Protocol Cover Page

Study Title: Phase 2 Study to Assess the Safety, Efficacy and Immunogenicity of Na-GST-1/Alhydrogel® Co-administered with Different Toll-Like Receptor Agonists in Hookworm-Naïve Adults

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Phase 2 Study to Assess the Safety, Efficacy and Immunogenicity of *Na*-GST-1/Alhydrogel® Co-administered with Different Toll-Like Receptor Agonists in Hookworm-Naïve Adults

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03 October 2025

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed: _____ Date: _____

Name: David Diemert, MD
Title: Principal Investigator

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
APR-1	Aspartic Protease-1
BCM	Baylor College of Medicine
CBC	Complete Blood Count
cGMP	Current Good Manufacturing Practices
CHHI	Controlled Human Hookworm Infection
CIOMS	Council for International Organizations of Medical Sciences
CpG	Cytosine-phosphate-Guanine Oligodeoxynucleotide
CRF	Case Report Form
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
ELISA	Enzyme linked immunosorbent assay
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GLA-AF	Gluco-Pyranosylphospho-Lipid A Aqueous Formulation
GMP	Good Manufacturing Practice
GST-1	Glutathione S-Transferase
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
hCG	Human choriogonadotropin
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IM	Intramuscular
IND	Investigational New Drug Application
IP	Investigational Product
IRB	Institutional Review Board
ISM	Independent Safety Monitor
MAAE	Medically-Attended Adverse Event
MedDRA®	Medical Dictionary for Regulatory Activities
MBC	Memory B Cell
MOP	Manual of Procedures
N	Number (typically refers to subjects)
Na	*Necator americanus*
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
ODN	Oligodeoxynucleotide
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure

SVI	Sabin Vaccine Institute (Albert B Sabin Vaccine Institute; Sabin)
US	United States
WBC	White Blood Cell

PROTOCOL SUMMARY

Title:	Phase 2 Study to Assess the Safety, Efficacy and Immunogenicity of *Na*-GST-1/Alhydrogel® Co-administered with Different Toll-Like Receptor Agonists in Hookworm-Naïve Adults
Phase:	2
Population:	48 healthy male and non-pregnant female volunteers aged 18-45 years, inclusive.
Number of Sites:	1 (The George Washington University, Washington, DC)
Study Duration:	22 months
Subject Participation Duration:	14 months
Description of Interventions:	a) The ***Na*-GST-1** candidate vaccine contains the recombinant *Na*-GST-1 adsorbed onto Alhydrogel® and suspended in a solution containing 10mM imidazole and 10% glucose. The final concentrations of *Na*-GST-1 and Alhydrogel® in the drug product are 0.1mg/ml and 0.8mg/ml respectively. Only one dose of *Na*-GST-1 will be tested (100µg), at a volume of 1.0ml delivered IM to the deltoid. b) **CpG 10104** is an unmethylated cytosine-phosphate-guanine oligodeoxydinucleotide (CpG) that is a Toll-like Receptor-9 agonist. The *Na*-GST-1/Alhydrogel® plus CpG 10104 formulation will be prepared immediately prior to vaccination by adding an appropriate volume of CpG 10104 to *Na*-GST-1/Alhydrogel® and withdrawing an appropriate volume to administer the desired amount of *Na*-GST-1 plus 500µg CpG 10104. c) Gluco-Pyranosylphospho-Lipid A Aqueous Formulation (GLA-AF; referred to as **AP 10-701** by the manufacturer) is a Toll-like Receptor-4 agonist. Point-of-injection formulations with this immunostimulant will be prepared immediately prior to vaccination by adding an appropriate volume of AP 10-701 to *Na*-GST-1/Alhydrogel® and withdrawing an appropriate volume to administer the desired amount of *Na*-GST-1 plus 5µg AP 10-701. d) To maintain the study blind by ensuring that all subjects receive an injection at each vaccination point, sterile normal **saline** (0.9%) for injection will be administered to those

subjects randomized to be infectivity controls for the controlled human hookworm infection arm.

e) Infectious ***Necator americanus*** **larvae (L3)**. *N. americanus* eggs will be obtained from the feces of a chronically infected human volunteer, who is negative for HIV, HBV, and HCV. Fecal material is processed following a qualified standard procedure, and after hatching from eggs, the *N. americanus* larvae are stored in the dark at ambient temperature (19-25°C) until use. Controls for the manufacturing process are tests for viability (motility), species identification, and microbial bioburden of the larvae.

Objectives:

Primary:

1. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on controlled human hookworm infection (CHHI) with *N. americanus* larvae in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.
2. To evaluate the safety and reactogenicity of *Na*-GST-1/ Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on Days 0, 56 and 112, in healthy, hookworm-naïve adults.

Secondary:

3. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on fecal egg counts, as determined by the McMaster method, after controlled human hookworm infection (CHHI) with *N. americanus* larvae in healthy, hookworm-naïve adults.
4. To assess the relationship between antibody responses to *Na*-GST-1 induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.
5. To assess the duration of antibody responses to *Na*-GST-1 induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104.

Exploratory:

1. To assess the relationship between the functional capacity of vaccine-induced antibodies that neutralize the *in vitro*

activity of native *Na*-GST-1 enzyme and responses to CHHI in healthy, hookworm-naïve adults.

2. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on levels of hookworm DNA as detected by real-time PCR in healthy, hookworm-naïve adults challenged with CHHI, as determined by the presence of eggs using a qualified flotation technique.
3. To assess the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on the affinity of antibody-antigen interactions, and how affinity relates to responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.
4. To assess the relationship between *Na*-GST-1 specific memory B cells induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.
5. To assess the relationship between innate immune responses to *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults.

Description of Study Design:

Double blind, randomized, controlled, Phase 2 clinical trial in hookworm-unexposed adults living in the metropolitan area of Washington, DC. Subjects will receive three doses of the assigned vaccine formulation, or saline placebo, delivered intramuscularly on approximately Days 0, 56, and 112.

Subjects will be challenged with 50 infectious *N. americanus* larvae 4 weeks after 3rd vaccination. Subjects will be evaluated weekly starting 5 weeks post-challenge to assess infection status. Albendazole will be administered 20 weeks post-challenge to cure infections. Subjects will be followed until 10 months after their final vaccination.

Safety of vaccination will be measured from the time of each study vaccination (Day 0) through 14 days after each study vaccination by the occurrence of solicited injection site and systemic reactogenicity events. Safety of CHHI will be measured from the time of larval application (Day 140) through the first day of treatment with albendazole (Day 280).

Unsolicited non-serious adverse events (AEs) will be collected until approximately 1 month following each study vaccination and from study Day 140 (day of CHHI) through Day 297.

New-onset chronic medical conditions, Serious Adverse Events (SAEs), and Adverse Events of Special Interest (AESIs) will be collected from the time of the first study vaccination through approximately 10 months after the third study vaccination (final visit). Clinical laboratory evaluations for safety will be performed on venous blood collected approximately 14 days after each vaccination and CHHI.

Immunogenicity testing will include IgG antibody responses to *Na*-GST-1, by a qualified indirect enzyme-linked immunosorbent assay (ELISA), on serum obtained prior to each study vaccination and CHHI, and at time points after each vaccination and after CHHI (see Appendix A); the affinity of vaccine-induced antibodies against *Na*-GST-1 using Surface Plasmon Resonance; the functional activity of vaccine-induced antibodies via *in vitro* enzyme neutralization assay; antigen-specific memory B cell responses; and, the innate immune responses to each of the TLR receptor immunostimulants.

Parasitological testing will include microscopic fecal egg detection by a qualified saline flotation technique, fecal egg counts by the McMaster method, fecal PCR for hookworm DNA, and peripheral eosinophil counts.

Recruitment and enrollment into the study will occur on an ongoing basis, with each group being recruited and vaccinated in sequence.

48 subjects will be enrolled into 4 groups of 12:

Subjects will be enrolled sequentially and upon enrollment will be randomized to one of the following IP assignments in a double-blind fashion:

- Group 1 IP allocation (n=12 subjects):
    - 12 subjects will receive 100µg *Na*-GST-1/Alhydrogel® delivered by IM injection in the deltoid muscle.

- Group 2 IP allocation (n=12):
    - 12 subjects will receive *Na*-GST-1/Alhydrogel® plus 500µg CpG 10104 delivered by IM injection in the deltoid muscle.

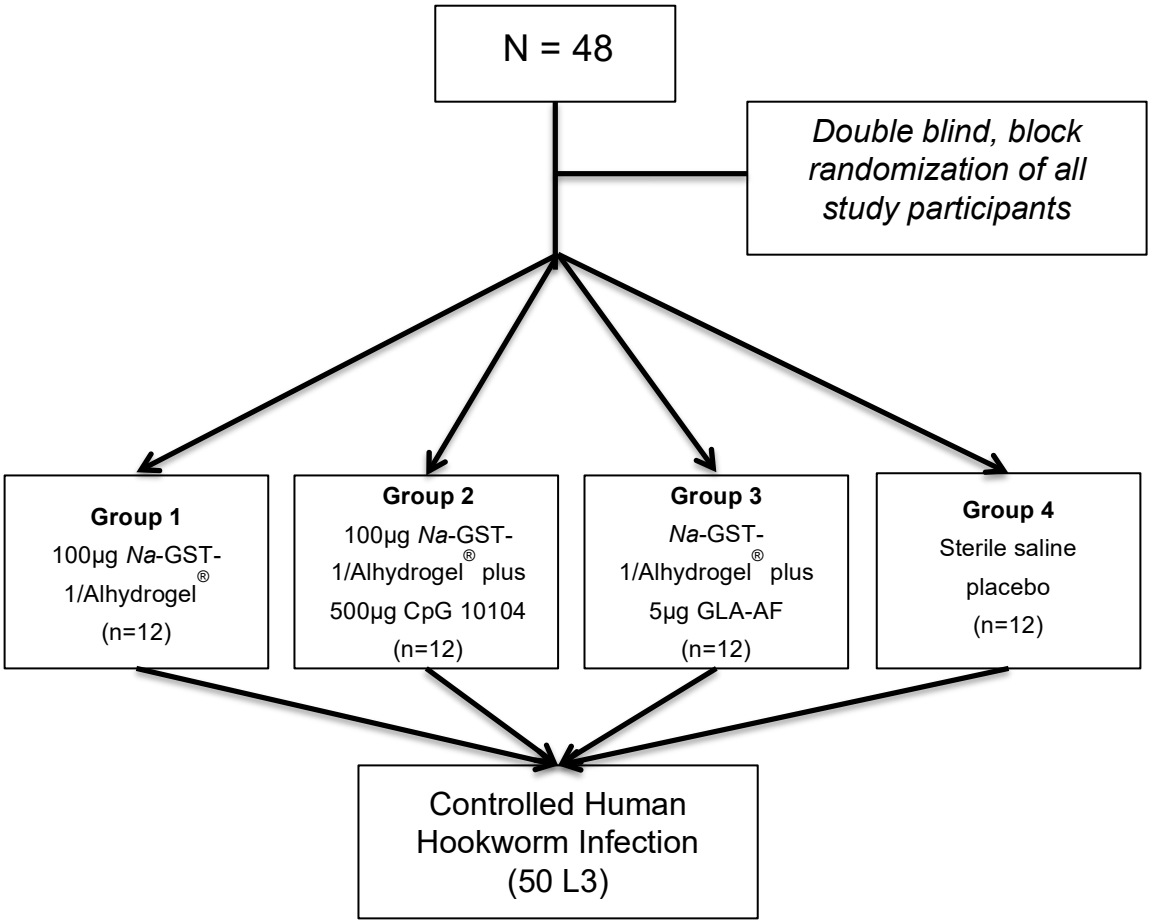
- Group 3 IP allocation (n=12):

- 12 subjects will receive *Na*-GST-1/Alhydrogel® plus 5µg AP 10-701 delivered by IM injection in the deltoid muscle.

- Group 4 IP allocation (n=12):
  - 12 subjects will receive sterile saline delivered by IM injection in the deltoid muscle

Estimated Time to Complete Enrollment:	6 months

Schematic of Study Design:



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Hookworm Infection and Justification for Vaccine Development

There is an urgent need for new tools to control human hookworm infection and to reduce its burden of disease in developing countries. This is especially important for children and women of reproductive age who represent populations that are highly vulnerable to the effects of hookworm disease. Up to 65,000 deaths annually have been attributed to human hookworm infection (1). However, the mortality figures pale in comparison to global disease burden estimates that suggest that hookworm may account for the loss of up to 22 million Disability Adjusted Life Years annually (2). With the exception of malaria, hookworm is the most important parasitic disease of humans.

Human hookworm infection is a soil-transmitted helminth infection caused by the nematode parasites *Necator americanus* and *Ancylostoma duodenale*. It is one of the most common chronic infections of humans, affecting up to 740 million people in the developing nations of the tropics (2). The largest number of cases occurs in impoverished rural areas of sub-Saharan Africa, Southeast Asia, China, and the tropical regions of the Americas. Approximately 3.2 billion people are at risk for hookworm infection in these areas. *N. americanus* is the most common hookworm worldwide, whereas *A. duodenale* is more geographically restricted (3).

Hookworm transmission occurs when skin comes into contact with infective third-stage larvae (L3) in fecally contaminated soil. The L3 have the ability to penetrate the skin, usually of the hands, feet, arms, buttocks and legs. The L3 invade human tissues and enter the gastrointestinal tract where they molt to the adult stage approximately 5-9 weeks following initial host entry. Adult hookworms are approximately 1 cm long parasites that cause host injury by attaching to the mucosa and submucosa of the small intestine to produce intestinal blood loss. There is a direct relationship between hookworm intensity (as determined by fecal egg counts) and host blood loss; typically the presence of between 40 and 160 adult hookworms in the intestine results in blood loss sufficient to cause anemia and malnutrition. The term “hookworm disease” refers primarily to the iron deficiency anemia and protein losses that occur in moderate and heavy infections (4). When host iron stores become depleted, there is a direct correlation between hookworm intensity and reduced host hemoglobin, serum ferritin, and protoporphyrin. Because of their low iron stores, children and women of reproductive age are the populations considered the most vulnerable to hookworm-associated blood loss (4-11).

In children, chronic hookworm infection and the resultant iron deficiency anemia have been shown to impair physical and intellectual development (3, 12, 13). Preschool children are particularly vulnerable to the effects of hookworm anemia and disease (8). In addition to its health impact on children, hookworm infection also affects adults. Unlike other soil-transmitted helminth infections such as ascariasis and trichuriasis, in which the highest intensity infections occur almost exclusively in school-aged children, it has been shown that high-intensity hookworm infections may also occur in adults (14-16).

The primary approach to hookworm control worldwide has been the frequent and periodic mass administration of benzimidazole anthelminthics to school-aged children living in high-prevalence areas. In 2001, the World Health Assembly adopted Resolution 54.19, which urges member states to provide regular anthelminthic treatment to high-risk groups with the target of regular treatment of at least 75% of all at-risk school-aged children. However, cure rates for a single dose of a benzimidazole are sub-optimal, particularly for mebendazole (17-20). These concerns have prompted interest in developing alternative tools for hookworm control (3, 21, 22). Vaccination to prevent the anemia associated with moderate and heavy intensity hookworm infection would alleviate the public health deficiencies of drug treatment alone.

The feasibility of developing a hookworm vaccine is based on the previous success of using live, irradiated hookworm larvae (L3 stage) as a vaccine for canine hookworm infection. This provided the experimental basis for the commercial development of a canine hookworm vaccine, which was marketed in the United States during the early 1970s. However, it is not realistic to develop a live L3 vaccine for humans due to multiple reasons including high production costs, challenging storage requirements, a short shelf life, and a lack of sterilizing immunity.

Alternatively, the strategy being pursued is to identify key hookworm proteins to which protective immune responses are directed in the animal models for this infection (namely the canine model) and to produce these as recombinant proteins that could then be used as vaccine antigens. This effort focused initially on identifying antigens expressed by the invading larvae (L3). In addition, a separate strategy has been to identify targets of the adult stage of the hookworm lifecycle; since hookworms attach onto the intestinal lumen and ingest host blood, antibodies could also be ingested that if directed against key hookworm proteins, would interfere with their function, ultimately resulting in the death or reduced fecundity of the worm.

2.1.2 Study Site

The study will be conducted in healthy adult volunteers without history of hookworm infection at the George Washington University Medical Faculty Associates (GW MFA) and the George Washington University School of Medicine and Health Sciences, Department of Microbiology, Immunology, and Tropical Medicine (GW MITM), both in Washington, DC.

2.1.3 Prior Clinical Experience with Hookworm Vaccines

The first hookworm vaccine to be tested in humans was the *Na*-ASP-2 (Ancylostoma Secreted Protein-2 of *N. americanus*) Hookworm Vaccine, consisting of recombinant *Na*-ASP-2 expressed in *Pichia pastoris* and adsorbed to aluminum hydroxide gel (Alhydrogel®). *Na*-ASP-2 is an excretory/secretory product produced by infective *N. americanus* larvae upon penetration of human skin. In animal models, vaccination with this recombinant antigen was shown to result in reduced worm burdens after challenge infection. Accordingly, a Phase 1 clinical trial of several different dose concentrations of the vaccine was conducted in healthy, hookworm-naïve adults living in the United States, which showed the formulation to be safe, well tolerated and immunogenic (23).

However, upon testing the vaccine in adults who had previously been infected with hookworm in Americaninhas, several volunteers in the lowest dose cohort to be vaccinated developed

generalized urticaria within 2 hours of immunization (24). Due to these immediate-type hypersensitivity reactions, vaccinations in this study were halted. Subsequent investigations revealed that the volunteers who developed urticaria upon their first dose of *Na*-ASP-2 had elevated levels of baseline (i.e., pre-vaccination) IgE to the vaccine antigen. Subsequently, a sero-epidemiological survey was conducted in an endemic region of Brazil; this study revealed that even in young children, a significant proportion of individuals have detectable levels of IgE to this protein, likely due to previous infection with *N. americanus*. In addition, similar findings were demonstrated for other larval proteins that were being considered as vaccine candidates.

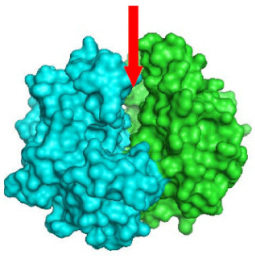
Due to these cumulative data, clinical development of the *Na*-ASP-2 and other larval-stage antigens as candidate vaccines was halted. Instead, the current strategy is to develop antigens expressed during the adult stage of the hookworm life cycle that play a role in digesting the host hemoglobin that is used by the worm as an energy source. These antigens do not induce specific IgE antibodies during natural infection, and hence have a low likelihood for inducing allergic reactions upon vaccination.

2.1.4 The *Na*-GST-1/Alhydrogel® Hookworm Vaccine

The nutritional and metabolic requirements of the adult hookworm living in the human intestine are dependent upon degradation of host hemoglobin that has been ingested by the worm. *N. americanus* hookworms depend on host hemoglobin for survival (25). Following hemolysis, adult hookworms use an ordered cascade of hemoglobinases to cleave hemoglobin into smaller molecules (25-30). Aspartic protease-1 of *N. americanus* (*Na*-APR-1) is responsible for initiating the proteolytic cascade in hookworms, as described below. After hemoglobin digestion, the freed heme generates toxic oxygen radicals that can be bound and detoxified by molecules such as glutathione S-transferase-1 (GST-1) (31-33). GST-1 of *N. americanus* (*Na*-GST-1) is a critical enzyme that plays a role in parasite blood feeding; used as a vaccine, we hypothesize that the antigen will induce anti-enzyme neutralizing antibodies that will interfere with parasite blood-feeding and cause parasite death or reduce worm fecundity.

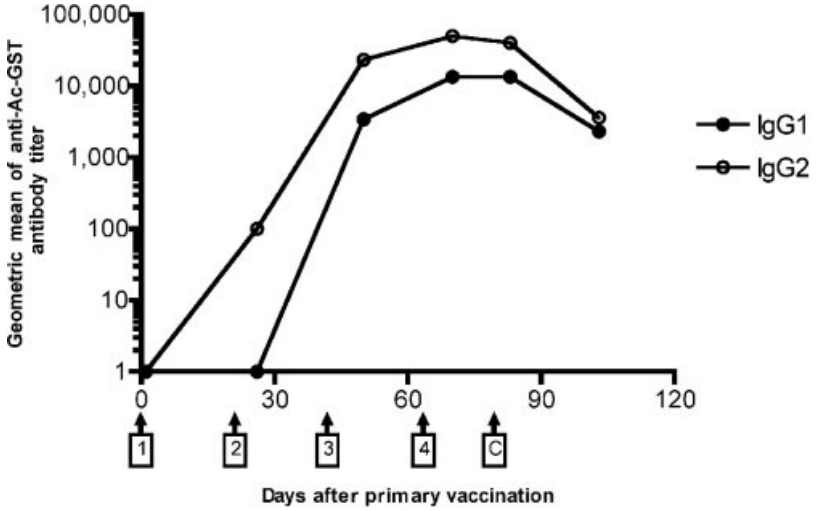
Na-GST-1 is a 24-kDa protein with peroxidase enzymatic activity that catalyzes the conjugation of reduced glutathione to a variety of electrophiles (31-33). This hookworm protein belongs to the Nu class of nematode GSTs, which also includes GSTs from the blood-feeding parasite of ruminants, *Haemonchus contortus*, and the rodent nematode *Heligomosoides polygyrus*. This class is characterized by diminished peroxidase activity relative to other classes of GSTs, but elevated binding capacity for heme and related products (31, 33-36). X-ray crystallography of *Na*-GST-1 demonstrates that the protein can form homodimers in solution, which create atypically large binding cavities accessible to a diversity of ligands, including heme (**Figure 1**) (33). *Na*-GST-1 binds heme at high affinity *in vitro* (31, 36). Because both heme and hematin contain oxidative iron, these molecules are potent generators of toxic reactive oxygen species that could potentially damage parasite macromolecules. *In vivo*, hookworm GSTs may therefore bind and detoxify the heme and hematin byproducts generated during the blood degradation process.

Figure 1: Three-dimensional surface plot of *Na*-GST-1. The path to the binding cavity is indicated by the red arrow (33).



Based on their putative role in hookworm blood feeding, both *Na*-GST-1 and its orthologue from the canine hookworm *Ancylostoma caninum* (*Ac*-GST-1) were tested as experimental vaccines in laboratory animals models of infection. In dogs, vaccination with recombinant *Ac*-GST-1 resulted in high levels of antigen-specific antibody (**Figure 2**); following challenge with *A. caninum* infective larvae, significantly lower host worm burdens and fecal egg counts were observed compared to control animals vaccinated only with adjuvant (31). In hamsters, vaccination with recombinant *Ac*-GST-1 also resulted in substantially lower worm burdens (51-54%) following heterologous challenge with *N. americanus* infective larvae compared to controls, as did vaccination with recombinant *Na*-GST-1 followed by homologous larval challenge (31, 32, 37). Because of these encouraging preclinical results, *Na*-GST-1 was manufactured according to current good manufacturing practices (cGMP) and formulated on Alhydrogel® in preparation for clinical trials.

