A Phase 1, Open-Label, Dose-Escalation Trial to Evaluate the Safety, Reactogenicity, and Immunogenicity of the Sm-p80 + GLA-SE (SchistoShield®) Vaccine in Healthy Adults

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DMID Clinical Project Manager: Ranjodh Gill, BSN, MPH

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human subjects research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

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

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance 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 United States (US) federal regulations and ICH E6 GCP guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

Lisa A. Jackson, MD, MPH

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ADCC	Antibody-dependent cellular cytotoxicity
AE	Adverse event
ALT	Alanine aminotransferase
ASC	Antibody secreting cell
BMI	Body mass index
CFR	Code of Federal Regulations
CI	Confidence interval
CMS	Clinical Material Services
CRP	C reactive protein
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GLA-SE	**G**lucopyranosyl **L**ipid **A**djuvant **S**table oil-in-water **E**mulsion
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HEENT	Head, ears, eyes, nose, and throat
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Intracellular cytokine staining

IDRI	Infectious Disease Research Institute
IEC	Independent or Institutional Ethics Committee
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
MAAE	Medically attended adverse events
MOP	Manual of Procedures
MPL	Monophosphoryl Lipid A
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NOCMC	New onset chronic medical condition
OHRP	Office for Human Research Protections
PBMC	Peripheral blood mononuclear cell
PHA	Phytohemagglutinin
PHI	Protected health information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SCRI	Seattle Children's Research Institute
SDCC	Statistical Data Coordinating Center
SE	Stable emulsion
SMC	Safety Monitoring Committee
Sm-p80	**S. m**ansoni calpain **p**rotein with a mass of approximately **80** kDa
TTUHSC	Texas Tech University Health Sciences Center

US	United States
VTEU	Vaccine and Treatment Evaluation Unit
WBC	White blood cell
WFI	Water for Injection

PROTOCOL SUMMARY

Title:	A Phase 1, Open-Label, Dose-Escalation Trial to Evaluate the Safety, Reactogenicity, and Immunogenicity of the Sm-p80 + GLA-SE (SchistoShield®) Vaccine in Healthy Adults
Design of the Study:	The study is a Phase 1 dose-escalation clinical trial to evaluate the safety, reactogenicity, and immunogenicity of the Sm-p80 + GLA-SE vaccine candidate in healthy adults. Five treatment groups, each including nine subjects, will receive three intramuscular (IM) injections of 0.5 mL of the designated study product on either Days 1, 29, and 57 or on Days 1, 29, and 180 (Table 1). Group A (unadjuvanted comparator) will receive 100 µg Sm-p80 alone on Days 1, 29, and 57, Group B (low dose standard schedule) will receive 10 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29 and 57, Group C (mid dose delayed booster) will receive 30 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29 and 180, Group D (mid dose standard schedule) will receive 30 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29, and 57, and Group E (high dose standard schedule) will receive 100 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29 and 57.
Study Phase:	1
Study Population:	45 healthy adults 18 through 55 years of age in the United States.
Number of Sites:	1
Description of Study Product:	Sm-p80 + GLA-SE, SchistoShield®, consists of Sm-p80, a recombinant protein produced in *Escherichia coli*, and Glucopyranosyl Lipid Adjuvant (GLA) formulated in a stable emulsion (GLA-SE) as an immunological adjuvant. The Sm-p80 + GLA-SE vaccine has not yet been studied in humans. The study will evaluate an antigen only formulation with 100 µg Sm-p80 and three adjuvanted formulations with varying antigen

	content (10 µg, 30 µg, or 100 µg Sm-p80, all with 5 µg GLA-SE). An injected volume of 0.5 mL will be administered for all three doses. All formulations will be evaluated on a Day 1, 29 and 57 administration schedule; the 30 µg + 5 µg GLA- SE formulation will also be evaluated on a Day 1, 29, and 180 delayed boost schedule.
Study Objectives:	Primary: • To assess the safety and reactogenicity following receipt of three doses of 1) 100 µg Sm-p80 (unadjuvanted), 2) 10 µg Sm-p80 + 5 µg GLA-SE, 3) 30 µg Sm-p80 + 5 µg GLA-SE, and 4) 100 µg Sm-p80 + 5 µg GLA-SE administered intramuscularly on Days 1, 29, and 57 and 5) 30 µg Sm-p80 + 5 µg GLA-SE administered on Days 1, 29, and 180. Secondary: • To assess anti- Sm-p80 IgG antibody responses from samples collected at specified time points. Exploratory: • To assess innate and adaptive immune signatures from samples collected at specified time points.
Duration of Individual Subject Participation:	Approximately 15 months (screening visit through last study visit) for subjects with the Day 1, 29 and 57 administration schedule and approximately 19 months for subjects with the Day 1, 29 and 180 administration schedule.
Estimated Time to Last Subject/Last Study Day:	Approximately twenty months: four weeks initial screening, six weeks to progress to enrollment of the delayed booster dose cohort (Group C) (Table 2), and study follow-up through day 545 after enrollment of the last Group C subject.

