mL	64 mL	11 mL	11 mL	217.5 mL	64 mL
Cumulative Volume		110.5 mL	121.5 mL	132.5 mL	196.5 mL	207.5 mL	218.5 mL	436 mL	500 mL at first follow up,1152mL over the course of the whole study