Figure 2: Geometric mean titers of the IgG1 and IgG2 antibody responses of vaccinated dogs against recombinant *Ac*-GST-1 formulated with GlaxoSmithKline's AS03 adjuvant. Vaccination time points (1, 2, 3, and 4) and challenge day (C) are marked with arrows (31).



Most importantly, extensive studies have been conducted to test for sensitization to the *Na*-GST-1 protein in individuals living in a hookworm endemic area who have been repeatedly

exposed and infected with *N. americanus*. Over 1000 individuals of all ages from a hookworm endemic area of Brazil have been tested for serum IgE antibodies to *Na*-GST-1 using an indirect ELISA. In addition, a subset of these serum samples stratified by age and infection status (n = 179) underwent confirmatory testing at the Johns Hopkins Dermatology, Allergy and Clinical Immunology Reference Laboratory (Baltimore, Maryland) using a custom ImmunoCAP assay. The ImmunoCAP method is considered the standard for measuring specific IgE to antigens in serum. This confirmatory testing demonstrated that none of the samples had *Na*-GST-1 IgE values above the clinical cut-off of 0.35 kU_A/L. Therefore, the likelihood of inducing immediate-type hypersensitivity reactions by vaccinating individuals living in hookworm-endemic areas with *Na*-GST-1 is low and likely not more than that associated with any new vaccine antigen entering clinical trials. The situation with *Na*-GST-1 is therefore very different from that of *Na*-ASP-2 in that repeated infection with hookworm does not induce an IgE response to the antigen, most likely due to the fact that it is a protein found in the digestive tract of adult hookworms and is therefore relatively hidden from the human immune system. This lack of antigen-specific IgE in people living in an area of high transmission has served as a major justification for advancing development of *Na*-GST-1 as a candidate vaccine antigen.

Na-GST-1 has been successfully manufactured and tested in the laboratory and in animals with both Alhydrogel®, Alhydrogel® plus AP 10-701, and Alhydrogel® plus CpG 10104. *Na*-GST-1 has been shown to be pure, potent, and stable in both of these three formulations.

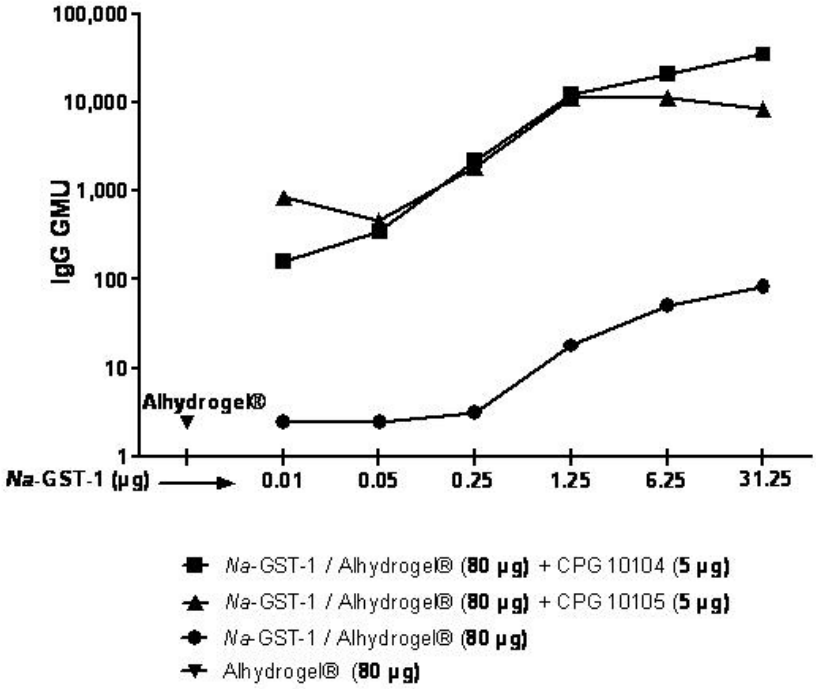
The *Na*-GST-1 vaccine formulation to be tested in this clinical trial consists of the 24-kDa recombinant protein *Na*-GST-1, adsorbed to an adjuvant, Alhydrogel® (aluminum hydroxide suspension) with or without the addition of one of two Toll-Like Receptor (TLR) agonists: a) Gluco-Pyranosylphospho-Lipid A Aqueous Formulation (AP 10-701); or, b) CpG 10104. AP 10-701 or CpG 10104 will be added to the Alhydrogel® formulation within 24 hours of immunization. The active ingredient is the recombinant *Na*-GST-1 protein that is derived by fermentation of *Pichia pastoris* yeast cells genetically engineered to express *Na*-GST-1.

2.1.5 Immunogenicity Studies with *Na*-GST-1

Several preclinical animal studies have been conducted in both mice and rats to assess the immunogenicity of *Na*-GST-1 in combination with different adjuvants. First, a study conducted in Sprague-Dawley Rats demonstrated that the addition of an adjuvant to recombinant *Na*-GST-1 was necessary, since administration of the recombinant protein without an adjuvant resulted in minimal specific antibody responses.

A second study was conducted in BALB/c mice to assess the effect of co-administering CpG 10104 with recombinant *Na*-GST-1/Alhydrogel® (**Figure 3**). In this study, mice were vaccinated with *Na*-GST-1/Alhydrogel® at antigen doses ranging from of 0.01 to 31.25 µg *Na*-GST-1 with or without CpG 10104 (5 µg) or CpG 10105 (5 µg). CpG 10105 is a CpG oligodeoxynucleotide sequence that is similar to CpG 10104 but that is not being proposed to be tested in the study described in this protocol. Mice were vaccinated twice intramuscularly at a 3-week interval, with blood collected for anti-*Na*-GST-1 IgG ELISA two weeks after the second immunization. This study demonstrated a large, highly significant increase in IgG specific for *Na*-GST-1 in the group administered *Na*-GST-1/Alhydrogel®/CpG 10104 compared to that administered only *Na*-GST-1/Alhydrogel® as shown in **Figure 3**.

Figure 3: Geometric mean anti-*Na*-GST-1 IgG antibody units 2 weeks after the 2nd Immunization of BALB/c mice with *Na*-GST-1/Alhydrogel® with or without co-administration of CpG 10104 or CpG 10105.

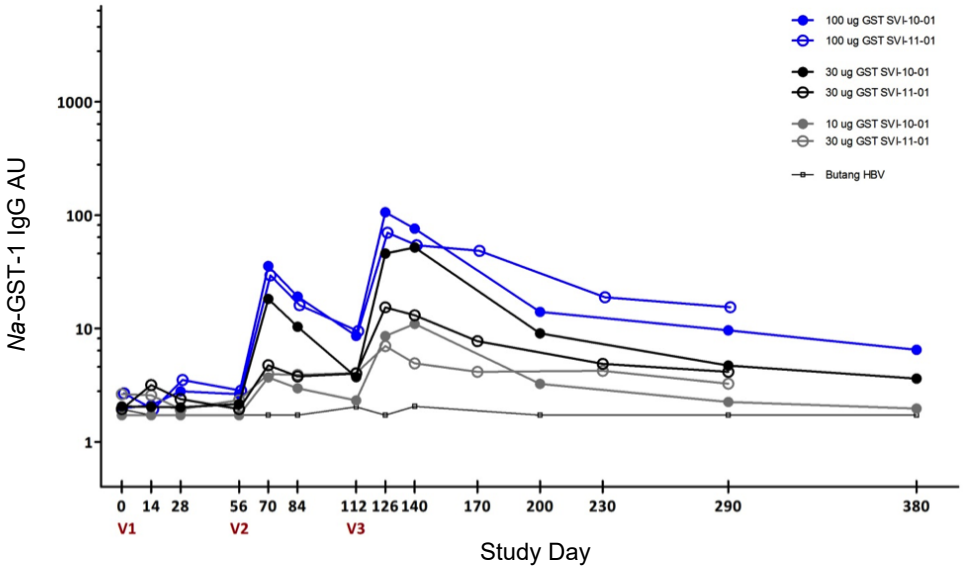


2.1.6 Clinical Experience with *Na*-GST-1/Alhydrogel® Hookworm Vaccine

<u>*Na*-GST-1/Alhydrogel® administered with or without AP 10-701</u>

The *Na*-GST-1/Alhydrogel® hookworm vaccine has been tested with or without AP 10-701 in two completed and two ongoing Phase 1 clinical trials in humans in the United States (n=40), Brazil (n=162) and Gabon (n=32) either alone or co-administered with the *Na*-APR-1 (M74)/Alhydrogel® hookworm vaccine. To date, no significant reactogenicity or safety issues have been observed in any of these trials. In study SVI-11-01 in healthy, hookworm-naïve American adults conducted in Washington, DC, 40 volunteers received three vaccinations with up to 100 µg *Na*-GST-1/Alhydrogel® administered with or without up to 5 µg AP 10-701 (NCT01385189). Mild to moderate injection site pain and tenderness were observed in a minority of study subjects; other common adverse events included mild to moderate headache and nausea. No vaccine-related SAEs or Adverse Events of Special Interest occurred and the vaccine was well tolerated. The incidence of vaccine-related adverse events was not related to either dose of *Na*-GST-1 or co-administration status with AP 10-701. Antigen-specific IgG antibodies were induced in a dose-dependent fashion after the second and third vaccinations (**Figure 4**).

Figure 4: Mean anti-*Na*-GST-1 IgG antibody units induced by vaccination with *Na*-GST-1/Alhydrogel® (with or without AP 10-701) in healthy American (SVI-11-01) *vs*. Brazilian (SVI-10-01). Butang HBV=hepatitis B vaccine (comparator); V=vaccination.



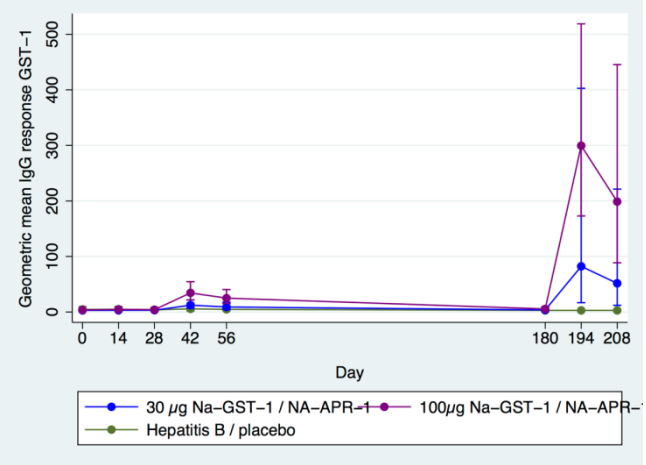
The trial of *Na*-GST-1 conducted in Brazil (SVI-10-01) has tested *Na*-GST-1/Alhydrogel® in both hookworm-unexposed (n=36) and hookworm-exposed (n=66) healthy adults (NCT01261130). This study was initiated in Belo Horizonte, a large urban area in the Brazilian state of Minas Gerais, in healthy, hookworm-unexposed adults (n=36) who received up to 100 µg *Na*-GST-1/Alhydrogel® with or without 2.5 µg AP 10-701. After no significant adverse events were observed in these volunteers, vaccinations were begun at the hookworm-endemic region of Americaninhas in Minas Gerais; this second part of the trial was a randomized, controlled, double-blind study in which subjects received up to 100 µg *Na*-GST-1/Alhydrogel® with or without 2.5 µg AP 10-701 (n=60), or the recombinant hepatitis B vaccine (n=6). All volunteers in this study received three intramuscular injections at 0, 2, and 4 months and were followed for 12 months after the final vaccination. In this study, *Na*-GST-1/Alhydrogel® (with or without AP 10-701) was safe and well tolerated in both hookworm-unexposed and hookworm-exposed adults. No vaccine-related SAEs or Adverse Events of Special Interest occurred and the vaccine was well tolerated. The most commonly observed solicited adverse events were mild injection site pain and tenderness. The most common systemic solicited adverse event was mild headache. Neither the incidence nor the severity of these adverse events was related to the number of injections received. Similar to SVI-11-01, antigen-specific IgG antibodies were induced in a dose-dependent fashion (**Figure 4**).

<u>*Na*-GST-1/Alhydrogel® co-administered with *Na*-APR-1 (M74)/Alhydrogel®</u>

Na-GST-1/Alhydrogel® is currently being tested in <u>two</u> Phase 1 clinical trials in which it is being co-administered with the *Na*-APR-1 (M74)/Alhydrogel® hookworm vaccine. In the first of these studies, 32 healthy adults living in a hookworm-endemic area of Gabon received 3 vaccinations of up to 100 µg *Na*-GST-1/Alhydrogel® co-administered with *Na*-APR-1 (M74)/Alhydrogel®

(n=24), compared to a licensed hepatitis B vaccine (n=8). All subjects randomized to receive the hookworm vaccines received an injection of *Na*-GST-1 in one arm and an injection of *Na*-APR-1 (M74) in the other arm, on each day of vaccination. Both *Na*-GST-1/Alhydrogel® and *Na*-APR-1 (M74)/Alhydrogel® were mixed with 5 µg AP 10-701 prior to vaccination. Vaccinations in this trial (NCT02126462) were administered according to a 0, 1, 6-month schedule and were completed in September 2015. Interim results from this trial indicate that co-administration of *Na*-GST-1/Alhydrogel/AP 10-701 when co-administered with *Na*-APR-1/Alhydrogel/AP 10-701 was well tolerated, with minimal reactogenicity. An interim analysis of anti-*Na*-GST-1 specific IgG antibody levels up to one month after the third set of vaccinations indicates that subjects in this hookworm-endemic area of Africa develop a humoral immune response to vaccination after the second vaccination that was boosted upon vaccination at 6 months after the first set of vaccinations (**Figure 5**).

Figure 5: Geometric mean anti-*Na*-GST-1 IgG antibody units in healthy Gabonese adults vaccinated with *Na*-GST-1/Alhydrogel® plus AP 10-701 and co-administered with *Na*-APR-1 (M74)/Alhydrogel® at 0, 1, and 6 months.



Vertical bars are 95% confidence intervals

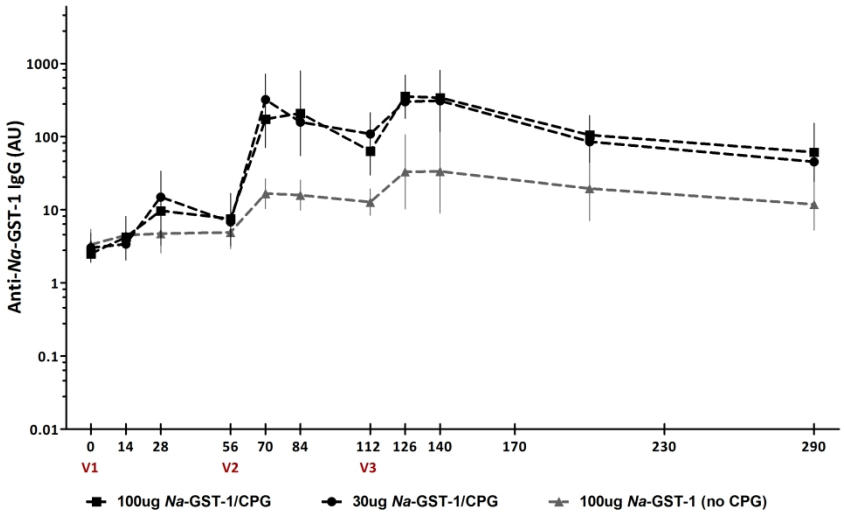
In the second currently ongoing Phase 1 trial, 60 healthy adults are being vaccinated with the *Na*-APR-1 (M74)/Alhydrogel hookworm vaccine either with or without co-administration of *Na*-GST-1/Alhydrogel in a hookworm endemic area of Brazil (NCT02476773). This study is being conducted with funding from a U01 grant from the National Institute of Allergy and Infectious Diseases (NIAID Grant #1U01AI116414-01). The aim of this trial is to determine if there is any immunological interference when the two hookworm vaccine antigens are co-administered to subjects chronically exposed to hookworm infection. Vaccinations in this trial were initiated in January 2016 and are currently ongoing. No Serious Adverse Events or Adverse Events of Special Interest have been reported in this study. Interim results from this clinical trial indicate that co-administration of the two vaccines is well tolerated; mild to moderate injection site pain and tenderness were the most common vaccine-related adverse events.

<u>*Na*-GST-1/Alhydrogel® administered with or without CpG 10104</u>

The *Na*-GST-1/Alhydrogel® hookworm vaccine is currently being tested with or without CpG 10104 in one ongoing Phase 1 clinical trial in humans in the United States (n=24). To date, no

significant reactogenicity or safety issues have been observed in this trial. In study SVI-GST-03 in healthy, hookworm-naïve American adults conducted in Washington, DC, 24 volunteers received three vaccinations with up to 100 µg *Na*-GST-1/Alhydrogel® administered with or without 500 µg CpG 10104 (NCT02143518). Vaccinations were completed in October 2015 and extended follow-up is ongoing. In this study, the most common reported adverse events have been mild to moderate injection site pain and tenderness; other common adverse events have included mild to moderate headache, nausea, myalgia and arthralgia. Immunogenicity conducted on serum samples collected up to and including 1 month following the final vaccination demonstrates that the addition of CpG 10104 to *Na*-GST-1/Alhydrogel® significantly improved antigen-specific IgG antibody responses over those induced by *Na*-GST-1/Alhydrogel® alone (**Figure 6**).

Figure 6: Anti-*Na*-GST-1 IgG antibody responses as measured by indirect ELISA in hookworm-naïve adults vaccinated with *Na*-GST-1/Alhydrogel® with or without co-administration of CpG 10104. AU = arbitrary antibody units; V=vaccination.



2.1.7 Clinical Experience with AP 10-701

In the clinical trial described in this protocol, *Na*-GST-1/Alhydrogel® will be tested in combination with the Toll-like Receptor 4 (TLR4) agonist, Gluco-Pyranosylphospho-Lipid A in Aqueous Formulation (AP 10-701, Infectious Diseases Research Institute [IDRI], Seattle, WA). AP 10-701 contains a synthetic monophosphoryl lipid A (MPL) molecule that has TLR4 agonist activity. MPL is itself derived from the lipopolysaccharide (LPS) of *Salmonella minnesota*, a natural TLR4 agonist that is pyrogenic and can induce toxic shock. LPS, and more specifically, its lipid A component, has long been known for its strong adjuvant effects; however, its high toxicity has precluded its use in a vaccine formulation. Ribi et al showed that the monophosphorylated form of lipid A retains its adjuvant function and almost completely loses its endotoxin effects (38).

There have been many clinical trials involving thousands of subjects in which MPL or a derivative have been administered as vaccine adjuvants to adults and children, including vaccines for human papillomavirus, malaria (39-41), leishmaniasis (42), and hepatitis B (43). In

general, these trials have demonstrated that administering MPL to humans is safe and well tolerated; when compared to formulations of vaccine that do not contain MPL, those adjuvanted with MPL may result in a minor increase in the incidence and/or severity of local injection site reactions. However, the addition of MPL also often results in a much improved specific antibody response to the vaccine antigen(s).

Of note, MPL is one of the components of the licensed Cervarix® vaccine (GlaxoSmithKline, Research Triangle Park, NC) for the prevention of cervical cancer due to human papillomavirus serotypes 16 and 18. The adjuvant for this vaccine consists of MPL adsorbed to aluminum hydroxide salt and is therefore similar to the combination of AP 10-701 and Alhydrogel® that we propose testing in combination with *Na*-GST-1 and *Na*-APR-1 (M74) in the study described in this protocol. The Cervarix® vaccine has been shown to have a very favorable safety profile after having been tested in tens of thousands of healthy individuals (44, 45).

As mentioned previously, up to 5 µg of AP 10-701 has been administered to over 100 human volunteers in combination with both *Na*-GST-1/Alhydrogel® and *Na*-APR-1 (M74)/Alhydrogel®. In addition, an oil-in-water emulsion of GLA (GLA-SE) has been used in combination with the Fluzone® trivalent killed influenza vaccine in a Phase 1 trial. In this study, doses up to 2.5 µg of GLA-SE were safe and well-tolerated and significantly enhanced influenza-specific antibody responses (46).

2.1.8 Clinical Experience with CpG 10104

Unmethylated cytosine-guanine dinucleotides (CpGs) are found in bacterial DNA in the expected frequency predicted by random usage, whereas their occurrence is suppressed 4-fold in vertebrate DNA. In vertebrate DNA CpG motifs are also usually methylated. Bacterial CpG-DNA motifs are recognized by the human innate immune system via Toll-like Receptor-9 (TLR-9), a pathogen-associated molecular pattern (PAMP) receptor that is expressed, in particular, by antigen-presenting dendritic cells. Interactions between CpG-DNA and TLR9 rapidly activate antigen-presenting dendritic cells to upregulate co-stimulatory molecules and to produce Th1-polarizing cytokines such as interleukin-12 and interferon gamma (47). CpG 10104 is a short synthetic oligodeoxynucleotide of the following sequence: 5'-TCG TCG TTT CGT CGT TTT GTC GTT-3'.

CpG 10104 has been tested in humans in combination with *Na*-GST-1/Alhydrogel in one Phase 1 trial to date, as described above in **Section 2.1.6**. In addition, ProMune® (CpG 7909), a human TLR9 agonist similar to CpG 10104, has been evaluated in clinical trials in combination with the BioThrax® anthrax vaccine(48), Engerix-B® hepatitis B virus vaccine(49), AMA1-C1/Alhydrogel® and BSAM-2/Alhydrogel® candidate malaria vaccines(50, 51), a number of therapeutic cancer vaccines, cancer chemotherapies, and other therapeutic interventions.(52) No serious adverse events related to ProMune® have been reported. Phase 3 evaluation of the HEPLISAV® Hepatitis B vaccine formulated with CpG 1018 ISS (a different CpG sequence than will be tested in the study described in this protocol) has also been completed, with results indicating improved immunogenicity as compared to currently available hepatitis B vaccines.(53) The US FDA has requested additional safety information with trials of sufficient size to evaluate rare autoimmune events following administration of HEPLISAV®.

2.1.9 Clinical Experience with Controlled Human Hookworm Infection (CHHI)

A total of nine experimental human hookworm infection studies have been reported in the literature and two more (EudraCT 2008-005008-24 and NCT01940757) are currently underway in the United Kingdom and Washington, DC, respectively (54-61). The completed studies were conducted to investigate the natural history of hookworm infection in humans but also to test the therapeutic efficacy of experimental infection on various inflammatory and allergic diseases such as celiac disease, inflammatory bowel disease and allergic rhinoconjunctivitis. The important points regarding these previous hookworm challenge studies are that: a) experimental infections were overall well-tolerated, with minimal significant adverse events; b) hookworm infection is curable with well-tolerated licensed medications; and, c) the time to patency (as determined by detection of egg in feces) is predictable. These studies, conducted under approvals from ethical review committees in the US, Australia and the UK, have enrolled a total of over 100 adults who have been challenged with between 10-100 infectious *N. americanus* larvae.