Table 1: Treatment Arms.

Study Group	N	Study Product	Administration Schedule
A	9	100 µg Sm-p80 (unadjuvanted)	Day 1, 29, 57
B	9	10 µg Sm-p80 + 5 µg GLA-SE	Day 1, 29, 57
C	9	30 µg Sm-p80 + 5 µg GLA-SE	Day 1, 29, 180 (delayed booster)
D	9	30 µg Sm-p80 + 5 µg GLA-SE	Day 1, 29, 57
E	9	100 µg Sm-p80 + 5 µg GLA-SE	Day 1, 29, 57

Dose escalation. The study will be initiated by enrollment of Study Groups A and B, which both evaluate first-in-human formulations. Group A will receive high antigen unadjuvanted product and Group B will receive low antigen adjuvanted product (Table 1). The safety profile of either formulation does not necessarily predict that of the other. Therefore, Groups A and B will be enrolled before the first safety data review (Table 2). For each of those Groups, only one subject will receive vaccine on the first day of administration of that formulation.

To progress to the next dose level, the Principal Investigator (PI) will review safety data collected through at least seven days after the first injection of the Group A and Group B subjects and assess this data according to pre-specified criteria for halting dose escalation (Section 8.6.3). If dose escalation halting criteria are not met, Study Groups C and D subjects, who will receive the next highest dose of 30 µg Sm-p80 + 5 µg GLA-SE on different schedules, will be enrolled. On the first day of administration of that formulation no more than one subject from Groups C and D will be vaccinated. Dose escalation to Group E, the highest dose group, will follow the same procedures for review of the safety data collected through at least seven days after the first injection of the Group C and D subjects. On the first day of administration of that formulation no more than one Group E subject will be vaccinated. For both dose escalation safety reviews, to allow progression to Groups C and D and then to Group E, if dose escalation halting criteria are met, all vaccine administrations will pause. The Safety Monitoring Committee (SMC) will then review the safety data and must approve dose escalation before vaccinations can resume.

Table 2: Dose Escalation Schedule.

Week	Group A	Group B	Group C	Group D	Group E
	Number of subjects receiving first study product injections by week				
1	9				
2		9			
3					
4	Review of safety data collected through day 7 after 1st study product injection for Groups A and B				
5			9		
6				9	
7					
8	Review of safety data collected through day 7 after 1st study product injection for Groups C and D				
9					
10					9

1 KEY ROLES

Lead Principal Investigator:	Lisa A. Jackson, MD, MPH Kaiser Permanente Washington Health Research Institute
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Statistical and Data Coordinating Center:	The Emmes Company, LLC

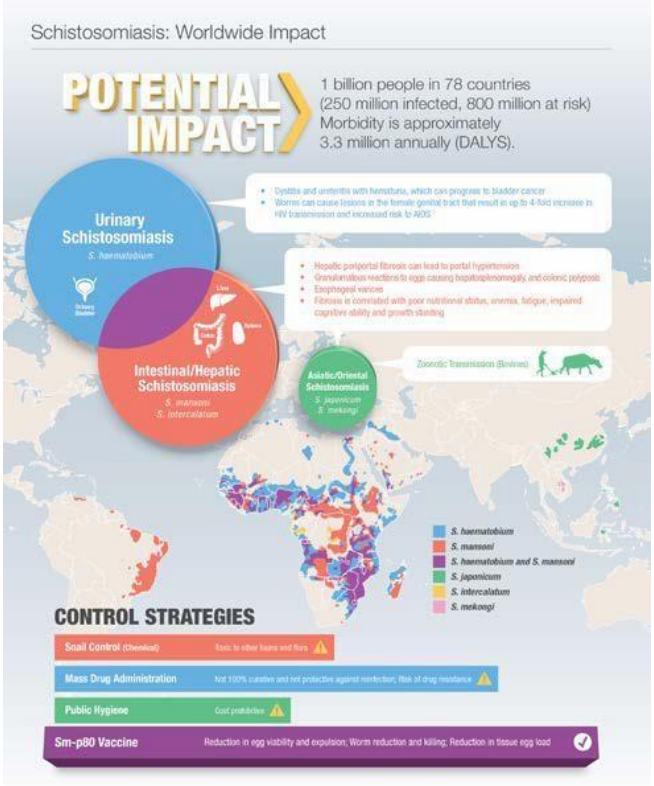
2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