In the previous studies, the time until hookworm eggs were detectable by microscopic examination of feces was consistently between 4 and 8 weeks after challenge. All experimental infections were completely cured by administration of a benzimidazole anthelminthic drug (either albendazole or mebendazole) orally. The most common adverse effects of challenge infection in these studies included transient skin rash at the site of larval application and gastrointestinal discomfort beginning approximately 4 to 6 weeks post-challenge that was characterized by flatulence, abdominal pain, nausea, vomiting, and diarrhea.

2.1.9.1 Experience with CHHI at George Washington University

A Phase 1 clinical trial of CHHI is currently underway at George Washington University (GWU) in Washington, DC, the same study site as will be used for the study described in this clinical protocol. This feasibility study (NCT01940757) is being conducted by Dr. David Diemert serving as study Principal Investigator. In it, 20 volunteers between the ages of 18 and 45 years who have no history of infection or exposure to hookworm were enrolled into 2 cohorts in a staggered fashion. In the first cohort volunteers received 25 *N. americanus* larvae whereas in the second cohort they received 50 larvae. All subjects (except 2 who were enrolled into a donor sub-study, see below) were cured of their infections by administering albendazole between 12 and 18 weeks post-infection.

In this study, adverse events have been mostly related to papulovesicular rash and pruritus at the site of larval application on the skin, and mild-to-moderate gastrointestinal symptoms most likely related to development of adult hookworms in the gastrointestinal tract. The skin reactions occurred immediately after application of larvae, have been graded by the study subjects as mild or moderate in most cases, and these have in some cases lasted for up to one month, sometimes with residual hyperpigmentation that gradually fades over time. Gastrointestinal symptoms consisting principally of mild to moderate abdominal bloating, flatulence and episodic nausea begin to occur approximately 4-6 weeks after infection. Skin reactions were more common in the 50 L3 cohort compared to the 25 L3 cohort, although there were no significant differences between the cohorts in incidence or severity of gastrointestinal complaints.

In the first cohort (25 L3), 3 of 10 subjects developed hookworm infections with eggs detectable in fecal samples by microscopy and PCR, whereas in the second (50 L3) cohort, 9 of 10 did. Mean egg counts, as estimated by the McMaster Method using all collected fecal samples, varied from 0 to 33 eggs per gram (epg) of feces in the 25 L3 cohort and from 0 to 133 epg in the 50 L3 cohort. Given the high rate of infection in the 50 L3 cohort, this will be the dose used in the Phase 2 clinical trial described in this protocol.

The larvae that were used in this initial study at GWU were obtained from the University of Nottingham. Two of the study subjects in the 50 L3 group have enrolled into a sub-study in which they remain untreated and are followed monthly to provide fecal samples that can be used to harvest hookworm eggs (donors). The same production method used at the University of Nottingham has been instituted at GWU to produce *N. americanus* larvae from these hookworm eggs that can then be used to infect future human subjects. Therefore, the larvae that will be used to experimentally infect study volunteers in the proposed Phase 2 trial at GWU will be supplied by the Department of MITM at GWU. The Baylor College of Medicine (BCM) currently holds an IND to the US FDA for the *N. americanus* hookworm larvae (IND#015752) to permit their use in the U01 clinical trial described herein, in which healthy hookworm-naïve volunteers are vaccinated with the *Na*-GST-1/Alhydrogel® hookworm vaccine and then challenged with an experimental hookworm infection.

2.2 Rationale

2.2.1 Rationale for the Study

A product that combines *Na*-GST-1 shows promise as an effective hookworm vaccine because vaccination of laboratory animals with recombinant *Na*-GST-1 results in significant protection from challenge infections (29, 37). Therefore, vaccination of humans with recombinant *Na*-GST-1 holds promise for inducing protection against this infection, particularly the moderate and heavy intensity infections that are associated with clinical sequelae such as intestinal blood loss and iron-deficiency anemia. The Sabin Vaccine Institute has sponsored a series of Phase 1 trials of the *Na*-GST-1 candidate antigen in healthy adult volunteers from hookworm endemic areas (rural Brazil and Gabon) and non-endemic areas (urban Brazil and USA). As of May 1, 2017, regulatory sponsorship of these vaccine candidates has been transferred to the Baylor College of Medicine (Houston, TX). These trials have tested three different formulations of *Na*-GST-1: adsorbed on Alhydrogel®, plus the addition of two different TLR agonist immunostimulants to the Alhydrogel® formulation, AP 10-701 and CpG 10104. These Phase 1 trials have demonstrated that all three formulations are well tolerated, have not resulted in significant adverse events, and induce antigen-specific IgG antibodies.

Since there are no known immune correlates of protection against natural hookworm infection, it is unclear how the immunogenicity observed in the Phase 1 trials of *Na*-GST-1 will translate into efficacy. Following the traditional pediatric vaccine clinical development pathway, the next steps would be to conduct age de-escalation Phase 1 trials into children, followed by large pediatric Phase 2 and 3 field trials. Given the transmission dynamics of hookworm even in highly endemic areas, it is estimated that it would take at least two years and require over 1000 children to be enrolled into a Phase 2 proof-of-concept trial of a single vaccine candidate in which the main endpoint would be mean fecal egg count. Given the length of time, the effort, and the cost involved, it would not be feasible to conduct multiple such Phase 2 trials of the

different vaccine formulations that have progressed past the Phase 1 stage. Therefore, it is critical that a new paradigm is developed in which an estimate of efficacy can be made early in development so that different candidate formulations can be compared and the most promising selected for advancement into pediatric testing.

In addition to use of the CHHI model in this trial to test proof-of-efficacy of various formulations of the *Na*-GST-1 vaccine candidate, we will utilize several state-of-the-art immune-profiling technologies (see **Section 8.2**) to characterize and compare the immune responses to *Na*-GST-1 induced by the different adjuvant preparations and immunostimulants (e.g., AP 10-701 and CpG 10104) and how these responses relate to the impact on CHHI. In particular, we will compare conventional indirect enzyme-linked immunosorbent assay (ELISA) techniques with cutting-edge antibody (Surface Plasmon Resonance or SPR), innate immunity and memory B cell (MDB) techniques that offer a more comprehensive profiling of the humoral immune response when administering vaccine antigens using a “systems vaccinology” approach. This will enable us to identify immune variables that are associated with, and can discriminate between, the different immunization groups. The introduction of these novel assays and systems analysis methodology early in vaccine clinical development will enable decision-making on optimal vaccine composition during clinical development, saving time, expense, and the required number of human research subjects.

2.2.2 Rationale for *Na*-GST-1 Doses and Dose Schedule to be Studied

For the study proposed in this clinical protocol, each subject will be vaccinated three times, on Days 0, 56, and 112, by intramuscular injection in the deltoid muscle. This vaccination schedule was selected to coordinate with the Expanded Program on Immunization as the human hookworm vaccine is intended to target children, including infants, and it is the schedule that has been tested in all but one of the Phase 1 trials of *Na*-GST-1, to date. Local subcutaneous nodules, believed to be granulomatous reactions to aluminum hydroxide, have been observed with use of aluminum hydroxide-based adjuvants. Thus, most aluminum hydroxide-adsorbed vaccines are injected intramuscularly (IM) rather than subcutaneously, and intramuscular injection in the deltoid muscle will be used for administration of all investigational products in the study. The doses of *Na*-GST-1, AP 10-701 and CpG 10104 are the maximum doses that have been tested in the human clinical trials conducted to date, as described above. This is justified since these doses were all well tolerated and there appears to be a dose effect for *Na*-GST-1 in terms of immunogenicity.

2.2.3 Rationale for Controlled Human Hookworm Infection Model (CHHI)

There have been several attempts to create permissive animal models for hookworm infection, but without much success (62). The two major laboratory animal models for studying hookworm infection are *A. caninum* infections in dogs, which closely resembles human hookworm infection, and *A. ceylanicum* and *N. americanus* in the golden hamster (*Mesocricetus auratus*) (63, 64). While both are better than other animal models, neither canines nor hamsters adequately reproduce the endpoints of human hookworm disease--specifically, including host eggs counts and adult worm recovery. Hence, an experimental hookworm infection model is being developed to provide early proof-of-concept that a hookworm vaccine targeting the blood-feeding pathway of adult hookworms is feasible and efficacious. This model consists of vaccinating healthy, hookworm-naïve adults with candidate hookworm vaccine formulations,

followed by challenging them with third stage infective *N. americanus* larvae (L3) to assess the effect of vaccination on infection. Such a model will provide an indication of potential efficacy of the vaccine antigens under development, prior to age de-escalation studies into children as described above.

A number of human challenge models have been established to evaluate investigational clinical products under development. Among these, thousands of volunteers around the world have received experimental dengue, malaria (65), influenza (66), enterotoxigenic *E. coli* (67), and *Shigella* infections (68), to name a few. Model development includes identification of the optimal dose and route of administration of an appropriate challenge strain or challenge organism, which results in safe but measurable infection, mirroring natural infection as closely as possible. Through the development and use of these models, preliminary assessment of vaccine or drug efficacy against targeted bacteria, viruses, and parasites can be obtained earlier in clinical development, for more rapid evaluation of go/no-go criteria, and better deployment of resources toward the most promising vaccine or drug candidates. Recent vaccine candidates evaluated via human challenge models include the RTS,S *Plasmodium falciparum* malaria vaccine and the influenza vaccine (66, 69).

The controlled human hookworm infection (CHHI) model is being developed as a tool to assess the potential efficacy of novel hookworm vaccine candidates early in their clinical development. The prior studies described above demonstrate that experimental infection with *N. americanus* can be induced in a safe and controlled manner, with predictable and manageable adverse effects. *N. americanus* is an easily curable infection using readily available licensed medications that result in rapid resolution of symptoms should they occur and be intolerable to study participants.

2.2.4 Inclusion of Placebo

Placebo recipients are included in order to maintain blinding, provide safety reference data, provide controls for the immunogenicity assays, and to confirm the infectivity of the *N. americanus* Larval Inoculum (i.e., infectivity controls) during the CHHI stage of the trial.

2.2.5 Clinical Development Plan for the Human Hookworm Vaccine

The target population for the human hookworm vaccine is children less than 10 years of age living in hookworm endemic areas, since this is the age group that is most at risk of disease from this infection. Prior to conducting clinical trials in this age group, however, *Na*-GST-1 will be tested in healthy, hookworm-naïve adults living in area where hookworm is not endemic, to provide proof-of-efficacy before large-scale, long-duration and expensive pediatric Phase 2 and 3 field trials are undertaken in endemic areas.

The decision to proceed to Phase 2 and 3 testing in children will be taken after the Sponsor has reviewed the final clinical study report of the study described in this protocol. Phase 1 testing of *Na*-GST-1/Alhydrogel is currently planned to start in the third quarter of 2016 in children aged 5-10 years living in a hookworm endemic area of Gabon. After safety and immunogenicity of this product is shown in children, and if the results of the Phase 2 trial described in this protocol indicate efficacy of at least one of the *Na*-GST-1 formulations, further and more extensive testing will be pursued, ultimately culminating in Phase 2 and 3 trials in this target population in which protection against natural infection will be the primary endpoint.

In the first pediatric Phase 2 trial, the hookworm vaccine will be compared to a licensed comparator vaccine to evaluate the impact of mean fecal egg counts as well as a variety of clinical and parasitological endpoints. Assuming that an impact on infection is shown in the Phase 2 trial, that there are no new safety issues that arise due to co-formulation of the antigens, and that combining them into one product does not adversely affect the immunogenicity of either, this product will be tested in a pivotal multi-center Phase 3 trial in children. The primary endpoint of the Phase 3 trial will be the incidence of moderate and heavy hookworm infection (as determined by fecal egg counts) following administration of an anthelminthic and vaccination.

2.3 Potential Risks and Benefits

2.3.1 Potential Risk

Risks to subjects are those associated with venipuncture, intramuscular injection of the *Na*-GST-1 vaccine formulations, possible reactions to the vaccine formulations, possible reactions to CHHI, possible reactions to albendazole, and breach of confidentiality.

2.3.1.1 Risks of Venipuncture

Risks occasionally associated with venipuncture include pain and bruising at the site of venipuncture, lightheadedness, and syncope (rarely). Intramuscular (IM) injection also may cause transient discomfort and fainting.

2.3.1.2 Risks of Vaccination

Possible local vaccine reactions include pain, swelling, erythema, induration, transient limitation of limb movement, lymphadenopathy, or pruritus at the injection site. Local subcutaneous nodules, believed to be granulomatous reactions to aluminum hydroxide, have been observed with use of aluminum hydroxide-based adjuvants. Thus, most aluminum hydroxide-adsorbed vaccines are injected intramuscularly rather than subcutaneously. Systemic reactions such as fever, headache, malaise, myalgia, and joint pain, may also possibly occur. Immediate hypersensitivity reactions including urticaria, anaphylaxis, or other IgE-mediated responses are possible as with any vaccine. As with any investigational vaccine, there is a theoretical possibility of risks about which we have no present knowledge. Subjects will be informed of any such risks should further data become available.

Transient, clinically insignificant decreases in white blood cell counts and platelet counts have been observed in the two weeks following receipt of other CpG ODN adjuvant preparations.

CpG 10104 is an immune activator, and has the potential to overcome the normal tolerance of the immune system for self antigens. In a number of murine models, administration of CpG ODN has been associated with development of certain autoimmune phenomenon. However, the relevance of this finding to humans is unclear because while TLR9 receptors are widely distributed among immune cells in mice, they appear to be restricted to B cells and plasmacytoid dendritic cells in humans. No subject in any study to date with CpG 7909 oligonucleotides, a closely related CpG sequence to CpG 10104, has developed signs or symptoms of autoimmune disease.

In animal studies, CpG ODN given by themselves only rarely induce the production of anti-double-stranded DNA antibodies. However, if the CpG 7909 ODN is administered as a complex together with a foreign protein or other antigen that can elicit T cell help, then it is likely that antibodies against the CpG 7909 ODN will be produced, and these may cross-react with double-stranded DNA. Vaccination of humans with a CpG 7909 ODN together with an antigen capable of binding to the ODN could be expected to result in the production of IgG anti-ODN antibodies, which could cross react with self DNA.

This phenomenon has been observed in some clinical studies with CpG ODNs involving use of CpG 7909 as an antineoplastic agent, in which study subjects have developed detectable antibody to double-stranded DNA. These antibodies have generally declined to baseline after cessation of CpG 7909, and have not been associated with other autoimmune phenomenon, and their possible clinical significance is unknown. Subjects in the study described in this protocol will be closely monitored for laboratory evidence (e.g., anti-double stranded DNA antibodies or rheumatoid factor) or clinical signs or symptoms of autoimmunity

Female subjects will be cautioned of the unknown risk of the *Na*-GST-1/Alhydrogel® vaccine administered with or without AP 10-701 or CpG 10104, and of hookworm infection (and of the albendazole used to treat hookworm infection) to the fetus. A female of childbearing potential, unless surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy), at least 2 years postmenopausal, practices abstinence, or has a vasectomized partner, must practice effective contraception (including oral, intra/transdermal, intravaginal, or implanted hormonal contraceptives; intrauterine device (IUD); double barrier methods [condom plus foam or spermicide, diaphragm plus foam or spermicide], or the Essure procedure) until documented clearance of hookworm infection following treatment with albendazole. Female subjects will be counseled by a study team member, or referred to the health provider of their choice, for evaluation and institution of an appropriate contraceptive method.

Female subjects will be cautioned of the theoretical risk of albendazole during pregnancy. Per the FDA's categorization of drug risks to a fetus, albendazole is Category C, indicating that animal reproduction studies have shown an adverse effect on the fetus. However, teratogenicity in humans has not been observed, and a study of over 800 women treated with albendazole during the second and third trimesters demonstrated no adverse effects (70). Use in the first trimester, however, is still not recommended, but potential benefits may warrant use of the drug in pregnant women despite potential risks. Albendazole has been shown to cause embryotoxicity and skeletal malformations in pregnant rats and rabbits. Female subjects will be counseled on the potential risks of albendazole use during pregnancy at each weekly visit.

2.3.1.3 Risks of Experimental Infection with *N. americanus* Larval Inoculum

Expected adverse effects of being inoculated with *N. americanus* infective larvae are local skin reactions at the site of application of the larvae and gastrointestinal symptoms upon maturation of the parasites into adult hookworms in the small intestine of the host. Reported skin reactions include localized maculopular or papulovesicular rash that may be significant and prolonged (up to a month) when higher doses (≥50) of larvae are applied. Occasional respiratory symptoms may be experienced during the migration of larvae through the lungs, including dry cough, shortness of breath, and wheeze. Reported gastrointestinal symptoms include abdominal bloating and pain, flatulence, diarrhea, nausea, and vomiting.

As with any investigational product, there is a theoretical possibility of risks of CHHI about which we have no present knowledge. Subjects will be informed of any such risks should further data become available during the course of this or other clinical studies. Risks associated with chronic hookworm infection include iron deficiency, iron-deficiency anemia (associated with fatigue and malaise) and chronic gastrointestinal symptoms including flatulence, abdominal bloating, abdominal pain, diarrhea, nausea, and vomiting. However, these usually only occur after several months or years of infection, which will not occur during this study.

Volunteers in this study who are experimentally infected with the *N. americanus* Larval Inoculum may develop patent infections and shed hookworm eggs in their feces. It is extremely unlikely that a bystander individual – for example, someone living in the same household as the study subject – could become infected with *N. americanus* due to exposure to these hookworm eggs that are shed, for several reasons. First, to become infectious to humans, hookworm eggs must hatch to release larvae into the environment. Hatching and development into infective larvae require a moist and warm environment (optimally between 23-33˚C) and a certain period of time, generally estimated to be at least a week. Second, *N. americanus* larvae can infect humans only by coming into contact with skin; they are not infectious by oral ingestion.

Therefore, in order for a study subject to transmit their hookworm infection to another person, they would have to defecate such that their feces did not get disposed in a toilet and were incubated at a warm temperature in adequate moisture for over a week. The incubated feces would then have to come into contact with another person's skin. Since almost all residents of the Washington, DC, area have access to flush toilets, the prospect of them depositing their feces outside of a toilet and into a warm, moist environment such that the hookworm eggs could incubate and hatch is quite far-fetched. However, to prevent this extremely unlikely event from occurring, all study subjects will be counseled to practice good hygiene and to always defecate in a flush toilet and to dispose of all fecally contaminated matter immediately into a toilet.

2.3.1.4 Risks of Albendazole

Albendazole is a licensed anthelminthic medication that is used ubiquitously throughout the world to treat intestinal nematode infections. Expected adverse effects associated with administration of three daily doses of albendazole include abdominal pain, nausea, vomiting, and diarrhea.

2.3.2 Precautions Taken to Minimize Risks

In order to minimize the risk to subjects, all subjects will be monitored closely during their participation in this study. The study vaccines, AP 10-701, and CpG 10104 have been produced according to current Good Manufacturing Practices (GMP). The *N. americanus* larvae that will be used in this study have been produced according to the procedure outlined in the IND for this product. The vaccine products and *N. americanus* larvae will be administered by experienced investigators with drugs and equipment available for the treatment of anaphylaxis and other potential adverse reactions. All vaccine doses will be given by intramuscular injection to minimize injection site reactions such as pain.

Subjects will be monitored throughout their infection for the development of anemia, by means of regular complete blood counts. Any subject who develops significant anemia during the study

that meets one of the halting rules (see **Section 9.5**) will be treated with albendazole for the infection, according to the procedure outlined in **Section 7.7** of the protocol.

Maintenance of Confidentiality
Subjects will be asked to provide personal health information. All attempts will be made to keep this information confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subjects' personal health information. All records will be kept in a locked file cabinet or maintained in a locked room at the study site. Electronic files will be password protected. Only people who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the personal health information that is collected. Any publications from this study will not use information that will identify subjects by name.

2.3.3 Known Potential Benefits

Subjects may not receive any direct benefit from participation in this study. It is hoped that information gained in this study will contribute to the development of a safe and effective hookworm vaccine.

3 OBJECTIVES

3.1 Study Objectives

Primary:

1. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on controlled human hookworm infection (CHHI) with *N. americanus* larvae in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.
2. To evaluate the safety and reactogenicity of *Na*-GST-1/ Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on Days 0, 56 and 112, in healthy, hookworm-naïve adults.

Secondary:

1. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on fecal egg counts, as determined by the McMaster method, after controlled human hookworm infection (CHHI) with *N. americanus* larvae in healthy, hookworm-naïve adults.
2. To assess the relationship between antibody responses to *Na*-GST-1 induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.
3. To assess the duration of antibody responses to *Na*-GST-1 induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104.

Exploratory:

1. To assess the relationship between the functional capacity of vaccine-induced antibodies that neutralize the *in vitro* activity of native *Na*-GST-1 enzyme and responses to CHHI in healthy, hookworm-naïve adults.
2. To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on levels of hookworm DNA as detected by real-time PCR in healthy, hookworm-naïve adults challenged with CHHI, as determined by the presence of eggs using a qualified flotation technique.
3. To assess the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on the affinity of antibody-antigen interactions, and how affinity relates to responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.
4. To assess the relationship between *Na*-GST-1 specific memory B cells induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

5. To assess the relationship between innate immune responses to *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Efficacy

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. Proportion of subjects with detectable hookworm eggs, at any time point, in fecal samples, as determined by microscopy using the qualified saline flotation technique.

Safety

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. Frequency of solicited injection site and systemic reactogenicity, graded by severity, on the day of each study vaccination through 14 days after each study vaccination.
2. Frequency of solicited adverse events, graded by severity, on the day of CHHI through study Day 280.
3. Frequency of study vaccine-related Serious Adverse Events from the time of the first study vaccination through approximately 10 months after the last study vaccination.
4. Frequency of clinical safety laboratory adverse events.
5. Frequency of unsolicited adverse events, graded by severity, from the time of each study vaccination through approximately 1 month after each study vaccination; and from the time of CHHI through treatment with albendazole (Day 297).
6. Frequency of new-onset chronic medical conditions through approximately 10 months after the third study vaccination.
7. Frequency of Adverse Events of Special Interest through approximately 10 months after the third study vaccination.

3.2.2 Secondary Outcome Measures

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

Efficacy

1. Fecal egg counts as determined by microscopy using the McMaster method, during Weeks 5 through 20 post-CHHI.

Immunogenicity

2. The anti-*Na*-GST-1 IgG antibody response, by a qualified indirect enzyme-linked immunosorbent assay (ELISA) at approximately 14 days after each vaccination, and approximately 1, 2, 4, 6, 7, 8 and 10 months after the third dose.

3.2.3 Exploratory Outcome Measures

1. The functional capacity of vaccine-induced antibodies to neutralize the *in vitro* activity of the native *Na*-GST-1 enzyme.
2. Levels of *N. americanus* DNA in fecal samples, as measured by real-time PCR, during Weeks 5 through 20 post-CHHI.
3. The affinity of the antibody interactions with recombinant *Na*-GST-1 antigen at approximately 14 days after each vaccination, and approximately 1, 2, 4, 6, 7, 8 and 10 months after the third dose.
4. The production of memory B cells specific for *Na*-GST-1 on days of vaccination; approximately 7 and 14 days following each vaccination; on day of CHHI; and then 7 and 14 days, and 5, 7, 13, and 20 weeks post-CHHI.
5. Innate immune responses on days of vaccination approximately 7 and 14 days following each vaccination; on day of CHHI; and then 7 and 14 days, and 5, 7, 13, and 20 weeks post-CHHI.

4 STUDY DESIGN

The study will be a randomized, double blind, placebo-controlled Phase 2 clinical trial in healthy hookworm-naïve adult volunteers. This study is designed to evaluate the efficacy, safety, reactogenicity, and immunogenicity of *Na*-GST-1/Alhydrogel® administered with or without the point-of-injection addition of the immunostimulants AP 10-701 or CpG 10104. To assess the impact of vaccination with these different vaccine formulations on infection, subjects will be challenged with 50 infectious *N. americanus* larvae by CHHI 4 weeks after the 3rd vaccination. Safety parameters will be monitored throughout the study.

Volunteers will be invited to participate in the study by means of verbal, email, and listserve announcements and poster, newspaper, and online advertisements (including ResearchMatch). After providing written informed consent, volunteers will undergo eligibility screening, including a complete medical history; physical examination; hematology testing; liver and renal function testing; anti-double stranded DNA (anti-dsDNA) and rheumatoid factor (Rf) testing; Human Immunodeficiency Virus (HIV), Hepatitis B, and C testing; fecal occult blood testing; fecal examination for ova and parasites; and, urinalysis (for protein and glucose). Urine pregnancy testing will be performed on all female volunteers. All clinically significant abnormalities will be reviewed with volunteers and they will be referred for follow-up care if appropriate. After screening, those volunteers determined to be eligible, based on the inclusion and exclusion criteria described in **Section 5** in this protocol, will be invited to participate in the study. No exemptions from the inclusion or exclusion criteria will be granted on inclusion/exclusion.

In the study, 48 volunteers will be enrolled and randomized into 4 groups. Groups will not be enrolled separately; rather, eligible volunteers will be randomly assigned to a group upon enrollment in a double-blind fashion (i.e., neither the subject nor the investigators will know to which group the subject has been assigned). In the first group, volunteers will receive 100µg *Na*-GST-1/Alhydrogel®. In the second group, volunteers will receive either 100µg *Na*-GST-1/Alhydrogel® co-administered with 500µg CpG 10104. In the third group, volunteers will receive either 100µg *Na*-GST-1/Alhydrogel® co-administered with 5µg AP 10-701. In the fourth group, volunteers will receive sterile saline.

As with other aluminum hydroxide-adsorbed vaccines, hypersensitivity reactions would be expected to occur within the first 24 hours after vaccination, and other severe local or systemic reactions within 72 hours of vaccination. Subjects will therefore be observed for immediate reactions following each vaccination for at least 1 hour, and will be assessed on Days 3, 7, 14 and 28 following each vaccination either in the study clinic or by telephone. See **Appendix A** for a detailed description of the scheduled clinical and laboratory evaluations.

Subjects will be challenged by CHHI with 50 infectious *N. americanus* larvae 4 weeks after 3rd vaccination. After CHHI, subjects will be observed for immediate reactions following the application of larvae for at least 1 hour. Fecal samples will be collected at least every other week starting 5 weeks post-CHHI until 2 weeks following treatment with albendazole at 20 weeks post-challenge to cure infections.

Subjects will be followed until 10 months after their final vaccination, for a total of 14 months of study participation. See **Appendix A** for a detailed description of the scheduled clinical and laboratory evaluations during the Vaccination and CHHI phases of the trial.

The Safety Monitoring Committee (SMC) and Independent Safety Monitor will have access to the randomization code for the study, as they may wish to review the data in an unblinded fashion should significant safety questions arise prior to the final unblinding. The trial will not proceed if any of the halting criteria listed in **Section 9.5** are met or, in the clinical judgment of the SMC, Independent Safety Monitor, and/or DMID Medical Monitor, continuation would pose an unacceptable safety risk to the subjects.

4.1 Substudies (if applicable)

Not Applicable.

5 STUDY ENROLLMENT AND WITHDRAWAL

Only subjects who meet all of the inclusion and none of the exclusion criteria will be eligible for enrollment into this study. No exemptions will be granted.

A total of 48 subjects will be enrolled. The study population will be enrolled from the metropolitan Washington, DC area.

Volunteers agreeing to participate will first provide written informed consent. Signing the informed consent form will provide consent for both screening procedures and study procedures following enrollment. The original signed informed consent form for each volunteer will be maintained as part of that volunteer's study records. A copy of the informed consent form will be provided to every volunteer.

Screening can occur up to 90 days prior to enrollment; enrollment/randomization, and administration of first dose of study products will occur on the same day. Volunteers will not be considered enrolled in the study until they have received their first dose of vaccine.

5.1 Subject Inclusion Criteria

1. Males and non-pregnant females between 18 and 45 years, inclusive.
2. Good general health as determined by means of the screening procedures[1].

   Existing medical diagnoses or conditions (except those in the Subject Exclusion Criteria below) must be deemed as stable chronic medical conditions. A stable chronic medical condition is defined as no change in prescription medication, dose, or frequency of medication in the last 90 days and health outcomes of the specific disease are considered to be within acceptable limits in the last 180 days. Any change due to change of health care provider, or that is done for financial reasons, as long as in the same class of medication, will not be considered a violation of this inclusion criterion. Any change in prescription medication due to improvement of a disease outcome, as determined by the Principal Investigator or a co-investigator, will not be considered a violation of this inclusion criterion. Individuals may be taking chronic or as needed medications if, in the opinion of the Principal Investigator or co-investigator, they pose no additional risk to safety or assessment of reactogenicity and immunogenicity. *Topical, nasal, and inhaled medications (with the exception of corticosteroids as outlined in the Subjects Exclusion Criteria [Section 5.2]), vitamins, and contraceptives are permitted.*

3. Available for the duration of individual subject study participation (14 months).
4. Willingness to participate in the study as evidenced by signing the informed consent document.
5. Able to understand and comply with planned study procedures.

5.2 Subject Exclusion Criteria

1. Pregnancy as determined by a positive urine human choriogonadotropin (hCG) test (if female).
2. Subject unwilling to use effective contraception for a minimum of 30 days prior to vaccination and up until documentation of clearance of hookworm infection post-CHHI (if female and not surgically sterile, abstinent from intercourse with a male partner, in a monogamous relationship with a vasectomized partner, at least 2 years post-menopausal, or determined otherwise by medical evaluation to be sterile).
3. Currently lactating and breast-feeding or plans to breastfeed at any given time from the first study vaccination until clearance of hookworm infection post-CHHI (if female).
4. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, rheumatologic, autoimmune, gastrointestinal, diabetes, or renal disease by history, physical examination, and/or laboratory studies.[2]

   [2]*Includes the conditions and diagnoses defined as AESIs in Section 9.3.4.*

5. Has a diagnosis of schizophrenia, bipolar disease or other major psychiatric condition that would make compliance with study visits/procedures difficult (e.g., subject with psychoses or history of suicide attempt or gesture in the 3 years before study entry, ongoing risk for suicide).
6. Known or suspected immunodeficiency or immunosuppression as a result of an underlying illness or treatment.[3]

   [3]*Causes for immunosuppression may include, but are not limited to, poorly-controlled diabetes mellitus, chronic liver disease, renal insufficiency, active neoplastic disease or a history of hematologic malignancy, connective tissue disease, organ transplant.*

7. Laboratory evidence of liver disease (alanine aminotransferase [ALT] greater than 1.25-times the upper reference limit).
8. Laboratory evidence of renal disease (serum creatinine greater than 1.25-times the upper reference limit, or urine dipstick testing positive for glucose or more than trace protein).
9. Laboratory evidence of hematologic disease (hemoglobin <11.1 g/dl [females] or <12.5 g/dl [males]; absolute leukocyte count <3400/mm^3 or >10.8 x 10^3/mm^3; absolute eosinophil count >500/mm^3; or platelet count <140,000/mm^3).
10. Other condition that in the opinion of the investigator would jeopardize the safety or rights of a volunteer participating in the trial or would render the subject unable to comply with the protocol.
11. Planned participation in another investigational vaccine or drug trial within 30 days of starting this study or until the last study visit.[4]

    [4]*This may include other licensed or unlicensed vaccines, drugs, biologics, devices, blood products, or medications.*

12. Volunteer has had medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 24 months.

13. Positive fecal occult blood test at screening.
14. Infection with a pathogenic intestinal helminth as determined by stool examination for ova and parasites at screening.
15. History of iron deficiency anemia or laboratory evidence of iron deficiency (serum ferritin concentration below the lower reference limit).
16. History of hypoalbuminemia.
17. History of a severe allergic reaction or anaphylaxis.
18. Severe asthma as defined by the need for daily use of corticosteroid and/or long-acting beta agonist inhalers, or emergency clinic visit or hospitalization within 6 months of the volunteer's expected first vaccination in the study.
19. Positive test for hepatitis B surface antigen (HBsAg).
20. Positive confirmatory test for HIV infection.
21. Positive confirmatory test for hepatitis C virus (HCV) infection.
22. Using or intends to continue using oral or parenteral corticosteroids, high-dose inhaled corticosteroids (>800 µg/day of beclomethasone dipropionate or equivalent) or other immunosuppressive or cytotoxic drugs within 30 days of the volunteer's expected first vaccination in this study or planned use during the study.
23. Receipt of a live vaccine within 4 weeks or a killed vaccine within 2 weeks prior to the volunteer's expected first vaccination in the study.
24. Receipt of immunoglobin or other blood products (with exception of Rho D immunoglobulin) within 90 days of the planned first study vaccination.
25. Known allergy to albendazole, amphotericin B or gentamicin.
26. History of previous infection with hookworm or continuous residence for more than 6 months in a community where hookworm is endemic.
27. Current or past scars, tattoos, or other disruptions of skin integrity at the intended site of larval application.
28. Previous receipt of the *Na*-GST-1/Alhydrogel® hookworm vaccine.
29. History of a surgical splenectomy.
30. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosis, rheumatoid arthritis, multiple sclerosis, Sjogren's syndrome, autoimmune thrombocytopenia; or laboratory evidence of possible autoimmune disease determined by a positive anti-dsDNA titer, positive rheumatoid factor, and/or proteinuria (greater than trace protein on urine dipstick testing).
31. Two or more first-degree family members with autoimmune or antibody-mediated disease including but not limited to those listed in exclusion criterion #30.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Eligible volunteers will be asked to come to the clinic on their scheduled day of enrollment into the study (Day 0). After undergoing a clinical interview and directed physical examination to ensure that they remain eligible for participation in the study, that they have had blood collected for safety clinical laboratory and baseline immunogenicity assessments, and that females have had a urine pregnancy test performed that is documented to be negative, volunteers will be randomized and enrolled in the order that they present for vaccination.

Within each group, randomization will be done through use of a randomization code, furnished to the study vaccine manager by the study statistician. Access to the randomization list will be exclusively limited to the study vaccine manager and assistant. Between vaccination days, the randomization list will be stored in a locked cabinet in the GW MFA Investigational Drug Service pharmacy or the office of the study vaccine manager. The study vaccine manager and assistant will be unblinded, but will not be involved in further evaluation of study subjects or assessment of adverse events. Volunteers will not be considered enrolled in the study until they have received their first dose of vaccine. In the event that a volunteer is randomized but not enrolled on the day of first vaccination, they will be replaced with an eligible alternate. Enrolled subjects that leave the study for any reason following first vaccination will not be replaced.

Vaccine/placebo assignment will be done by block randomization. That is, for each block of 4 subjects who are sequentially enrolled into the trial, 1 will be assigned to receive *Na*-GST-1/Alhydrogel®, 1 to *Na*-GST-1/Alhydrogel® plus CpG 10104, 1 to *Na*-GST-1/Alhydrogel® plus AP 10-701, and 1 to sterile saline placebo.

5.3.2 Masking Procedures

Due to the double-blind nature of this Phase 2 clinical trial, neither study subjects nor study team personnel will know to which group an individual subject has been assigned. Investigators and subjects will be blinded to the vaccine/placebo allocation until all subjects have completed their final study visit (i.e., Visit #39 at Day 380), the primary (efficacy and safety) and secondary IgG immunogenicity outcomes (i.e., anti-*Na*-GST-1 IgG antibody results by ELISA) have been monitored and entered into the database, and the database has been locked for analysis.

The study vaccine manager will also prepare all investigational product doses (vaccine or sterile saline placebo) in a separate room, and will hand filled syringes to the vaccinator(s). Since the doses of the *Na*-GST-1 formulations are of different opacity than that of sterile saline (slightly turbid *vs*. clear, respectively), the contents of all syringes will be disguised using opaque tape. As a further precaution, the vaccinator(s) will not be involved in assessments of reactogenicity or adverse events.

5.3.3 Reasons for Withdrawal and Discontinuation of Vaccinations

Subjects are free to withdraw from the study at any time and for any reason. Subjects who have received vaccine, regardless of the number of doses received, or who developed an AE or SAE will be encouraged to remain in the study to be followed for safety purposes.

Subjects may voluntarily withdraw their consent for study participation at any time and for any reason, without penalty.

A subject may withdraw or be withdrawn from the study for any of the following reasons:

- Medical disease or condition, or any new clinical findings for which continued participation, in the opinion of the Principal Investigator or appropriate co-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of the study, or would interfere with the evaluation of responses.

- Subject no longer meets eligibility criteria.
- As deemed necessary by the Principal Investigator or appropriate co-investigator for noncompliance or other reasons.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Termination of the study.
- New information becomes available that makes further participation unsafe.

The second or third study vaccination, or the CHHI, may not be administered to a subject if any of the following criteria are met:

- Medical condition (including pregnancy) for which continued participation, in the opinion of the Principal Investigator or appropriate co-investigator, would pose a risk to the subject or would be likely to confound interpretation of the results.
- Presence of signs or symptoms that could confound or confuse assessment of study vaccine reactogenicity. For subjects with injection site or systemic signs or symptoms, or with an acute illness, including an oral temperature greater than 38.0°C, the study vaccination should/may be postponed/deferred until signs, symptoms, or acute illness have resolved and if within the acceptable protocol-specified window for that visit. If outside this window, the Sponsor must first approve the second or third study vaccination and the documentation of approval should be filed in the subject's chart.
- Any unresolved or continuing solicited or unsolicited Grade 3 adverse event. An unresolved or continuing Grade 1 or Grade 2 adverse event is permissible unless, in the opinion of the Principal Investigator or appropriate co-investigator, it would render study vaccination unsafe or interfere with the evaluation of responses.
- Grade 3 clinical safety laboratory value that does not decrease to Grade 2 or less prior to the second or third study vaccination or prior to planned CHHI. Any clinical safety laboratory parameter may be re-evaluated only once at the central (clinical) laboratory prior to the second or third study vaccination, or CHHI. If the clinical safety laboratory value decreases to Grade 2 or less, the subject may receive the second or third study vaccination, or CHHI. The second or third study vaccination, or the CHHI, should be scheduled to occur within the acceptable protocol-specified window for that visit. If outside this window, the Sponsor must first approve the study vaccination or CHHI and the documentation of approval should be filed in the subject's chart.
- Severe or sustained reaction or disability related to a preceding study vaccination.
- New onset of illness or condition that meets exclusion criteria.
- Subject no longer meets eligibility criteria.

- As deemed necessary by the Principal Investigator or appropriate co-investigator for noncompliance or other reasons.
- Subject refusal of further study vaccination.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Termination of the study.
- New information becomes available that makes further participation unsafe.

5.3.4 Handling of Withdrawals

The primary reason for withdrawal from the study will be recorded on the appropriate data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in **Section 7.5**. Although subjects are free to withdraw at any time or may be withdrawn by the Principal Investigator or appropriate co-investigator at any time, subjects who receive at least one dose of study vaccine will be encouraged to remain in the study for follow-up safety assessments and collection of venous blood samples for immunogenicity testing and fecal samples for parasitological testing. Every attempt will be made to follow all adverse events, including solicited injection site and systemic reactions, serious adverse events, and new-onset chronic medical conditions ongoing at the time of early withdrawal to resolution.

Subjects who have received *N. americanus* challenge by CHMI who withdraw or are withdrawn before Day 280 post-CHHI and who have not been treated with albendazole for hookworm infection must receive early presumptive treatment for hookworm or else agree to continue be followed until Day 297 (Visit #37) post-CHHI. Subjects who elect to withdraw from the study will not be subjected to additional research portions of the study (research blood collections, fecal collections, etc.) although they will be strongly advised to continue with the safety portions (i.e., fecal testing for hookworm eggs, safety laboratory tests) of the study.

Although subjects are free to withdraw at any time or may be withdrawn by the Principal Investigator or appropriate co-investigator at any time, subjects will be encouraged to remain in this study for safety follow-up assessments (at a minimum, by telephone if not in person) continuing through approximately study Day 140 for subjects who do not receive CHHI, or study Day 189 (approximately 6 weeks post-CHHI) for subjects who receive CHHI. In the latter case, volunteers will be encouraged to remain in the study until Day 189 to ensure that treatment with albendazole is given after the point where the hookworms would have become adult worms, since the medication is not effective against the immature forms of the worm.

If voluntary withdrawal occurs, the subject will be asked for permission to continue scheduled safety evaluations and to complete an end-of-study evaluation. If the withdrawal is due to an AE or an SAE, appropriate medical care and supervision of the subject should continue until the AE or SAE resolves or the subject's condition becomes stable.

If it is felt that inclusion of the study subject's data for analysis is compromised, the study subject will be terminated from the study, and the relevant study data may not be included in analysis. However, all data generated before withdrawal that is not compromised will be included. This does not preclude the ethical responsibility of the investigators to ensure the safety of the subject and offer curative therapy for hookworm if they have received CHHI.

If possible, subjects who leave the study area will be traced and visited by clinical investigators to collect safety follow-up data. In the case of subjects who fail to appear for a follow-up assessment, extensive efforts (i.e., at least three documented phone calls and certified mail) will be made to locate or recall them, or at least to determine their health status and provide treatment with albendazole if CHHI has been performed. These efforts will be documented in the subjects' records. Any subject who does not adhere to the protocol requirements must be classified (e.g., non-compliant, AE, lost-to-follow-up, or other) and the classification will be recorded on the appropriate Case Report Form.

Pregnancies occurring in study subjects will be reported on the study Pregnancy Report Form. No further study vaccinations or CHHI will be administered to pregnant subjects.

Subjects who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after being randomized will not be replaced. Subjects who have signed the informed consent form and who meet all inclusion/exclusion criteria after the screening visit and procedures will be randomized to vaccine formulation/placebo assignment until the study sample size for randomized subjects has been met.

5.3.5 Termination of Study

Any recommendation of the Independent Safety Monitor, DMID Medical Monitor, and SMC to resume or suspend further injections (either for an individual subject or an entire dose Group) will be communicated in writing to the sponsor and Principal Investigator. All communications from the SMC will subsequently be forwarded by the investigators to the IRB of the George Washington University.

The study Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Incidence or severity of AEs indicating a potential health hazard
- Data recording is inaccurate or incomplete
- The Investigator has not adhered to the protocol or applicable regulatory guidelines in conducting the study

In addition, the study may be discontinued at the discretion of the U.S. FDA or the study site.

6 STUDY INTERVENTIONS/INVESTIGATIONAL PRODUCTS

Investigators will receive the current version of the Investigator's Brochures for *Na*-GST-1/Alhydrogel® (with or without AP 10-701), *Na*-GST-1/Alhydrogel® (with or without CpG 10104), and *Necator americanus* Larval Inoculum, which comprehensively describe all available preclinical and human experience with the experimental vaccine formulations and CHHI. If relevant new information becomes available during the course of the trial, the investigators will receive the revised Investigator's Brochure(s).

6.1 Study Product Description

_Na_-GST-1/Alhydrogel®

Na-GST-1 contains recombinant *N. americanus* Glutathione-S-Transferase-2 (*Na*-GST-1), which is expressed in modified *Pichia pastoris*. *Na*-GST-1 from the human hookworm *N. americanus* is a 24 kDa protein with peroxidase enzymatic activity that catalyzes the conjugation of reduced glutathione to a variety of electrophiles, including toxic compounds generated as products of blood degradation by the adult hookworm. When recombinant Na-GST-1 is used as a vaccine, it is hypothesized that it will induce neutralizing antibodies that will interfere with parasite blood-feeding and cause parasite death or reduce worm fecundity. The three formulations that will be used in this study consist of:

(a) *Na*-GST-1 adsorbed to Alhydrogel®;

(b) *Na*-GST-1 adsorbed to Alhydrogel® and administered with the novel immunostimulant AP 10-701, or Gluco-Pyranosylphospho-Lipid A Aqueous Formulation (GLA-AF), a synthetic lipid A molecule that is a Toll-Like Receptor (TLR)-4 agonist;

(c) *Na*-GST-1 adsorbed to Alhydrogel® and administered with the novel immunostimulant CPG 10104, a synthetic oligodeoxynucleotide molecule that is a Toll-Like Receptor (TLR)-9 agonist.

AP 10-701

AP 10-701, previously referred to as Gluco-Pyranosylphospho-Lipid A Aqueous Formulation (GLA-AF), is a TLR-4 agonist. Point-of-injection formulations with this immunostimulant will be prepared immediately prior to vaccination by adding an appropriate volume of AP 10-701 to *Na*-GST-1/Alhydrogel® and withdrawing an appropriate volume to administer the desired amount of *Na*-GST-1/Alhydrogel® plus 5mcg AP 10-701.

CPG 10104

CPG 10104 is a TLR-9 agonist. Point-of-injection formulations with this immunostimulant will be prepared immediately prior to vaccination by adding an appropriate volume of CPG 10104 to *Na*-GST-1/Alhydrogel® and withdrawing an appropriate volume to administer the desired amount of *Na*-GST-1/Alhydrogel® plus 500mcg CPG 10104.

Normal Saline, 0.9% Sodium Chloride

Normal saline will be used as the placebo. It consists of a sterile 0.9% physiologic solution of sodium chloride that has no pharmacologic activity.

6.1.1 Acquisition

Na-GST-1/Alhydrogel® for this study will be supplied to the study site by the Baylor College of Medicine. Vials of CpG 10104 and AP 10-701 will be purchased from the Infectious Diseases Research Institute (IDRI).