2.1.1 Schistosomiasis

Schistosoma species have infected humans since 1500 BC and continue to threaten global public health with the highest burden in sub-Saharan Africa (Figure 1). Eight hundred million people are at risk in 78 countries, and 240 million are already infected. Seroprevalence studies are scarce, but available data indicate that up to 90% of persons living in high-risk areas acquire a schistosome infection at some point, usually before 10 years of age, resulting in a high prevalence in school-aged children. Of deaths caused by parasites, schistosomiasis ranks second only to *Plasmodium falciparum* malaria, killing 280,000 people annually in sub-Saharan Africa alone.

Figure 1: Infographic on the potential impact of the SchistoShield® vaccine.



Schistosomiasis is caused by a trematode worm of the *Schistosoma* genus, a parasitic blood fluke.[1] During the infectious stages, they are released from snails in fresh water. Prolonged infection can result in complex organ manifestations. Intestinal schistosomiasis is caused by *S. mansoni* and occurs in sub-Saharan Africa, the Mediterranean, the Caribbean, and South America. *S. intercalatum* causes intestinal schistosomiasis and occurs in Central Africa. Oriental or Asiatic schistosomiasis is caused by the *S. japonicum* group of parasites (including *S. mekongi*) and is endemic to Southeast Asia. Finally, *S. haematobium* causes urinary schistosomiasis and is endemic to Africa and the Mediterranean. The life cycle of schistosomes starts with the release of cercariae by freshwater snails. Human infection occurs when cercariae actively penetrate the skin and enter the body. The larvae invade host blood vessels, develop into adult schistosomes and multiply. Female schistosomes release eggs into the bloodstream. Eggs may be excreted through urine or feces and contaminate water sources, completing the schistosome life cycle. Eggs that are not excreted can become lodged in human tissue and trigger immune reactions that result in clinical disease.

Clinical misclassification of schistosomiasis is common. The current diagnostic gold standard is microscopic examination of stool or urine for eggs but is not sensitive enough to identify mild infections (i.e., adult worms may not have produced detectable eggs) and 20-30% of infections may be missed due to intermittent excretion of eggs in stool or urine resulting in a systematic underestimation of the burden.[2,3] Adjusting for diagnostic sensitivity, the global burden of schistosomiasis estimated in 2007 was 391-587 million cases.[4] The mainstay of schistosomiasis control is preventative and therapeutic administration of the anthelminthic praziquantel. However, repeated treatment is needed for curative parasite clearance. A single infection does not prevent future infection; as such, the cost-effectiveness of mass-treatment programs is compromised when reinfection is common.

To combat the schistosomiasis burden, large-scale prevention strategies (e.g., mass vaccination campaigns) that complement treatment programs are necessary. According to the World Health Organization, target groups for large-scale, periodic treatment include school-aged children and high-risk adults (e.g., fishermen, farmers, irrigation workers, and other persons who have increased contact with infested water) or entire communities living in highly endemic areas. Periodic praziquantel treatment has been shown to reduce clinical symptoms; however, there remains a gap between those who require and those who receive treatment. According to a 2015 estimate, only 28% of overall cases requiring treatment received care. An alternative or supplementary control method is the reduction of intermediate snail hosts using molluscicides, but this is even more challenging and costly since frequent application of molluscicides is necessary and logistically challenging.[5]

Therefore, an efficacious and safe vaccine, giving long-lasting protection against all forms of schistosomiasis, will have a profound impact on infection control. Induction of sterile immunity is not a prerequisite for a highly effective schistosome vaccine, as schistosome worms do not replicate within their definitive hosts. A vaccine that reduces morbidity or even partially reduces worm burden will have a major public health impact.[6,7] A combined control approach that couples mass treatment with vaccination will significantly reduce worm loads and break the transmission cycle, allowing for the eventual elimination of schistosomiasis.[8-10]

2.1.2 SchistoShield®

The proposed vaccine candidate, SchistoShield® (Sm-p80 [antigen] + GLA-SE [adjuvant formulation]), targets the Sm-p80 surface membrane antigen, which is the heavy chain of the *S. mansoni* calcium activated neutral protease (calpain), and includes a Toll-like receptor 4 agonist-based adjuvant formulation (GLA-SE). Sm-p80 stands for ***S**. **m**ansoni* calpain **p**rotein with a mass of approximately **80** kDa and GLA stands for **G**lucopyranosyl **L**ipid **A**djuvant-**S**table oil-in-water **E**mulsion. The vaccine antigen is an *E. coli* – produced recombinant Sm-p80 calpain protein from *S. mansoni*, manufactured, filled, and released by PAI Life Sciences Inc. (Seattle, WA). The vaccine adjuvant formulation, GLA-SE, is a synthetic Lipid A – like molecule and Toll-like receptor 4 agonist formulated in a squalene emulsion manufactured and released by the Infectious Disease Research Institute (IDRI) (Seattle, WA).