Normal saline (0.9% sodium chloride injection, USP) and albendazole will be purchased commercially by the GWU Investigational Drug Service.

The *Necator americanus* Larval Inoculum that will be used for CHHI in this study will be supplied by the Department of Microbiology, Immunology, and Tropical Medicine of GWU under a current Investigational New Drug application to the US FDA (IND#015752). Briefly, *N. americanus* eggs will be obtained from the feces of chronically infected human volunteers who are subjects in a GWU IRB-approved clinical trial of CHHI (NCT#01940757). These individuals are regularly tested to ensure that they are negative for HIV, HBV, and HCV. Fecal material is processed following a qualified standard procedure, and after hatching from eggs, the *N. americanus* larvae are stored in the dark at ambient temperature (19-25^{o}C) until use. Controls for the manufacturing process are tests for viability (motility), species identification, and microbial bioburden of the larvae. Donors are tested for HIV, HBV, and HCV prior to release of each new batch of larvae and release will not be accepted if any of these tests are positive.

Na-GST-1/Alhydrogel®, CpG 10104, AP 10-701, and the sterile saline placebo will be transported to the Investigational Drug Service at GWU MFA at 0.5^{o}C to 10^{o}C; temperature recording devices will accompany the vaccines at all times during transport to ensure temperature limits have not been violated.

6.1.2 Formulation, Packaging, and Labeling

6.1.2.1 Na-*GST-1/Alhydrogel®*

Na-GST-1/Alhydrogel® is supplied as a sterile milky-white suspension (when shaken slightly). Each 2.0 ml vial contains 1.35 ml of a 0.1 mg/ml suspension of *Na*-GST-1 adsorbed to 0.8 mg/ml of Alhydrogel® in a buffer consisting of 10% glucose and 10 mM imidazole, pH 7.4. Glucose acts as an excipient and imidazole as the buffer based on evidence that these components specifically enhance the stability and solubility of *Na*-GST-1. The dose that will be administered in this study is 100 µg of *Na*-GST-1, or 1.0 ml of the final drug product. This volume contains the equivalent of approximately 400µg aluminum. *Na*-GST-1/Alhydrogel® was manufactured, formulated and vialed at Walter Reed Army Institute of Research.

6.1.2.2 *CpG 10104*

CpG 10104 will be supplied as a clear to slightly hazy, colorless aqueous solution at a concentration of 2mg/mL, in multi-dose vials containing 0.8 ml each. Appropriate volumes of

CpG 10104 will be withdrawn and added to a vial containing *Na*-GST-1/Alhydrogel® (as described in the study Pharmacy Manual). The mixture must be administered not more than 4 hours after mixing the CpG 10104 with *Na*-GST-1/Alhydrogel®. CpG 10104 has been manufactured according to cGMP by the Infectious Diseases Research Institute (IDRI).

6.1.2.3 Glucopyranosyl-Lipid A Aqueous Formulation (AP 10-701)

AP 10-701 will be supplied as a 0.5 mL clear to slightly hazy, colorless aqueous solution in multi-dose vials containing 25 µg/mL of GLA without preservative. Appropriate volumes of AP 10-701 will be withdrawn from the multi-dose vials using a syringe and added to a vial containing *Na*-GST-1/Alhydrogel® (described in the Investigator's Brochure and study Pharmacy Manual). The mixture must be administered not more than 24 hours after mixing the AP 10-701 with *Na*-GST-1/Alhydrogel®. AP 10-701 has been manufactured according to cGMP by the Infectious Diseases Research Institute (IDRI).

6.1.2.4 Placebo (Normal Saline, 0.9% Sodium Chloride, USP)

Normal saline (0.9% sodium chloride injection, USP) is a sterile, nonpyrogenic, isotonic solution. Each mL contains sodium chloride 9 mg and contains no preservatives, bacteriostatic, antimicrobial agent, or added buffer. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3, range 4.5-7.0).

6.1.2.5 N. americanus *Larval Inoculum*

N. americanus larval inoculum will be supplied as a clear, colorless suspension in individual cryotube vials containing 50 infectious *N. americanus* larvae each in sterile water.

6.1.3 Product Storage and Stability

The vaccine, CpG, and AP 10-701 will be stored at the site in a refrigerator at 2°C to 8°C until just prior to administration and will not be frozen. Normal saline placebo will be stored at the site at room temperature (20°C to 25°C). All study product vials will be stored in the upright position.

N. americanus Larval Inoculum will be maintained at 19°C to 25°C until just prior to administration.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

Doses of *Na*-GST-1/Alhydrogel® administered without AP 10-701 or CpG 10104 will be prepared the site Investigational Pharmacist by withdrawing appropriate volumes into syringes of appropriate size. Study product doses will be prepared using aseptic technique in a biosafety cabinet. The *Na*-GST-1/Alhydrogel® plus AP 10-701 formulation will be prepared by adding appropriate volume of AP 10-701 solution to vials of *Na*-GST-1/Alhydrogel® within 24 hours of vaccination. The *Na*-GST-1/Alhydrogel® plus CpG formulation will be prepared by adding appropriate volume of CpG solution to vials of *Na*-GST-1/Alhydrogel® within 4 hours of vaccination. Doses of the sterile saline control will be prepared by withdrawing the appropriate volume into syringes of appropriate size.

Doses of vaccine/placebo will be administered by qualified study personnel in the deltoid muscle of the appropriate arm after disinfecting the skin with an alcohol swab and allowing it to dry.

Individual doses of *N. americanus* larvae will be prepared at MITM and transported to the study site at ambient temperature, immediately prior to application on a study subject's skin. Doses of the *N. americanus* larval inoculum will be prepared by pipetting the entire contents of the dose vial containing 50 motile larvae onto a sterile absorbent pad under a fume hood. The absorbent pad will then be directly applied to the subject's forearm and immobilized there using a transparent sterile adhesive dressing for at least one hour before removing and placing the pad back into the dose vial for counting of residual larvae at MITM.

6.3 Modification of Study Intervention/Investigational Product for a Subject

There will be no dose modifications. If a subject's second and/or third dose of study vaccination or CHHI is deferred, it should be rescheduled to occur within the acceptable protocol-specified window for that visit. If this period elapses, the site must obtain prior approval from the Sponsor to administer the second and/or third study vaccination, or the CHHI, and the documentation of approval should be filed in the subject's chart. Subjects who do not receive the second and/or third study vaccination or CHHI will be asked to return for safety assessments and for scheduled venous blood sample collections for immunogenicity testing and will be followed for the duration of the study.

Unblinding can occur upon request of the Independent Safety Monitor or the SMC at any time during the study.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

Study vaccine, AP 10-701, CpG 10104 and sterile saline placebo supplies must be received by a designated person at the GWU MFA Investigational Drug Service pharmacy, handled and stored safely and properly, and kept in a secure location to which only the designated individuals have access. Study-site personnel are responsible for maintaining accurate records of the vaccine supplies (i.e., *Na*-GST-1/Alhydrogel®, the sterile saline placebo, CpG10104, and AP 10-701) received, the quantities administered to study subjects, and the amounts remaining at the conclusion of the study.

After administration of vaccine or AP 10-701 or CpG 10104 doses, the empty or wasted vials will be accounted for and stored at the study site until monitoring by the study Sponsor or their designee. At the conclusion of the study, all used and unused *Na*-GST-1/Alhydrogel®, CpG 10104, and AP 10-701 vials will be returned to the Sponsor or destroyed on site upon direction from the Sponsor, or maintained at 2 to 8°C until further notice from the Sponsor regarding their disposition.

Supplies of *N. americanus* larval inoculum will be prepared and stored at the Department of MITM at GWU. Individual doses of larvae will be prepared on the planned day of inoculation and

transported to the study clinic for administration to study subjects. At the study clinic, the larval doses must be received by a designated person, handled and stored safely and properly and kept in a secure location prior to administration. MITM personnel will be responsible for maintaining accurate records of the supplies of the *N. americanus* larval inoculum, the quantities administered to study subjects, and the amounts remaining at the conclusion of the study

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

Not Applicable.

6.6 Concomitant Medications/Treatments

Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all medications taken within the 30 days prior to enrollment, 28 days after each dose of study vaccine, from study Day 140 to 297, and for new-onset chronic medical conditions through the end of participation in the study for each subject. Prescription and over-the-counter drugs will be included as well as vitamins and supplements.

Use of new medication will prompt an evaluation for the presence of a new diagnosis of chronic medical disease or chronic medical condition.

Medications that might interfere with the evaluation of the investigational vaccine formulations should not be used unless absolutely necessary. Medications in this category include, but are not limited to, glucocorticoids, i.e., high dose oral, parenteral and daily inhaled steroids, and immunosuppressive or cytotoxic drugs. Other than from participation in this study, subjects should not receive experimental agents, including vaccines, for the duration of the study.

The administration of licensed vaccines, should be delayed until 28 days after the last administration of investigational vaccine, with the exception of the inactivated influenza vaccine that should not be administered within 2 weeks of receipt of study vaccine (i.e., 2 weeks prior to planned study vaccination and 2 weeks after study vaccination).

7 STUDY SCHEDULE

The following sections provide a detailed listing of the procedures and studies to be performed in this protocol at designated time points. The total volume of blood (approximately 1091 mL) to be collected from each volunteer over 14 months is less than the volume collected when donating three units of blood and should not compromise the health of trial subjects.
See **Appendix A** for the schedule of study procedures.

7.1 Screening

Volunteers aged 18-45 years, inclusive, will be invited to undergo screening for the study. During this initial screening visit, a member of the study team will review the consent form with the volunteer. The study team member will explain the study to the volunteer and will clarify all of the volunteer's questions. Volunteers will be encouraged to ask questions. The volunteer may either sign the consent form immediately or later after further consideration.

The following procedures will be performed upon initial screening (note that all procedures might not be performed on the same day):

1. Explain the study and Informed Consent to the volunteer.
2. Elicit a complete medical history, including medication history, and for female subjects, a menstrual and contraceptive history and/or history of surgical sterility.
3. Administer a complete physical examination.
4. Obtain blood for hematology, biochemistry, anti-dsDNA, rheumatoid factor, serum ferritin, and tests for HIV and viral hepatitis (B and C).
5. Obtain urine for urine dipstick testing for glucose and protein, as well as urine pregnancy testing in females.
6. Counsel females to avoid becoming pregnant during the study.
7. Obtain feces for fecal occult blood testing and baseline parasitology.
    - Volunteers unable to provide a fecal sample during the screening visit may provide a sample prior to the planned Day 0 study visit. A fecal sample must be obtained prior to determination of eligibility and larval administration.
    - Explain procedures for fecal sample collection, storage, and transport to the clinic.
8. Explain the procedure for at-home fecal sample collection, storage, and transport to the clinic on or before Day 0.

Screening steps 2-8 must be performed within 90 days of the planned enrollment into the study. Should this screening window be exceeded before the first vaccination, screening procedures (not including administration of the informed consent form) may be repeated to ensure continued eligibility for the study (blood screening tests can be repeated a maximum of one time for this reason). Abnormal screening test results may be repeated to confirm the result, at the discretion of the investigator.

7.2 Enrollment/Baseline

Volunteers will not be considered enrolled in the study until they have received their first dose of vaccine. In the event that a volunteer is randomized but not enrolled on the day of first vaccination, they will be replaced with an eligible alternate.

Study Day 0 (Visit #1: Day of First Vaccination)

1. Verify that Informed Consent was obtained.
2. Verify that all applicable eligibility criteria have been met.
3. Perform abbreviated history (including concomitant medications) and physical exam, focusing on any acute complaints.
4. Obtain blood for hematology, biochemistry, rheumatoid factor, anti-dsDNA, anti-*Na*-GST-1 antibody and cellular immunology assays.
5. For females, obtain a urine sample for pregnancy testing. Test must be negative to proceed; a positive test will exclude the volunteer from the trial.
6. Record vital signs (blood pressure, oral temperature, and heart rate). Vital signs recorded pre-vaccination on Day 0 will be considered as baseline.
7. Subjects will be enrolled and randomly assigned to a study group by the vaccine manager prior to the first study vaccination.
8. Administer the vaccine in the deltoid muscle. Record the site of injection (i.e., right or left arm) on the appropriate data collection form.
9. Observe for at least 1 hour after vaccination to evaluate for immediate adverse reactions. During the 1-hour post-immunization wait period, study staff will discuss signs and symptoms of potential AEs, describe proper use of digital thermometers, injection-site reaction measurement tools, and subject symptom diaries.
10. Record vital signs at the end of the 1-hour post-immunization wait period.

7.3 Follow-up

Study Day 3 (Visit #2) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the post vaccination symptom memory aid).
2. Collect concomitant medication information.

Study Day 7 (Visit #3)

1. Perform basic history and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (oral temperature, pulse, and blood pressure).
3. Review and collect subject post vaccination symptom memory aid.
4. Obtain blood for cell-mediated immunity (CMI) assays.

Study Day 14 (Visit #4)

1. Perform basic history and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (oral temperature, pulse, and blood pressure).
3. Review and collect subject post vaccination symptom memory aid (if not collected on Visit #3/Study Day 7).

4. Obtain blood for hematology, biochemistry, rheumatoid factor, anti-dsDNA, anti-*Na*-GST-1 antibody and CMI assays.

Study Day 28 (Visit #5) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the post vaccination symptom memory aid).
2. Collect concomitant medication information.

Study Day 56 (Visit #6: Day of second vaccination)

1. Perform basic history (including concomitant medications) and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Obtain blood for hematology, biochemistry, rheumatoid factor, anti-dsDNA, anti-*Na*-GST-1 antibody and CMI assays.
3. For females, obtain a urine sample for pregnancy testing. Test must be negative to proceed; a positive test will exclude the volunteer from further vaccinations.
4. Record vital signs.
5. Administer the vaccine.
6. Observe for at least 1 hour after vaccination to evaluate for immediate adverse reactions. During the 1-hour post-immunization wait period, study staff will discuss signs and symptoms of potential AEs, describe proper use of digital thermometers, injection-site reaction measurement tools, and subject symptom diaries.
7. Record vital signs at the end of the 1-hour post-immunization wait period.

Study Day 59 (Visit #7) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the post vaccination symptom memory aid).
2. Collect concomitant medication information.

Study Day 63 (Visit #8)

1. Perform basic history and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Review and collect subject post vaccination symptom memory aid.
4. Obtain blood for CMI immunology assays.

Study Day 70 (Visit #9)

1. Perform basic history and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Review and collect subject post vaccination symptom memory aid (if not collected on Day 63 visit).
4. Obtain blood for hematology, biochemistry, rheumatoid factor, anti-dsDNA, anti-*Na*-GST-1 antibody and CMI assays.

Study Day 84 (Visit #10) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the post vaccination symptom memory aid).

2. Collect concomitant medication information.

Study Day 112 (Visit #11: Day of Third Vaccination)

1. Perform basic history (including concomitant medications) and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Obtain blood for hematology, biochemistry, rheumatoid factor, anti-dsDNA, anti-*Na*-GST-1 antibody and CMI assays.
3. For females, obtain a urine sample for pregnancy testing. Test must be negative to proceed; a positive test will exclude the volunteer from further vaccinations.
4. Record vital signs.
5. Administer the vaccine.
6. Observe for at least 1 hour after vaccination to evaluate for immediate adverse reactions. During the 1-hour post-immunization wait period, study staff will discuss signs and symptoms of potential AEs, describe proper use of digital thermometers, injection-site reaction measurement tools, and subject symptom diaries.
7. Record vital signs at the end of the 1-hour post-immunization wait period.

Study Day 115 (Visit #12) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the post vaccination symptom memory aid).
2. Collect concomitant medication information.

Study Day 119 (Visit #13)

1. Perform basic history and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Review and collect subject post vaccination symptom memory aid.
4. Obtain blood for CMI assays.

Study Day 126 (Visit #14)

1. Perform basic history and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Review and collect subject post vaccination symptom memory aid (if not collected during Day 119 visit).
4. Obtain blood for hematology, biochemistry, rheumatoid factor, anti-dsDNA, anti-*Na*-GST-1 antibody and cellular immunology assays.
5. Obtain fecal sample for parasitological testing.

Study Day 140 (Visit #15: Application of *N. americanus* Larval Inoculum)

1. Verify that Informed Consent was obtained.
2. Verify that all applicable eligibility criteria have been met.
3. Perform abbreviated history (including concomitant medications) and physical exam, focusing on any acute complaints.
4. Obtain blood for hematology, biochemistry, antibody and cellular immunology assays.
5. Obtain a urine sample for pregnancy testing (females only). Pregnancy test must be negative to proceed; a positive test will exclude the volunteer from the receiving the CHHI.

6. Record vital signs (blood pressure, oral temperature, and heart rate).
7. Administer the *N. americanus* Larval Inoculum.
8. Observe for at least 1 hour after application of the *N. americanus* Larval Inoculum to evaluate for immediate adverse reactions. During the 1-hour post-application wait period, study staff will:
   - Discuss signs and symptoms of potential Adverse Events (AEs);
   - Discuss proper use of the digital thermometer, skin reaction measurement tool, and subject post-CHHI symptom memory aid, and;
   - Review procedures for fecal sample collection, storage, and transport to the clinic.
9. At the end of the observation period, remove absorbent pad used to apply the Larval Inoculum and place in a screw-top tube for transport to GWU MITM.

Study Day 143 (Visit #16) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the post vaccination symptom memory aid).
2. Collect concomitant medication information.

Study Day 147 (Visit #17)

1. Perform basic history and physical exam (including larval application site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain blood for antibody and cellular assays.
4. Review subject post-CHHI symptom memory aid.

Study Day 154 (Visit #18)

1. Perform basic history and physical exam (including larval application site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Review and collect post-CHHI symptom memory aid.
4. Give subject two weekly post-CHHI symptom memory aids.
5. Obtain blood for hematology, biochemistry, antibody and cellular assays.

Study Day 175 (Visit #19)

1. Perform basic history and physical exam (including larval application site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Collect prior weeks' weekly symptom memory aids and give a weekly GI symptom memory aid.
4. Obtain blood for hematology, antibody and cellular assays.
5. Obtain fecal sample for parasitological testing.

Study Day 182 (Visit #20)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Collect prior week's weekly GI symptom memory aid and give new weekly GI symptom memory aid.
4. Obtain fecal sample for parasitological testing.

Study Day 189 (Visit #21)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior week's weekly GI symptom memory aid and give new weekly GI symptom memory aid.
5. Obtain blood for hematology, antibody and cellular assays.

Study Day 196 (Visit #22) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the GI symptom memory aid).
2. Collect concomitant medication information.

Study Day 203 (Visit #23)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior week's weekly GI symptom memory aid and give new weekly GI symptom memory aid.
5. Obtain blood for hematology.

Study Day 210 (Visit #24) - Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the GI symptom memory aid).
2. Collect concomitant medication information.

Study Day 217 (Visit #25)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior weeks' GI symptom memory aids and give two new weekly GI symptom memory aids.
5. Obtain blood for hematology.

Study Day 224 (Visit #26) - Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the GI symptom memory aid).
2. Collect concomitant medication information.

Study Day 231 (Visit #27)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior weeks' GI symptom memory aids and give two new weekly GI symptom memory aids.

5. Obtain blood for hematology, antibody and cellular assays.

Study Day 238 (Visit #28) - Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the GI symptom memory aid).
2. Collect concomitant medication information.

Study Day 245 (Visit #29)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior weeks' GI symptom memory aids and give two new weekly GI symptom memory aids.
5. Obtain blood for hematology.

Study Day 252 (Visit #30) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the GI symptom memory aid).
2. Collect concomitant medication information.

Study Day 259 (Visit #31)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior weeks' GI symptom memory aid and give two new weekly GI symptom memory aids.
5. Obtain blood for hematology.

Study Day 266 (Visit #32) – Telephone Call

1. Review interim medical history emphasizing any acute signs or symptoms (including entries on the GI symptom memory aid).
2. Collect concomitant medication information.

Study Day 273 (Visit #33)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior weeks' GI symptom memory aid and give new weekly GI symptom memory aid.

Study Day 280 (Visit #34)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain fecal sample for parasitological testing.
4. Collect prior week's weekly GI symptom memory aid.
5. Obtain blood for hematology, antibody and cellular assays.
6. For females, obtain a urine sample for pregnancy testing. Test must be negative to proceed with albendazole administration.

7. Dispense 3-dose treatment of albendazole 400 mg once a day by mouth.
8. Observe administration of 1st dose of albendazole, and provide 2 remaining doses to subject for at-home use.

Study Day 290 (Visit #35)

1. Obtain fecal sample #1 collected approximately 1 week **post-treatment** with albendazole, for test of cure.

Study Day 294 (Visit #36)

1. Obtain fecal sample #2 collected approximately 1.5 weeks **post-treatment** with albendazole, for test of cure.

Study Day 297 (Visit #37)

1. Obtain fecal sample #3 collected approximately 2 weeks **post-treatment** with albendazole, for test of cure.
2. Obtain blood for antibody and cellular assays.
3. Only for subjects with laboratory evidence of eosinophilia post-CHHI: obtain blood for hematology testing (complete blood count with white blood cell differential).
4. For females, obtain a urine sample for pregnancy testing (follow-up post albendazole treatment).

Study Day 320 (Visit #38)

1. Perform basic history and physical exam (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs.
3. Obtain blood for rheumatoid factor, anti-dsDNA, anti-*Na*-GST-1 antibody assays.

7.4 Final Study Visit

Study Day 380 (Visit #39)

1. Perform basic history and physical exam, emphasizing examination of any acute complaints.
2. Obtain blood for anti-*Na*-GST-1 antibody assays.

7.5 Early Termination Visit

Every attempt will be made to retain subjects once they have been enrolled. However, if for safety reasons, withdrawal of consent, or loss to follow-up, a subject is deemed by the investigators and/or SMC to be not eligible to receive the study product as per protocol, he/she will discontinue vaccinations. Volunteers will be encouraged to continue attending scheduled study visits to be followed for safety. Study subjects who discontinue the vaccinations or study participation early will not be replaced. Withdrawal from the study for any reason will not impact the subject's medical care.

The following activities will be performed at the early termination visit for subjects who withdraw, or are withdrawn or terminated from the study:

- Obtain interim medical history by interview of subjects and note any changes since the previous visit.
- All concomitant medications will be recorded on the appropriate data collection form (if within 28 days of subject's last vaccination or during the CHHI phase of the study).
- Information regarding adverse events/Serious Adverse Events will be assessed and recorded on the appropriate data collection form.
- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the Principal Investigator or co-investigator, if indicated based on review of interim medical history.
- Approximately 10 mL of venous blood may be collected for safety labs and performed by the local clinical laboratory (if prior to Visit 15 and if not recently obtained per study schedule).
- Approximately 40 mL of venous blood may be collected for antibody and cellular assays (if prior to Visit 15 and if not recently obtained).

Subjects who have undergone CHHI and who insist upon terminating the study prematurely and prior to completion of Day 197 will be recommended to take albendazole for hookworm infection (400 mg orally for three consecutive days). Follow-up care will be offered regardless of study participation, if so desired by the subject.

7.6 Unscheduled Visit

Unscheduled visits may occur at any time during the study. Procedures conducted at these visits may include any of the procedures detailed in **Appendix A** as appropriate at the discretion of the investigator depending on the nature of the unscheduled visit. Any of the following activities may be performed:

- Review concomitant medications (if prior to 28 days after a study vaccination or between study Days 140 to 297).
- Review solicited adverse events (if prior to 28 days after a study vaccination or between study Days 140 to 280).
- Review serious adverse events and new-onset chronic medical conditions.
- Obtain medical history by interview of subjects and note any changes since the previous visit (if indicated).

- A targeted physical examination may be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator, if indicated based on review of medical history.

- Examine study vaccination site (if within 14 days after a study vaccination).

- Approximately 10 mL of venous blood will be collected for safety labs and performed by the study clinical laboratory (if indicated).

7.7 Clearance of Infection

In order to confirm that treatment with albendazole results in cure of experimental hookworm infection, participants will be asked to provide fecal samples 1, 1½ and 2 weeks following treatment (Days 290, 294 and 297), which will be examined for the presence of eggs by microscopy. Three negative samples are required as confirmation of successful treatment. As treatment with even a single dose of albendazole is currently highly effective (20, 71), and as this study is being conducted in a hookworm non-endemic area, cure following a 3-dose course of treatment is expected for all subjects (72). Nevertheless, if eggs are detected in post-treatment fecal samples, the participant will be given a second course of albendazole, with the same procedure for confirmation of successful treatment.

In addition, treatment with albendazole will be offered to any study participant who wishes to withdraw from the study (whether due to the occurrence of adverse events or for personal reasons) prior to study Day 280. In such an instance, the participant will be strongly encouraged to provide post-treatment fecal samples to ensure cure of the infection. Fecal samples at 1 week, 2 weeks and 60 days following albendazole treatment will be requested for subjects who terminate the study early.

If a female becomes pregnant during the course of the study, provisions for treatment of hookworm infection will be offered (i.e., albendazole treatment after the first trimester of pregnancy).

7.8 *N. americanus* Donor Sub-Study

Transition to and enrollment into the Donor Sub-Study of protocol SVI-CH-01 will be scheduled at or before Study Day 280. In this sub-study participants will agree to continue being infected with *N. americanus* and to provide routinely scheduled fecal samples of not more than three per month. Participants will not receive the 3-dose treatment of albendazole 400 mg scheduled at Study Day 280, but will be permitted to remain infected initially for up to 6 years. During the donor sub-study, participants will be tested for infection with HIV and viral hepatitis (B and C) when they provide a fecal sample that may be used to prepare larvae for application to human study subjects, if this testing has not been done in the previous 30 days. If any participant in the donor sub-study develops new infection with HIV, hepatitis B or hepatitis C, they will be withdrawn from the sub-study and provided treatment for the *N. americanus* infection as described in the protocol for study SVI-CH-01.

Study participants who enroll into the Donor Sub-Study of protocol SVI-CH-01 will remain as participants in protocol SVI-CH-02/DMID 16-0116 until Visit #39 as described above. Participants will complete all study procedures described in Sections 7.3 and 7.4 and will be considered to have completed protocol SVI-CH-02/DMID 16-0116 after Visit #39 (study day 380) is completed.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Subjects will be monitored for local and systemic adverse events during specific protocol-defined periods. As with other aluminum hydroxide-adsorbed vaccines, hypersensitivity reactions would be expected to occur within the first 24 hours after vaccination, and other severe local or systemic reactions within 72 hours of vaccination. Subjects will therefore be observed for immediate reactions following each vaccination for at least 1 hour, and will have a clinical assessment at the study clinic on Days 7 and 14 following each vaccination. A telephone call with the subject will take place on Days 3 and 28 following vaccinations 1 and 2 to monitor for adverse events. Day 28 following vaccination 3 falls on the day of CHHI and the subject will be assessed at the study clinic. Should a subject call on a study clinician to report an adverse event (AE), it will be fully documented in their study chart, and discussed with the Principal Investigator.

Following CHHI, local larval application site reactions are expected to occur within the first month after application of the larvae. Subjects will therefore be observed for immediate reactions following each larval application for at least 1 hour. Subjects will have a telephone call on day 3 following CHHI to monitor for adverse events. A clinical assessment at the study clinic will be performed on Days 7 and 14 following CHHI. Gastrointestinal AEs are expected to occur after approximately week 4 after CHHI; subjects will therefore be evaluated weekly from weeks 5 through 20 after CHHI, until treatment with albendazole.

All AEs will be graded for severity and relationship to study vaccine or larval application, captured on the appropriate case report form (CRF), and followed to resolution. All Serious Adverse Events (SAEs) will be reviewed by a study physician, recorded on the appropriate SAE form, reported according to applicable regulations and guidelines, and followed through to resolution or stabilization by a study clinician. Special attention will also be paid to monitoring for the occurrence of Adverse Events of Special Interest (AESIs), which include inflammatory and autoimmune disorders that may potentially be related to the use of an immunostimulatory adjuvant, and gastrointestinal, hematologic or respiratory disorders that may potentially be related to CHHI.

A DMID Medical Monitor and a local Independent Medical Monitor will be appointed and a Safety Monitoring Committee (SMC) formed to monitor subject safety and to advise the Principal Investigator and co-investigators on trial-related medical questions or problems. The SMC will periodically review data on safety and enrollment, and will review cumulative safety data for evidence of study related AEs, adherence to the protocol, and factors that may affect outcome or study data such as protocol violations and losses to follow up.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Using standard techniques, the tests listed below will be performed at the site's clinical laboratory. One or more of the laboratory parameters may be repeated at any time during the

study as determined by the PI, if indicated by an AE. A clinically significant abnormal value should be repeated within 10 days if possible and followed up as clinically relevant.

1. Complete blood count plus white blood cell (WBC) differential*
2. Serum creatinine
3. Alanine aminotransferase (ALT)
4. Rheumatoid factor (RF)
5. Anti-double stranded DNA antibody (anti-dsDNA)
6. HBsAg ELISA
7. HCV serology
8. HIV serology
9. Serum ferritin

**The following CBC parameters will be assessed for safety throughout the trial: WBC count, absolute neutrophil count, absolute eosinophil count, hemoglobin concentration and platelet count.*

Urine pregnancy testing will be performed at the clinical trial site using test kits that have been approved by the FDA. Urine dipstick testing will also be performed at the trial site using an approved product.

Parasitological and immunology assays (see below) will be performed in the laboratories of the Department of MITM at the GWU, and at Baylor College of Medicine (fecal PCR for hookworm DNA).

8.2.2 Immunology Assays

Anti-*Na*-GST-1 IgG antibody, other humoral, innate immunity and cellular assays will be performed at the Department of MITM of the George Washington University School of Medicine and Health Sciences. All cryopreservation of plasma, sera and peripheral blood mononuclear cells (PBMCs) will be in liquid nitrogen.

8.2.2.1 Anti-*Na*-GST-1 Antibody Assays

Antibodies to *Na*-GST-1 will be measured in serum or plasma of study subjects using an indirect ELISA assay. Antigen-specific IgG and IgG1-4 antibody levels will be measured at baseline and at several time-points post-vaccination. For the indirect ELISA method, microwell plates will be adsorbed with purified recombinant *Na*-GST-1*,* blocked, and incubated with test sample sera or plasma, and then incubated overnight at approximately 4°C. Plates will then be washed and a horseradish peroxidase conjugated anti-human antibody (e.g., anti-human IgG) will be added and incubated with plates. Plates will be washed again, incubated with a chromogenic substrate, and then the Optical Density (OD) of the samples read using an automated ELISA plate reader. Levels of antibodies against *Na*-GST-1 in sera or plasma will be converted to Arbitrary Units of antibody by homologous interpolation of their OD readings from a standard reference curve derived from serial dilutions of a Standard Reference Serum unique to each antibody isotype.

Surface Plasmon Resonance (SPR) will be used to measure the affinity of antibody interactions with recombinant *Na*-GST-1 antigen. SPR determines the immunoreactivity and affinity of

antibody responses in numerous contexts as it measures rates of antibody-antigen association and dissociation based on the Law of Mass Action to assess antibody affinity.

8.2.2.2 Enzyme Neutralization Assays

An *in vitro* assay has been developed to measure the functional capacity of antibodies induced by vaccination with recombinant *Na*-GST-1 to inhibit or neutralize the catalytic activity of the native form of this protein. Serum or plasma samples collected from study subjects will be tested for their neutralizing capacity at several post-vaccination time points. These assays will be performed at the Clinical Immunology Laboratory of the Department of MITM of the George Washington University.

8.2.2.3 Memory B Cell Measurement

The ELISPOT method will be used to evaluate the induction of memory B cells (MBCs) after each vaccination and the maintenance of MBCs throughout the vaccination schedule to determine if MBCs act as surrogate markers (biomarkers) for later antibody production.

8.2.2.4 Cellular and Innate Immunity Assays

PBMCs will be isolated by Ficoll gradient and the circulating dendritic cell (DC) population isolated by affinity columns according to standard protocol. The DC population will be cultured *in vitro* and the cells will either remain “un-stimulated” or be “stimulated” with a TLR7/8 agonist (1 µg/ml, 4-amino-2-ethoxymethyl-α, α-dimethyl-1H-imidazoquinoline-1-ethanol). After 24 hours of stimulation, the supernatants will be collected and assayed for cytokine production (e.g., Interleukin (IL)-12, IFN-gamma, TNF-alpha, IL-4, IL-5 and IL-18) by commercially available ELISA kits. The pellet collected from the culture will be assayed for the cytokines by real-time (RT) PCR. In addition, purified DCs will be directly assayed for positive co-stimulatory molecules such as CD40, CD80, CD86 CD83, HLA-DR, PD-L1, and ICOSL by flow cytometry using commercially available labeled antibodies. The Natural Killer (NK) cell response generated in response to vaccination with *Na*-GST-1 administered with a TLR4 or TLR9 agonist will be compared to the response when *Na*-GST-1 is administered without these TLR agonists. At various points post-vaccination and post-CHHI, purified PBMCs will be stained with labeled anti-CD56 and the cells will be assayed for functionality by measuring cytokine production by intracellular staining. As functionality of NK cells can also be measured by their cytolytic activity, the cells will be analyzed for granzyme B and activation markers (e.g., NKG2D and NKP46) by flow cytometry. The cytotoxic activity of NK cells may also be assayed by using ^{51}Cr-labeled K562 targets.

In addition, circulating DC and purified T cell populations will be separated by affinity columns. The DC will be cultured ($1x10^5$ cells/well) overnight and pulsed with the recombinant *Na*-GST antigen. The following day, purified T cells from the same individual will be added to the culture and after 48 hours of incubation the T cells will be evaluated for proliferation (Ki67 labeling), cytokine production (IL-2, IL-4, IL-5, IFNγ, TNFα), co-stimulatory molecules (CD40, CD40L, CD80, CD86, and ICOS), and inhibitory receptors (PD-1, PDL-1, LG-3, 2B4) by polychromatic flow cytometry.

8.2.3 Parasitology Assays

8.2.3.1 Fecal Egg Detection by Microscopy

Fecal exams will begin with the super-saturated saline flotation technique to determine hookworm infection status (i.e., positive or negative). Fecal samples will be analyzed by the McMaster technique to determine the intensity of hookworm infection. To perform the McMaster examination, a fecal suspension is applied to a slide with counting chambers. Egg counts are performed under light microscopy.

8.2.3.2 Adult Worm Counts

During the 3-day albendazole treatment period at the end of the study, subjects will be asked to provide all fecal material passed, which will be examined for expelled worms. Worms will be washed in phosphate-buffered saline and stored in ethanol. The worms will be clarified in a phenol solution and the mouthparts examined under a microscope.

8.2.3.3 Polymerase Chain Reaction (PCR) Test for *N. americanus*

A quantitative PCR assay will be used to detect and quantify *N. americanus* DNA in fecal samples collected from study participants. DNA will be isolated from a small sample of feces (~50 mg), and *Necator* DNA amplified by real-time quantitative PCR using primers and probe specific for the internal transcribed spacer 2 (ITS2) of *N. americanus* (73).

8.3 Specimen Preparation, Handling, and Shipping

8.3.1 Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the protocol-specific Manual of Procedures (MOP), as appropriate.

Blood specimens for clinical screening and safety labs will be tested locally by the LabCorp® clinical laboratory.

Urine dipstick tests and urine pregnancy tests will be performed in the research clinic laboratory at the GW MFA.

Fecal specimens obtained for parasitology testing will be processed as specified in the MOP and transported to the Department of MITM at GWU for processing and storage.

Blood specimens for isolation of serum, plasma, and PBMCs will be collected as specified in the study MOP and transported to the Department of MITM at GWU for processing and storage.

8.3.2 Specimen Shipment

Specimen shipment (primarily fecal samples to Baylor College of Medicine, Houston, TX) will occur at intervals during the course of the study following all applicable International Air Transport Association (IATA) requirements and according to the specifics for storage temperature and documentation as detailed in protocol-specific SOPs, as appropriate.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Study product (vaccine or CHHI)-related serious adverse events occurring from the time of the first study vaccination through approximately 10 months after the last study vaccination.

2. Study vaccine-related solicited Adverse Events – reactogenicity events occurring on the day of each study vaccination through 14 days after each study vaccination:

    a) Injection site reactions including erythema (redness), induration (hardness)/swelling, pain, and tenderness.

    b) Systemic reactions including fever, myalgia (body aches/muscular pain exclusive of the injection site), arthralgia (joint pain exclusive of the injection site), headache, nausea, and vomiting.

3. CHHI-related solicited adverse events – reactogenicity events occurring on the day of CHHI through study Day 280 (time of treatment with first dose of albendazole):

    a) Application site reactions including pruritus, rash, induration (hardness)/swelling, pain, and tenderness.

    b) Gastrointestinal reactions including abdominal pain, abdominal bloating, flatulence, nausea, vomiting, and diarrhea.

    c) Respiratory reactions (possibly due to migration of larvae through the lungs) including cough and sore throat.

4. Clinical safety laboratory adverse events occurring throughout the study. Parameters to be evaluated include WBC, ANC, absolute eosinophil count, hemoglobin concentration, and platelet count; ALT; creatinine; rheumatoid factor; and, anti-ds DNA antibodies.

5. Unsolicited Adverse Events – non-serious adverse events occurring from the time of each study vaccination through approximately 28 days after each vaccination and from study Day 140 (day of CHHI) through Day 297.

6. New-onset chronic medical conditions occurring from the time of the first study vaccination through approximately 10 months after the last study vaccination.

7. Adverse Events of Special Interest occurring from the time of the first study vaccination through approximately 10 months after the last study vaccination.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

An adverse event (AE) includes any noxious, pathological or unintended change in anatomical, physiological or metabolic functions as indicated by physical signs, symptoms and/or laboratory-detected changes occurring in any phase of the clinical study, whether associated with the study product (i.e., vaccine, placebo or CHHI), and whether or not considered study product related. This includes an exacerbation of pre-existing conditions and intercurrent illnesses.

All AEs will be graded for severity and relationship to study vaccine or to CHHI. A licensed clinician (i.e. physician, nurse practitioner, physician assistant) listed on the Form FDA 1572 will be readily available for the duration of the trial to assess AEs. Should a subject call on a study clinician to report an AE, it will be fully documented in their study chart, and discussed with the Principal Investigator.

All AEs will be assessed by the investigator using the following protocol-defined grading system, as or described in **Tables 1-5**:

Grade 1:	**Mild** - No effect on activities of daily living; no medical intervention/therapy required
Grade 2:	**Moderate** - Partial limitation in activities of daily living (can complete ≥50% of baseline); no or minimal medical intervention/therapy required
Grade 3:	**Severe** - Activities of daily living limited to < 50% of baseline; medical evaluation/therapy required

All AEs will have their possible relationship to study vaccine or CHHI assessed using the following terms:

Definite:	Clear-cut temporal association, and no other possible cause.
Probable:	Clear-cut temporal association and a potential alternative etiology is not apparent.
Possible:	Less clear temporal association; other etiologies also possible.
Unlikely:	Temporal association between the AE and the study product or the nature of the event is such that the study product is not likely to have had any reasonable association with the observed illness/event (cause and effect relationship improbable but not impossible).
Not Related:	The AE is completely independent of study product administration; and/or evidence exists that the event is definitely related to another etiology.

The degree of certainty with which an AE can be attributed to administration of study product will be determined by how well the event can be understood in terms of one or more of the following:

1. The event being temporally related with vaccination (or CHHI) or reproduced on re-vaccination.

2. A reaction of similar nature having previously been observed with this study product.
3. The event having often been reported in the literature for similar types of vaccines or for CHHI.

9.2.2 Vaccine Reactogenicity

All subjects will be observed for at least 60 minutes after each study vaccination to detect and treat any immediate AEs. Subjects will also complete study memory aids after each vaccination beginning with the day of vaccination and through 14 days after vaccination. Injection site reactions including bruising, redness, induration (hardness)/swelling, pain, and tenderness will be recorded on the study aids. Systemic reactions including fever, myalgia (body aches/muscular pains exclusive of the injection site), arthralgia (joint pains exclusive of the injection site), headache, nausea, and vomiting will also be recorded.

Subjects will also be asked to record any medications taken and any emergency room or healthcare provider visits (other than routine check-ups). The subject memory aid will be reviewed with the subject at subsequent clinic visits.

Vaccine reactogenicity events are AEs that are known to occur with types of vaccine similar in composition to those being tested in this study. The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Table 1: Assessment of Solicited or Expected Adverse Event Severity – Vaccination Phase

Adverse Event	Grade	Severity
Pain at injection site	1	Aware of pain but it does not interfere with daily activity **and** no pain medication is taken
	2	Aware of pain; there is interference with daily activity **or** it requires use of pain medication
	3	Aware of pain **and** it prevents daily activity
Tenderness at injection site	1	Area immediately surrounding injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity
	2	Area immediately surrounding injection site hurts when touched or with arm motion, **and** it interferes with daily activity
	3	Area immediately surrounding injection site hurts when touched or with arm motion, **and** it prevents daily activity
Erythema at injection site	1	25 mm – 50 mm
	2	51 mm – 100 mm
	3	> 100 mm
Induration/swelling at injection site	1	25 mm – 50 mm and does not interfere with daily activity
	2	51 mm – 100 mm or interferes with daily activity
	3	>100 mm or prevents daily activity
Fever* (oral†)	1	38.0°C – 38.4°C
	2	38.5°C – 38.9°C
	3	>39.0°C
Headache	1	Easily tolerated, does not interfere with daily activity
	2	Repeated use of non-narcotic pain reliever for >24 hours or interferes with daily activity
	3	Any use of narcotic pain reliever or prevents daily activity

Nausea	1 2 3	Easily tolerated, does not interfere with daily activity Interferes with daily activity Prevents daily activity
Vomiting	1 2 3	1-2 episodes in 24 hours and does not interfere with activity > 2 episodes in 24 hours or interferes with daily activity Prevents daily activity or requires outpatient IV hydration
Myalgia**	1 2 3	Easily tolerated, does not interfere with daily activity Interferes with daily activity Prevents daily activity
Arthralgia**	1 2 3	Easily tolerated, does not interfere with activity Interferes with daily activity Prevents daily activity

* Oral temperature assessed on Day 0 (Visit 1) prior to the first study vaccination will be considered as baseline. Temperatures obtained during the clinic visits will be recorded.

† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

** Not at injection site.

Table 2: Assessment of Unsolicited Systemic Adverse Event Severity – Vaccination Phase

Systemic AE	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Anorexia	Loss of appetite without decreased oral intake lasting greater than 48 hours	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss
Diarrhea	3 loose stools/24 hours	4-5 loose stools/24 hours	>6 loose stools or requires outpatient IV hydration
Constipation	Not Applicable	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated
Fatigue	No interference w/activity	Some interference w/activity	Significant, prevents daily activity
Arthritis	Mild pain with inflammation, erythema or joint swelling – but not interfering with function	Moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	Severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living
Vasovagal episode (associated with a procedure of any kind)	Present without loss of consciousness	Present with transient loss of consciousness	Not Applicable
Vertigo	Causes no or minimal interference	Causes greater than minimal interference	Inability to perform daily activities

	with usual daily activities	with usual daily activities	
Cough	Transient- no treatment	Persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment
Bronchospasm, Acute	Transient; no treatment; 70% - 80% FEV_1 of peak flow	Requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	No normalization with bronchodilator; FEV_1 25% - 50% of peak flow; or retractions present
Dyspnea	Dyspnea on exertion	Dyspnea with normal activity	Dyspnea at rest
Hypersensitivity	Transient flushing or rash	Rash; flushing; urticaria; dyspnea	Symptomatic bronchospasm, with or without urticaria; parenteral medications(s) indicated; allergy-related edema/angioedema; hypotension
Urticaria	Requiring no medications	Requiring PO or topical treatment, or IV medication or steroids for <24 hours	Requiring IV medication or steroids for >24 hours

9.2.3 CHHI Reactogenicity

All subjects will be observed for at least 60 minutes after application of the *N. americanus* larvae during CHHI to detect and treat any immediate AEs. Subjects will also complete study memory aids after CHHI beginning with the day of larval application and through Day 280 when they will be treated with albendazole to cure their infections. For the first 4 weeks after application of the *N. americanus* Larval Inoculum, the memory aids will be used to record pruritus, pain, tenderness, rash and swelling at the site of larval application, as well as cough and sore throat (to capture symptoms that may possibly be related to larval migration through the lungs). Starting on Day 175 (approximately 5 weeks after CHHI) of the study until the participant receives their first dose of albendazole, the memory aids will be used to record nausea, vomiting, diarrhea, abdominal pain, abdominal bloating and flatulence. Female participants of childbearing potential will additionally capture on the memory aids the form of contraception they are using. The memory aids will include a printed reminder of the potential risks associated with use of albendazole during pregnancy.

Subjects will also be asked to record any medications taken and any emergency room or healthcare provider visits (other than routine check-ups) through Day 297. The subject memory aid will be reviewed with the subject at subsequent clinic visits.

CHHI reactogenicity events are AEs that are known to occur with hookworm infection. The following Toxicity Grading Scales will be used to grade solicited local (larval application site) and systemic (subjective and quantitative) reactions:

Table 3: Assessment of Solicited or Expected Adverse Event Severity – CHHI Phase

Adverse Event	Grade	Severity
Pain at site of larval application	1	Aware of pain but it does not interfere with daily activity **and** no pain medication is taken
	2	Aware of pain; there is interference with daily activity **or** it requires use of pain medication
	3	Aware of pain **and** it prevents daily activity
Tenderness at site of larval application	1	Area immediately surrounding application site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity
	2	Area immediately surrounding application site hurts when touched or with arm motion, **and** it interferes with daily activity
	3	Area immediately surrounding application site hurts when touched or with arm motion, **and** it prevents daily activity
Rash at site of larval application	1	25 mm – 50 mm and does not interfere with daily activity
	2	51 mm – 100 mm or interferes with daily activity
	3	> 100 mm or prevents daily activity
Induration/swelling at site of larval application	1	25 mm – 50 mm and does not interfere with daily activity
	2	51 mm – 100 mm or interferes with daily activity
	3	> 100 mm or prevents daily activity
Pruritus at site of larval application	1	Easily tolerated, does not interfere with daily activity
	2	Interferes with daily activity
	3	Prevents daily activity
Abdominal pain	1	Easily tolerated, does not interfere with daily activity
	2	Interferes with daily activity
	3	Prevents daily activity
Abdominal bloating	1	Easily tolerated, does not interfere with activity
	2	Interferes with daily activity
	3	Prevents daily activity
Nausea	1	Easily tolerated, does not interfere with daily activity
	2	Interferes with daily activity
	3	Prevents daily activity
Vomiting	1	1-2 episodes in 24 hours and does not interfere with activity
	2	> 2 episodes in 24 hours or interferes with daily activity
	3	Prevents daily activity or requires outpatient IV hydration
Diarrhea	1	2-3 loose stools/24 hours
	2	4-5 stools/24 hours
	3	> 6 loose stools or requires outpatient IV hydration
Flatulence	1	Easily tolerated, does not interfere with daily activity
	2	Interferes with daily activity
	3	Prevents daily activity
Cough	1	Easily tolerated, does not interfere with daily activity
	2	Interferes with daily activity
	3	Prevents daily activity
Sore throat	1	Easily tolerated, does not interfere with daily activity
	2	Interferes with daily activity
	3	Prevents daily activity

The severity of unsolicited adverse events post-CHHI will be graded as per Table 2 above.

9.2.4 Additional Adverse Event Severity Grading

Pulse and blood pressure will be graded as follows (Table 4):

Table 4: Assessment of Basic Body Function Adverse Event Severity

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Tachycardia – beats per minute (bpm)	101-115	116-130	>130
Bradycardia – beats per minute	50 - 54 or 45-50 bpm if baseline <60 bpm	45 - 49 or 40-44 if baseline <60bpm	< 45 or <40bpm if baseline <60bpm
Hypertension** (systolic, mm Hg)	141-150	151-155	>155
Hypertension** (diastolic, mm Hg)	91-95	96-100	>100
Hypotension** (systolic, mm Hg)	85-89 (and symptomatic)	80-84 (and symptomatic)	<80
Respiratory Rate – breaths per minute	18-20	21-25	>25

**Subject should be at rest for measurement of vital signs*
***With repeat testing at same visit*

Table 5: Assessment of Laboratory Adverse Event Severity

HEMATOLOGY	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)
Hemoglobin *Males* *Females*	 11.0 – 12.0 g/dL 10.3 – 11.3 g/dL	 9.5 – 10.9 g/dL 8.5 – 10.2 g/dL	 <9.5 g/dL <8.5 g/dL
Platelets	110,000 – 130,000/mm^3	90,000 – 109,999/mm^3	<90,000/mm^3
WBCs *(increase)*	10,800 – 15,000/mm^3	15,001 – 20,000/mm^3	<20,000/mm^3
WBCs *(decrease)*	2300 – 3200/mm^3	1400 – 2299/mm^3	<1400/mm^3
ANC *(decrease)*	750 – 1000/mm^3	500 – 749/mm^3	<500/mm^3
Absolute eosinophil count *(increase)*	700 – 1500/mm^3	1501 – 5000/mm^3	>5000/mm^3
CLINICAL CHEMISTRIES	**Grade 1**	**Grade 2**	**Grade 3**
Serum creatinine	1.5 – 1.7 mg/dL	1.8 – 2.0 mg/dL	>2.0 mg/dL or requires dialysis
ALT *Males* *Females*	 58 – 115 U/L 64 – 128 U/L	 116 – 230 U/L 129 – 255 U/L	 >230 U/L >255 U/L

9.2.5 Serious Adverse Events

An SAE is an AE, whether considered related to a study product or not, meeting one of the following conditions:

1. Death during the period of protocol-defined surveillance
2. Life threatening: defined as an event that places a subject at immediate risk of death at the time of the event and does not refer to an event that hypothetically might have caused death were it more severe
3. Hospitalization during the period of protocol-defined surveillance: defined as at least an overnight stay in the hospital or emergency ward for treatment that would have been inappropriate if administered in the outpatient setting
4. Results in a congenital anomaly or birth defect
5. Results in a persistent or significant disability or incapacity: defined as a substantial disruption of the study subject's ability to carry out normal life functions
6. Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious AE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

9.2.6 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The Principal Investigator or appropriate co-investigator is responsible for reporting all AE/SAEs that are observed or reported during the study, regardless of the relationship to study products. AE/SAEs, abnormal clinical laboratory test values, or abnormal clinical findings will be documented, reported, and followed appropriately.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

AEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

All SAEs will be reviewed by a study physician, recorded on the appropriate SAE form, and followed through to resolution or stabilization by a study physician. All SAEs will be reported by email, telephone or fax within 24 hours of notification of the SAE occurrence to the Principal Investigator, via a scanned PDF version of an SAE form to:

Dr. Maria Elena Bottazzi, PhD
Deputy Director
Texas Children's Hospital Center for Vaccine Development
Baylor College of Medicine and Texas Children's Hospital
1102 Bates St., Ste. 550, Houston, TX 77030
832-824-0504, Alt. 713-798-1199, Fax 832-825-0549
Email: bottazzi@bcm.edu

In addition, the Principal Investigator will report all SAEs that are determined to be definitely or probably related to study product to the Institutional Review Board (IRB) that approved the study (the IRB of the George Washington University) according to the IRB's reporting timelines and using the IRB-specified reporting forms, as the DMID Medical Monitor.

All local and systemic reactions not meeting the criteria for SAE will be captured on the appropriate case report form (CRF). These events will be followed to resolution.

In addition, all SAEs must be submitted within 24 hours of site awareness on an SAE form to the DMID pharmacovigilance contractor, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

The site will notify the study ISM when an SAE is provided to the Sponsor and the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the Principal Investigator or appropriate co-investigator becomes aware of an SAE that is suspected to be related to study product, the Principal Investigator or appropriate co-investigator will report the event to the Sponsor and the DMID Pharmacovigilance Group.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Not Applicable

9.3.3 Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND

Following notification from the Principal Investigator, the IND sponsor (Baylor College of Medicine) will report events that are both serious and unexpected that are possibly, probably, or definitely related to the study product, to the FDA within the required timelines: fatal and life-threatening events within 7 calendar days (by phone, fax, or internet) and all other SAEs in writing within 15 calendar days. All SAEs not listed as possibly, probably, or definitely related will be reported to the FDA at least annually in a summary format.

All Adverse Events of Special Interest (AESIs; see below) will be reported to Sponsor, and FDA according to the same procedure as for reporting SAEs, and according to the same timelines as described above.

9.3.4 Adverse Events of Special Interest (AESI)

Special attention will be paid to monitoring for the occurrence of certain adverse events termed, "**Adverse Events of Special Interest**" or AESIs. These include inflammatory and autoimmune disorders that may potentially be related to the use of an immunostimulatory adjuvant (although none have been associated with the use of *Na*-GST-1 in combination with either AP 10-701 or CpG 10104). The occurrence of the following AESI's will be closely monitored:

- Neuroinflammatory disorders (optic neuritis, multiple sclerosis, demyelinating disease, transverse myelitis, Guillain-Barre syndrome, myasthenia gravis, encephalitis, neuritis, Bell's palsy)
- Musculoskeletal disorders (systemic lupus erythematosus, cutaneous lupus, Sjogren's syndrome, scleroderma, dermatomyositis, polymyositis, rheumatoid arthritis, juvenile rheumatoid arthritis, polymyalgia, rheumatica, reactive arthritis, psoriatic arthropathy, ankylosing spondylitis, spondylarthropathy)
- Gastrointestinal disorders (Crohn's disease, ulcerative colitis, celiac disease)
- Metabolic diseases (autoimmune thyroiditis, Grave's or Basedow's disease, Hashimoto thyroiditis, insulin-dependent diabetes mellitus, Addison's disease)
- Skin disorders (psoriasis, vitiligo, Raynaud's phenomenon, erythema nodosum, autoimmune bullous skin diseases)
- Others (autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, antiphospholipid syndrome, temporal arteritis, Behcet's syndrome, pernicious anemia, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune glomerulonephritis, autoimmune uveitis, autoimmune cardiomyopathy, sarcoidosis, Stevens-Johnson syndrome).
- Vasculitides

In addition, disorders that may potentially be related to experimental hookworm infection will be defined as AESIs that will be closely monitored following CHHI, including:

- Gastrointestinal disorders (Crohn's Disease, Ulcerative Colitis, Irritable Bowel Syndrome)
- Gastrointestinal bleeding
- Anemia (greater than Grade 1 low absolute hemoglobin concentration as defined in **Table 5**)
- Pneumonitis

The new onset of AESI's during the study will be reported to the Sponsor, the DMID Medical Monitor, and the FDA in the same fashion as described above in **Section 9.3.1** for SAEs.

9.3.5 Medically-Attended Adverse Events (MAAEs)

Special attention will be paid to recording medically-attended adverse events, which are defined as any clinical symptom or diagnosis (including local symptoms at the injection site or systemic

symptoms) for which medical evaluation and/or care is sought from a qualified healthcare professional, outside of a regularly scheduled study visit. Note that MAAEs are not the same as SAEs. Whether or not an AE is an MAAE will be recorded on the appropriate Case Report Form.

9.3.6 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported on the study-specific Pregnancy Report form. No further study vaccinations or CHHI will be administered to pregnant subjects, but with the subject's permission all study mandated blood samples will be obtained and the subject will continue in follow-up for safety events. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome pending the subject's permission. If a woman becomes pregnant after CHHI but before study Day 280, management of her infection will be followed as per **Section 7.7** of this protocol (i.e., deferral of treatment with albendazole until after the first trimester due to the uncertain risk of this medication during the first trimester).

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be recorded if they start from the time of the first study vaccination (Day 0) up to 28 days after each dose of study vaccine, and from time of CHHI up to study Day 297 (to include the albendazole treatment period). AEs limited to AESI's and new-onset chronic medical conditions will be followed through approximately 6 months after the larval application (Day 380, approximately 10 months after the final vaccination).

SAEs will be followed from the time of the first study vaccination (Day 0 [Visit 1]) through resolution even if this extends beyond the study-reporting period (approximately 10 months after the third study vaccination [Day 380, Visit 39]). Resolution of an AE/SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5 Halting Rules

If the study vaccine or CHHI is considered significantly reactogenic or results in significant AEs (see below and **Section 9.2**), additional vaccinations or CHHIs will be suspended until reviewed by the SMC, Independent Safety Monitor, DMID Medical Monitor, and IND sponsor (BCM). Any recommendation of the Independent Safety Monitor, DMID Medical Monitor, and SMC to resume or suspend further injections or CHHIs (either for an individual subject or the full study) will be communicated in writing to the sponsor and Principal Investigator. All communications from the SMC will subsequently be forwarded by the investigators to the IRB.

The following criteria will be used to define significant reactogenicity:

- One or more participants experience a protocol defined SAE that is determined to be possibly, probably, or definitely related to a study vaccine or CHHI, **OR,**
- One or more participants experience a protocol defined AESI that is determined to be possibly, probably, or definitely related to a study vaccine or CHHI, **OR,**
- One or more participants experience a protocol defined Grade 3 hypersensitivity reaction that is possibly, probably or definitely related to a study vaccine or CHHI, **OR,**
- Two or more participants experience a protocol defined Grade 2 hypersensitivity reaction that is probably or definitely related to a study vaccine or CHHI, **OR,**
- Three or more participants experience the same objective physical finding or laboratory abnormality of Grade 3 or higher (with the exception of isolated Grade 3 erythema or swelling at the site of vaccination or Grade 3 erythema and/or rash at the site of application of the larvae), that is determined to be probably or definitely related to a study vaccine or CHHI, **OR,**
- Three or more participants experience the same Grade 2 or higher safety laboratory abnormality (excluding eosinophilia [or increased white blood cell count secondary to eosinophilia] after CHHI) or Grade 3 clinical AE that is possibly, probably or definitely related to a study vaccine or CHHI.

9.6 Unblinding

The study will be double-blinded until all study subjects have completed their final study visit. Prior to final study unblinding, a study subject's randomization code may be unblinded only for safety purposes. This is unlikely to occur, since once a vaccine is administered, knowing which vaccine was given is unlikely to influence the medical management of an AE. This procedure is therefore exceptional and any decision to unblind will be discussed with the sponsor, the Principal Investigator, the Independent Safety Monitor, and the SMC. If deemed necessary for urgent safety reasons, the Independent Safety Monitor, in consultation with the SMC (if possible in a timely manner), may unblind a specific subject without revealing the study blind to the investigators or the Sponsor. Any unblinding will be thoroughly documented. It is to be emphasized that the Independent Safety Monitor may put the study on hold at any time and discuss with the SMC. In the event that the investigators come to know the study code prior to final unblinding, the Principal Investigator must notify the Sponsor immediately. The reasons will be documented by the Principal Investigator and added to the study file.

The decision to completely unblind the study prior to the final study Day 380 visit or to permanently stop the study prior to Day 380 will take the form of a formal recommendation by the SMC to the study Sponsor. The Principal Investigator must then notify the IRB of this decision.

9.7 Safety Oversight

9.7.1 Independent Safety Monitor (ISM)

An independent Safety Monitor will be appointed for oversight of subject safety in this trial. The ISM will be a local, qualified physician who will be available to advise the investigators on trial-related medical questions or problems. The ISM will work with the DMID Medical Monitor to

ensure adequacy of adverse event monitoring and reporting. Should the ISM not be available, he/she will recommend an alternative to serve as a substitute ISM.

The ISM's primary responsibility will be to monitor subject safety. The Principal Investigator is responsible for ensuring that the ISM is aware of any new safety information that becomes available during the course of the trial.

9.7.2 Safety Monitoring Committee (SMC)

At least three individuals will be selected to serve as the study Safety Monitoring Committee (SMC) to advise the Sponsor and the study investigators on the trial. All SMC members will be independent from the Sponsor and study site. The SMC's primary responsibility will be to monitor subject safety. The Principal Investigator is responsible for ensuring that the SMC is aware of all new safety information. The SMC will periodically review data on safety and enrollment, and will review cumulative safety data for evidence of study related AEs, adherence to the protocol, and factors that may affect outcome or study data such as protocol violations and losses to follow up.

The SMC will review safety data at the following time points:

- Ad hoc when a study halting rule is met (see Section 9.5) or for immediate concerns regarding observations during this trial, or as needed.
- 1-2 weeks before the first CHHI application to review safety data collected to date.
- After the first 20 subjects have completed their study Day 175 (5 weeks after CHHI) visits.
- Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded safety data for the study.

Data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by the study Principal Investigator, the Sponsor, or DMID.

The SMC will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the SMC. At this time, each data element that the SMC needs to assess will be clearly defined. Procedures for SMC reviews and meetings will be defined in the charter. The SMC will review applicable data to include, but not limited to, study progress and subject, clinical, safety, and reactogenicity data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, subject compliance with study procedures/interventions, and solicited and unsolicited AE/SAEs. Additional data may be requested by the SMC, and interim statistical reports may be generated as deemed necessary and appropriate by the Sponsor. The SMC will receive data in aggregate and presented by group. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request vaccine assignment be unblinded for an individual subject if required for safety assessment. The SMC will review grouped and unblinded data in the closed session only. As an outcome of

each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations or CHHI (as applicable), and to continue, modify, or terminate this trial.

The study Sponsor, Principal Investigator or the SMC chair may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of this trial. The ISM and DMID Medical Monitor will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during this trial.

It is the Principal Investigator's (or designated agent) responsibility to ensure that the SMC reviews the current safety data (grouped by dose cohort), study protocol, and any other requested documents at its meetings. Occurrence of an SAE will be reported to the SMC at the same time that it is reported to the IRB. Additionally, any new information that may adversely affect the safety of the subjects or the conduct of the study will be submitted to the SMC as it becomes available.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

The Sponsor (or its designee) will monitor all aspects of the study, with respect to current Good Clinical Practices, and for compliance with applicable government regulations. Prior to the start of the study, the Principal Investigator will be informed of the frequency of monitoring visits and will be given reasonable notification prior to each visit. The objectives of a monitoring visit will be to verify the prompt reporting of SAEs, to check the availability of the signed Informed Consent for enrolled study subjects, to compare CRFs and spreadsheets with source data for completeness and accuracy, to verify compliance with the clinical protocol, and to check investigational product accountability. During the monitoring visit, the Principal Investigator (and/or designee) and other study personnel should be available to discuss the study. Study documents must be available for review throughout the course of the study.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

This study is not designed to test statistically significant small differences in efficacy between different adjuvant formulations of the *Na*-GST-1 hookworm vaccine as assessed in the CHHI model. Rather, it is intended to provide an indication of an impact on infection as assessed by CHHI, and to detect large differences in efficacy with acceptable power. Furthermore, the study intends to assess the safety, reactogenicity, and immunogenicity of three novel adjuvant formulations of *Na*-GST-1: *Na*-GST-1 formulated on Alhydrogel, *Na*-GST-1/Alhydrogel® administered with AP 10-701, and *Na*-GST-1/Alhydrogel® administered with CpG 10104.

The chosen sample size principally facilitates the assessment of efficacy (as estimated by infection status determined by the saturated saline flotation technique for hookworm egg detection in fecal samples following CHHI) as well as safety, as discussed in Section 11.2, *Sample Size Considerations.* Given that, this study will attempt to assess the following hypotheses:

1. Administration of the *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will result in a reduced proportion of volunteers infected with hookworm following CHHI, as determined by the saline flotation technique, compared to placebo controls.

2. Co-administration of *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will increase the IgG antibody response to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

3. Co-administration of *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will increase the affinity of induced IgG antibodies to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

4. Co-administration of the *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will increase the induction of specific MBCs to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

5. Co-administration of the *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 increases the neutralizing capacity of induced IgG antibodies to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

6. Co-administration of *Na*-GST-1/Alhydrogel® vaccine with either AP 10-701 or CpG 10104 will increase the avidity of induced IgG antibodies to *Na*-GST-1 compared to when *Na*-GST-1/Alhydrogel® is administered alone.

11.2 Sample Size Considerations

Sample size considerations are based on the proportion of subjects with detectable hookworm eggs at any time, as measured by the saturated saline flotation technique. The primary analysis of the proportions will consist of comparing the proportion in each of the three *Na*-GST-1

intervention groups to the proportion in the placebo control group (group 4) using a one-sided Fisher exact test, for a total of three comparisons. The Holm-Bonferroni procedure will be used to control the familywise error rate at 0.05. Based on the results from the initial CHHI trial that was conducted at GWU (NCT01940757), we expect an infected proportion of 90% in group 4 (i.e., infectivity controls), and a proportion less the 20% in at least one of the intervention groups. At alpha=0.05/3, this yields a power of 87.7%. The power is 73.2% for an infection rate of 30% in intervention and 96.3% for an infection rate of 10%.

11.3 Planned Interim Analyses

None planned.

11.4 Final Analysis Plan

The purpose of this trial is to estimate vaccine efficacy as assessed by CHHI, adverse event rates and patterns of immune responses as well as to compare these rates and patterns between the investigational *Na*-GST-1 vaccine formulations.

This section briefly describes the statistical methods to be used; a detailed analytical plan will fully describe the methods. The analytical plan will discuss the planned approaches to missing data. Deviations from the original analytical plan will be thoroughly documented and reported to the Sponsor.

Descriptive and hypothesis-testing approaches will be used to meet the protocol objectives as stated in **Section 3**. Estimates will be presented with their 95% confidence intervals. Formal statistical tests, as outlined below, will be used to compare the different *Na*-GST-1 vaccine formulations, as compared to saline placebo. Statistical tests will use a significance level of 5% (either two-sided or one-sided, depending on the analysis being conducted; this will be specified *a priori*).

Primary Objective #1: To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on controlled human hookworm infection (CHHI) with *N. americanus* larvae in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

Primary Outcome #1 Measure:

The following parameters will be evaluated for each formulation of *Na*-GST-1:

1. Proportion of subjects with detectable hookworm eggs, at any time point, in fecal samples, as determined by microscopy using the qualified saline float technique.

Analysis Plan:

a. The proportion of participants with detectable hookworm eggs in fecal samples by microscopy, at any time point post-CHHI, will be summarized as a descriptive measure by study group.

b. The proportion of participants with detectable hookworm eggs in fecal samples by microscopy will be summarized as a descriptive measure by study group at each time point.
c. The proportion of subjects with detectable hookworm eggs in each of the intervention groups (Groups 1, 2 and 3) will be compared to the proportion of subjects in the placebo control group (Group 4) using a one sided Fisher exact test. The p-values from the three tests will be adjusted using the Holm-Bonferroni procedure to control the familywise error rate at 0.05. Power calculations are based on this outcome.

Primary Objective #2: To evaluate the safety and reactogenicity of *Na*-GST-1/ Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on Days 0, 56 and 112, in healthy, hookworm-naïve adults.

AEs will be coded according to Medical Dictionary of Regulatory Activities (MedDRA™) preferred terms. The frequency, severity, and relationship of AEs per each vaccine formulation of *Na*-GST-1 will be presented in tabular form using the MedDRA™ coded term and organized by MedDRA™ System, Organ, and Class (SOC) designations.

1. The frequency of solicited local injection site and systemic AEs, and unsolicited AEs will be summarized by SOC and preferred term.
2. Line listings of clinical and laboratory AEs classified as solicited injection site, solicited systemic, or unsolicited and will be displayed in tables stratified by formulation group (i.e., *Na*-GST-1/Alhydrogel, *Na*-GST-1/Alhydrogel plus AP 10-701, *Na*-GST-1/Alhydrogel plus CpG 10104, or saline placebo).
3. AEs will be summarized by severity and relationship to vaccine by individuals and formulation of *Na*-GST-1.
4. The frequency of vaccine-related SAEs and AESIs will be tallied as well as summarized by body system, by vaccine formulation.

Primary Outcome #2 Measures:

The following summary parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to saline placebo control:

1. Frequency of solicited injection site and systemic reactogenicity, graded by severity, on the day of each study vaccination through 14 days after each study vaccination.

2. Frequency of solicited adverse events, graded by severity, on the day of CHHI through study Day 280.

3. Frequency of study vaccine-related Serious Adverse Events from the time of the first study vaccination through approximately 10 months after the last study vaccination.

4. Frequency of clinical safety laboratory adverse events.

5. Frequency of unsolicited adverse events, graded by severity, from the time of each study vaccination through approximately 1 month after each study vaccination; and from the time of CHHI through treatment with albendazole (Day 297).

6. Frequency of new-onset chronic medical conditions through approximately 10 months after the third study vaccination.

7. Frequency of Adverse Events of Special Interest through approximately 10 months after the third study vaccination.

Analysis Plan:

a. The proportion of subjects with at least one injection site AE will be compared by *Na*-GST-1 vaccine formulation. We will test the null hypotheses that the type and number of adverse events is the same across all groups by Fisher's exact test.

b. We will test the null hypothesis that the frequency of adverse events grouped by SOC term is the same in all groups using exact Poisson tests of the ratio of the rates. There will be no adjustment for multiple comparisons.

c. Laboratory results (hematological and clinical chemistry) will be examined for trends over time and any clinically significant values for individuals will be reported.

Secondary Objective #1: To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on fecal egg counts, as determined by the McMaster method, after CHHI with *N. americanus* larvae in healthy, hookworm-naïve adults.

Secondary Outcome #1 Measure:

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. Fecal egg counts as determined by microscopy using the McMaster method, during Weeks 5 through 20 post-CHHI.

Analysis Plan:

a. Fecal egg counts as determined by microscopy of the subjects in each of the intervention groups (Groups 1, 2 and 3) will be compared to the eggs counts of the subjects in Group 4 using a Wilcoxon-Mann-Whitney rank-sum test. The p-values from the three tests will be adjusted using the Holm-Bonferroni procedure to control the familywise error rate at 0.05.

Secondary Objective #2: To assess the relationship between antibody responses to *Na*-GST-1 induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

Secondary Objective #3: To assess the duration of antibody responses to *Na*-GST-1 induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104.

Secondary Outcome #2 and #3 Measures:

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. The anti-*Na*-GST-1 IgG antibody response, by a qualified indirect enzyme-linked immunosorbent assay (ELISA) at approximately 14 days after each vaccination, and approximately 1, 2, 4, 6, 7, 8 and 10 months after the third dose.

Analysis Plan:

a. The proportion of participants with detectable anti-*Na*-GST-1 IgG responses will be summarized as a descriptive measure.
b. IgG levels will be displayed graphically by study group using notched box plots at each time point.
c. Geometric mean antibody responses will be compared between vaccine formulation groups at each time point. Comparisons between groups will be made by a one-way analysis of variance (ANOVA) with pair-wise comparisons between groups made by contrasts.
d. Geometric mean antibody responses for each antigen will be compared using a regression model with main effects for the doses of *Na*-GST-1 (0 or 100µg), AP 10-701 (0 or 5 µg), and CpG 10104 (0 or 500µg).
e. For antigen-specific log IgG levels, a three-way ANOVA will be conducted, with *Na*-GST-1, CpG 10104 and AP 10-701 doses as factors.
f. Pair-wise comparisons of IgG levels on study Day 126 (2 weeks after the third vaccination) across all combinations of *Na*-GST-1 doses (i.e., 0 [placebo] or 100 µg), AP 10-701 doses (i.e., 0 or 5 µg), and CpG 10104 doses (0 or 500µg) will be assessed using Tukey's HSD.
g. Percent change in IgG antibody levels from days of vaccination to days 7,14 and 28 post-vaccination will be compared between vaccine formulation and dose groups. Values will be reported with 95% confidence intervals.
h. Percent change in IgG antibody levels from vaccination day #1 to #2, from vaccination day #2 to #3, and from vaccination day #3 to day of CHHI will be compared between vaccine formulation and dose groups to assess the sustainability of the response from trough to trough and post-CHHI. Values will be reported with 95% confidence intervals.
i. Percent change in IgG antibody levels from the peak following the third vaccination, to 1, 2, 4, 6, 7, 8 and 10 after the third vaccination. Values will be reported with 95% confidence intervals.
j. Percent change in IgG antibody levels from the day of CHHI to 1, 3, 5, 6, 7 and 9 months post-CHHI. Values will be reported with 95% confidence intervals.
k. A longitudinal model will be built to describe the IgG levels over time. Using a longitudinal panel model, differences in antibody isotype levels by formulation of *Na*-GST-1 will be explored. This will be accomplished using Proc Mixed in SAS 9.3 so the analysis will take account of the correlation between measurements on the same subject.
l. Logistic regression will be explored for the binary response of hookworm infection post-CHHI as a function of antibody response, and a Poisson regression of the event counts (i.e., infection) as a function of IgG antibody response. In these models, vaccination group will be a covariate, rather than just using the IgG responses.

Exploratory Objective #1: To assess the relationship between the functional capacity of vaccine-induced antibodies that neutralize the *in vitro* activity of native *Na*-GST-1 enzyme and responses to CHHI in healthy, hookworm-naïve adults.

Exploratory Outcome #1 Measure:

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. The functional capacity of vaccine-induced antibodies to neutralize the *in vitro* activity of the native *Na*-GST-1 enzyme.

Analysis Plan:

a. Percent inhibition of native enzyme activity will be displayed graphically for the various study groups at each time point.
b. Three-way ANOVAs with *Na*-GST-1, CpG 10104, and AP 10-701 as factors and percent inhibition as the outcome will be estimated.
c. A longitudinal model will be built to describe percent inhibition of native enzyme activity over time. Using a longitudinal panel model, differences in percent inhibition by formulation of *Na*-GST-1 will be explored. This will be accomplished using Proc Mixed in SAS 9.3, so the analysis will take account of the correlation between measurements on the same subject.

Exploratory Objective #2: To compare the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on levels of hookworm DNA as detected by real-time PCR in healthy, hookworm-naïve adults challenged with CHHI.

Exploratory Outcome #2 Measure:

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. Levels of *N. americanus* DNA in fecal samples, as measured by real-time PCR, during Weeks 5 through 20 post-CHHI.

Analysis Plan:

a. The proportion of participants with detectable hookworm DNA in fecal samples by PCR, at any time point post-CHHI, will be summarized as a descriptive measure by study group.
b. The proportion of participants with detectable hookworm DNA in fecal samples by PCR will be summarized as a descriptive measure by study group at each time point.
c. Estimated fecal egg counts as determined by PCR of the subjects in each of the vaccination groups (Groups 1, 2 and 3) will be compared to the eggs counts of the subjects in Group 4 using a Wilcoxon-Mann-Whitney rank-sum test. Current PCR hookworm spiking studies and previous egg/DNA correlations are used to determine estimated egg counts (74). The p-values from the three tests will be adjusted using the Holm-Bonferroni procedure to control the familywise error rate at 0.05.

Exploratory Objective #3 To assess the impact of vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 on the affinity of antibody-antigen interactions, and how affinity relates to responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

Exploratory Outcome #3 Measures:

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. The affinity of the antibody interactions with recombinant *Na*-GST-1 antigen at approximately 14 days after each vaccination, and approximately 1, 2, 4, 6, 7, 8 and 10 months after the third dose.

Analysis Plan:

a. Antibody affinity will be displayed graphically for the various study groups at each time point post-vaccination and post-CHHI.
b. Three-way ANOVAs with *Na*-GST-1, CpG 10104 and AP 10-701 as factors and overall affinity as the outcome will be estimated.
c. A longitudinal model will be built to describe increasing antibody affinity (affinity maturation) over time. Using a longitudinal panel model, differences in affinity maturation by formulation of *Na*-GST-1 will be explored. This will be accomplished using Proc Mixed in SAS 9.3, so the analysis will take account of the correlation between measurements on the same subject.
d. Logistic regression will be explored for the binary response of hookworm infection post-CHHI as a function of antibody affinity, and a Poisson regression of the event counts (i.e., infection) as a function of IgG antibody affinity. In these models, vaccination group will be a covariate, rather than just using the IgG affinity.

Exploratory Objective #4: To assess the relationship between *Na*-GST-1 specific memory B cells induced by vaccination with *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults, as determined by the presence of eggs using a qualified flotation technique.

Exploratory Outcome #4 Measures:

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. The production of memory B cells specific for *Na*-GST-1 on days of vaccination; approximately 7 and 14 days following each vaccination; on day of CHHI; and then 7 and 14 days, and 5, 7, 13, and 20 weeks post-CHHI.

Analysis Plan:

a. The amount of *Na*-GST-1 specific memory B cells will be expressed as a percentage of total lymphocytes present in the blood.
b. The percentage of specific memory B cells will be displayed graphically by study group at each time point.
c. Two separate longitudinal panel analyses will test the null hypothesis that average percentages for each of memory B cells are the same in the different *Na*-GST-1 formulation groups over time. This will be accomplished using Proc Mixed in SAS 9.3

so the analysis will take account of the correlation between measurements on the same subject.

d. Wilcoxon-Mann-Whitney tests will assess the null hypothesis that memory B cell levels are the same in the groups at the primary time point of Day 126, two weeks after final vaccination.
e. Logistic regression will be explored for the binary response of hookworm infection post-CHHI as a function of memory B cell production, and a Poisson regression of the event counts (i.e., infection) as a function of memory B cell counts. In these models, vaccination group will be a covariate, rather than just using the B cell counts.

Exploratory Objective #5 To assess the relationship between innate immune responses to *Na*-GST-1/Alhydrogel® delivered with or without AP 10-701 or CpG 10104 and responses to CHHI in healthy, hookworm-naïve adults.

Exploratory Outcome #5 Measure:

The following parameters will be evaluated for each formulation of *Na*-GST-1, in comparison to the saline placebo control:

1. Innate immune responses on days of vaccination approximately 7 and 14 days following each vaccination; on day of CHHI; and then 7 and 14 days, and 5, 7, 13, and 20 weeks post-CHHI.

Analysis Plan:

a. Changes in innate immune responses will be described using descriptive statistics.

A longitudinal panel analysis will test the null hypothesis that innate immune responses are the same among the different formulations of *Na*-GST-1 and examine for trends over time.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Complete source documentation (clinical evaluations and test results) will be collected for every study subject for the duration of the study, with supplementary documents (laboratory test reports, supplementary hospital or medical records, etc.) forming part of the source documentation. Case Report Forms (CRFs) will be used to record study-specific data for enrolled subjects, and study-specific data may be entered directly onto CRFs: in these cases, the documents will be both source and CRF. The Principal Investigator will responsible for the accuracy and completeness of the data reported in the CRFs and the source documents. Data reported in the CRFs that is derived from source documents should be consistent with source documents and the discrepancies should be explained.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written site clinical quality management plan that is accepted by the Sponsor and by DMID, the investigational site is responsible for conducting routine quality assurance and quality control activities to internally monitor study progress and protocol compliance. The PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The PI will ensure all study personnel are appropriately trained and applicable documentation is maintained on site.

Clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to the Sponsor and to DMID.

The data management vendor will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

The study will be conducted according to fundamental ethical principles including: The principle of respect for human dignity and the principles of non-exploitation, non-discrimination and non-instrumentalisation; The principle of individual autonomy (entailing the giving of free and informed consent, and respect for privacy and confidentiality of personal data); The principle of justice (the equitable distribution of burdens and benefits of research); The principle of beneficence and non-maleficence, namely with regard to the improvement and protection of health; and, The principle of proportionality (including that research methods are necessary to the aims pursued and that no alternative more acceptable methods are available).

14.1 Ethical Standard

The study will be conducted according to: the Declaration of Helsinki (amended in 2008); CIOMS (Council for International Organizations of Medical Sciences) International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002); International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (1996) Guideline for good clinical practice E6 (R1); and the US Code of Federal Regulations (Protection of Human Subjects [21 CFR 50], Institutional Review Boards [21 CFR 56], and Obligations of Clinical Investigators [21 CFR 312]).

14.2 Institutional Review Board

The investigators will be responsible for obtaining full IRB approvals for the study from the George Washington University IRB. Before the start of the study, the appropriate documents (including the protocol, Investigator's Brochures, and informed consent form) will be submitted to the IRB. The IRB will be informed by the Investigator of any new information that may adversely affect the safety of the subjects or the conduct of the study, an annual update and/or request for re-approval, and when the study has been completed.

14.3 Informed Consent Process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented prior to any protocol-specified procedures being conducted. Informed consent will be obtained in accordance with US 21 CFR 50.25.

Informed consent will be documented by the use of a written consent form approved by the GWU IRB. All relevant information will be provided in both oral and written form in a way that is understandable to the subject. Ample time and opportunity will be given for the subject to inquire about details of the study.

The Principal Investigator (or the Investigator's designee) will explain the nature of the study and will inform the subject that participation is voluntary and that they can withdraw at any time. The volunteer will be informed about the study's purpose, goals, expected benefits and risks, and potential risks that are currently unforeseeable. They will be provided with a description of the procedures and an estimated duration of time that will be required to participate in the study,

and they will be informed of alternatives to participation in the study. The volunteers will receive an explanation as to what options are available if injury occurs as a result of participation in the study and whom to contact in the event of a study-related injury. They will also be informed whom they should contact for answers to any questions relating to the study. The volunteer will be informed that his/her signature on the informed consent form indicates that he/she has decided to participate in the study having discussed the information presented.

The original signed informed consent form for each volunteer will be maintained as part of the volunteer's study records by the Principal Investigator. A copy of the signed informed consent form will be provided to every volunteer.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

Not Applicable

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

Healthy adults age 18 to 45 meeting all protocol defined inclusion and exclusion criteria will be invited to participate. Females of childbearing potential, unless surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy), at least 2 years postmenopausal, or practicing abstinence, must use an effective method of avoiding pregnancy (including oral, transdermal, or implanted contraceptives, intrauterine device, female condom with spermicide, diaphragm with spermicide, cervical cap, or use of a condom with spermicide by the sexual partner) until at least one month following clearance of their hookworm infection with albendazole.

This clinical trial will enroll healthy, non-pregnant volunteers from the general population of Washington, DC, without regard to gender or racial/ethnic group. That is, no specific racial/ethnic groups will be targeted or excluded from participation in these trials. In addition, both (non-pregnant) women and men will be equally encouraged to participate, and it is expected that roughly equal numbers of each gender will consent to participate in this Phase 2 study, based on the experience of the study team that will conduct the proposed clinical trial and which has enrolled subjects into similar vaccine trials at the same site.

There will not be any research conducted on children under the age of 18 years as part of this trial. Children between the ages of 18-21 years, inclusive, will be eligible to participate in this Phase 2 trial, given that the legal age of consent in the United States is 18 years.

14.5 Subject Confidentiality

All study-related information will be stored securely at the study site. All subject information will be stored in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study data collection, and administrative forms will be identified by coded number only, to maintain subject confidentiality. All computer data entry will be done by coded number only, and all databases will be secured with password-protected access systems.

Forms, lists, and any other documents that link subject identification numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

Subjects' study information will not be released without the written permission of the subject, except as necessary for monitoring by BCM and/or its designee, DMID, the GWU IRB and/or the FDA.

14.6 Study Discontinuation

If the trial is discontinued, subjects who sign the informed consent form, and are randomized and vaccinated will continue to be followed for safety assessments. No further study vaccinations or CHHI will be administered.

In the event that the study is discontinued, all subjects who received CHHI and who have been and who have not been treated for hookworm will be offered albendazole (3 daily oral doses of 400 mg). The subjects will be asked to follow up weekly for 2 weeks to ensure clearance of infection (see **Section 7.7**).

14.7 Future Use of Stored Specimens

Some of the biological samples collected from study subjects may be stored at the local site and at the MITM at GWU. Stored samples may be shared with other investigators at other institutions for the purposes of conducting the tests outlined in this study protocol. Stored samples will not be sold or used directly for production of any commercial product. No human genetic tests will be performed on samples. Each sample will be encoded (labeled) only with a unique code and a unique tracking number to protect subject's confidentiality. There are no benefits to subjects in the collection, storage and subsequent research use of samples. Reports about research done with subject's samples will NOT be kept in their health records.

15 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events must be recorded on the appropriate data collection form, assessed for severity and relationship to vaccination, and reviewed by the Principal Investigator or appropriate co-investigator.

Data collection is the responsibility of the study personnel at the study site under the supervision of the Principal Investigator. During the study, the Principal Investigator must maintain complete and accurate documentation for the study.

The data management vendor for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

CRFs will be used to record study-specific data for enrolled subjects, and study-specific data may be entered directly onto CRFs: in these cases, the documents will be both source and CRF. The Principal Investigator will responsible for the accuracy and completeness of the data reported in the CRFs and the source documents. Data reported in the CRFs that is derived from source documents should be consistent with source documents and the discrepancies should be explained.

15.3 Types of Data

Data for this study will include clinical, safety, and outcome measures (e.g., clinical laboratory values, reactogenicity, parasitology and immunogenicity data).

15.4 Timing/Reports

A final clinical study report suitable for submission to the FDA in support of an application for licensure will be prepared following the last subject visit and upon completion of assays related to the efficacy and immunogenicity endpoints.

15.5 Study Records Retention

Trial-related documents will be maintained by the Investigator for a period of 2 years after final marketing approval of the vaccine, or if 2 years have elapsed since the formal discontinuation of clinical development of all products being tested in this trial. The Sponsor is required to inform the Principal Investigator as to when such documents need no longer be retained. Storage of all trial-related documents will be such that confidentiality will be strictly maintained. During the study and while archived at the site all study-related information will be stored securely in locked file cabinets in areas with access limited to study staff. All laboratory specimens, reports, study

data collection, and administrative forms will be identified by coded number only, to maintain subject confidentiality. All computer entries will be done using a coded number only, and all local databases will be secured with password-protected access systems. Forms, lists, and any other listings that link subject ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. Subject study information will not be released without the written permission of the subject, except as necessary for monitoring, and required by the FDA.

15.6 Protocol Deviations

No revisions to this protocol will be permitted without documented approval from the GWU IRB. This does not apply to changes made to reduce discomfort or avert risk to study subjects. Furthermore, in the event of a medical emergency, the investigators shall perform any medical procedures that are deemed medically appropriate. The Principal Investigator must notify the Sponsor of all such occurrences. Any change to the protocol will be submitted to the GWU IRB as a protocol amendment, and changes not affecting risk to subjects may be expedited, as appropriate.

A protocol deviation is any noncompliance with the study protocol, GCP, or protocol-specific requirements. The noncompliance may be either on the part of the subject, the PI, or other study personnel. As a result of deviations, corrective actions will be developed by the site and implemented promptly. It is the responsibility of the PI and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to the study Sponsor.

Any deviation from the IRB-approved protocol will be documented in study subject data collection forms, including the date and detailed description of the deviation and all corrective actions taken. For any deviation determined to have potential or known impact on subject safety, an IRB problem report will be generated and submitted to the GWU IRB according to their guidelines and reporting timelines.

16 PUBLICATION POLICY

It is anticipated that results from this study will be published in peer-reviewed journals. If publication is sought, the identity of study subjects or any easily traceable identifiers will not be revealed. Authorship issues will be discussed and agreed upon between the Sponsor and collaborating partners prior to submission for publication. Additionally, the results of the study will be communicated to study subjects.

The Principal Investigator and all partners on this study will make publically available any final research data resulting from the trial, in a timely fashion following closure of the clinical trial (not more than 12 months after the last subject follow-up visit).

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SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS – VACCINATION PHASE

	Study Day	Pre[1]	0	3 (±1)	7 (±2)	14 (±2)	28 (±4)	56 (±7)	59 (±1)[3]	63 (±2)[3]	70 (±2)[3]	84 (±4)[3]	112 (±7)[3]	115 (±1)[4]	119 (±2)[4]	126 (±2)[4]
	Visit Number	Pre[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Blood Volume	**Procedures**															
	Complete History/Physical	X														
	Obtain Informed Consent	X														
	Interim Clinical Evaluation		X		X	X		X		X	X		X		X	X
	Telephone call			X			X		X			X		X		
	Urinalysis	X														
	Urine pregnancy test (females)	X	X					X					X			
2 mL	CBC[2]	X	X			X		X			X		X			X
5 mL	RF, anti-dsDNA	X	X			X		X			X		X			X
	Serum ferritin	X														
5 mL	ALT	X	X			X		X			X		X			X
	Creatinine	X	X			X		X			X		X			X
10 mL	HCV testing	X														
	HBsAg testing	X														
	HIV testing	X														
	Fecal sample collection	X														X
	VACCINATION		**1**					**2**					**3**			
10 mL	Anti-*Na*-GST-1 antibody assays		X			X		X			X		X			X
50 mL	Innate & Cellular immunity assays		X		X	X		X		X	X		X		X	X
	Blood Volume (mL)	22	72		50	72		72		50	72		72		50	72
	Total Blood Volume (mL)	22	94		144	216		288		338	410		482		532	604

[1]Completed within 90 days of first vaccination.
[2]CBC parameters to be assessed for safety: WBC, absolute neutrophil count, absolute eosinophil count, hemoglobin, and platelet count.
[3]Since Vaccination #2.
[4]Since Vaccination #3.

APPENDIX A: SCHEDULE OF EVENTS – CHHI PHASE

	Study Day	140 (±7)[4]	143 (±1)[5]	147 (±2)	154 (±2)	175 (±3)	182 (±3)	189 (±3)	196 (±3)	203 (±3)	210 (±3)	217 (±3)	224 (±3)	231 (±3)	238 (±3)	245 (±3)	252 (±3)	259 (±3)	266 (±3)	273 (±3)	280 (±3)	290 (±3)	294 (±3)	297 (±3)	320 (±14)	380 (±14)
	Visit Number	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Blood Volume	**Procedures**																									
	Interim Clinical Evaluation	X		X	X	X	X	X		X		X		X		X		X		X	X	X	X	X	X	X
	Telephone Call		X						X		X		X		X		X		X							
2 mL	CBC[1]	X			X	X		X		X		X		X		X		X			X			X[2]		
5 mL	ALT, Creatinine	X			X																					
5 mL	RF, anti-dsDNA																								X	
	Urine pregnancy test (females)	X																			X			X		
	LARVAL INOCULATION	**X**																								
	Fecal sample collection[3]					X	X	X		X		X		X		X		X		X	X	X	X	X		
10 mL	Antibody assays	X		X	X	X		X						X							X			X	X	X
50 mL	Innate & cellular immunity assays	X			X	X		X						X							X			X		
	Anthelminthic Rx																				X					
	Blood Volume (mL)	67		10	67	62		62		2		2		62		2		2			62			62	15	10
	Total Blood Volume (mL)	671		681	748	810		872		874		876		938		940		942			1004			1066	1081	1091

[1]CBC parameters to be assessed for safety: WBC, absolute neutrophil count, absolute eosinophil count, hemoglobin, and platelet count.
[2]Subjects with laboratory evidence of eosinophilia post-larval inoculation only.
[3]Subjects that withdraw from the study prior to study Day 280 will be strongly encouraged to provide post-treatment fecal samples to ensure cure of the infection.
[4]Since Vaccination #3.
[5]Since Larval Inoculation.