Development of effective recombinant protein vaccines for *S. mansoni* is possible for the following reasons:

- Unlike protozoan parasites, helminths such as *Schistosoma* do not undergo significant antigenic variation.
- *Schistosoma* live as two sexes in the circulation, where they are exposed to antibodies and their effector functions.
- While both sexes express Sm-p80 on their surface, the female worms – which cause pathology due to egg deposition – have a higher density of Sm-p80 on their surface, making them most vulnerable to the action of the vaccine.
- While some regions of the targeted deployment countries have both *S. mansoni*, from which Sm-p80 is derived, and *S. haematobium,* the causative agent of urinary schistosomiasis, our data suggest that cross-species efficacy is induced by the vaccine, likely due to the high level of homology between the proteins.

- Safe and effective adjuvants that preferentially induce T_H1 responses are now available; the proposed GLA-SE adjuvant has been extensively tested with Sm-p80, as well as in thousands of human subjects.

2.1.3 Preclinical and Safety Studies

Sm-p80 has been tested for its vaccine efficacy in different vaccine formulations and approaches. These include naked DNA, recombinant protein, and DNA prime/protein boost in three experimental animal models of infection and disease (mouse, hamster, and baboon). These Sm-p80-based vaccines provided comprehensive coverage against different stages of the parasite's life cycle, including its eggs, schistosomula, and adult worms. Sm-p80 is unique in that it has demonstrated desirable outcomes of Sm-p80 vaccination including: 1) prophylactic efficacy against *S. mansoni* (intestinal/hepatic schistosomiasis);[9]; 2) reduction in egg-induced tissue/organ pathology;[9] 3) post-exposure therapeutic efficacy by elimination of established adult worms in chronic disease;[11] 4) cross species-protection against *S. haematobium* (urinary schistosomiasis)[12] and *S. japonicum* (Asiatic/oriental disease);[13] 5) long lived immunity as Sm-p80 specific IgG titers are present in mice for up to 60 weeks and 5-8 years in baboons;[14] and 6) maternal transfer of Sm-p80-specific antibodies in baboons.[14] Sm-p80-specific IgE has not been detected in high-risk or infected populations from Africa and South America, thus reducing the possibility of hypersensitivity following vaccination with the Sm-p80 vaccine in humans. The protective immune response to *Schistosoma* is thought to be antibody mediated with cell-mediated help to skew the antibody isotypes to IgG1 and IgG3.

This section includes descriptions of preclinical and safety/immunogenicity experiments with the Sm-p80 recombinant protein antigen. Summaries of the following studies are included:

- Representative preclinical studies in murine models of schistosomiasis that demonstrate protective immunity induced by vaccination;
- Safety, immunogenicity, and efficacy studies of the SchistoShield® vaccine in a nonhuman primate model. The Sm-p80-based vaccine was evaluated in 40 baboons (*Papio anubis*), natural hosts of schistosomiasis and the most relevant nonhuman primate model of human clinical manifestations of both acute and chronic disease.

2.1.3.1 Potency of Sm-p80 in Murine Models

The rSm-p80 antigen was tested for its prophylactic and anti-pathology efficacy in murine challenge models. Briefly, groups of mice were immunized three times, four weeks apart. At week twelve, four weeks after the last boost, mice were challenged with 150 cercariae of *S. mansoni* via tail immersion. Serum samples were collected via tail bleed before every immunization and before challenge. The mice were sacrificed six to eight weeks after challenge.

Protection (P) was calculated by comparing worm burdens from vaccinated (V) and control (C) animals by a standard formula: %P = [(CV)/C x 100)]. After sacrifice, liver and intestine samples were collected from each animal and digested in 4% KOH. The number of eggs present in the tissues and percent reduction in egg production was determined. Antibody responses to Sm-p80 in immunized mice were estimated by an enzyme-linked immunosorbent assay (ELISA). In sera collected from these mice distinct Sm-p80-specific antibody titers were obtained for total IgG.

Animals immunized with adjuvant alone did not exhibit any significant IgG titers. However, robust antigen-specific (Sm-p80) IgG titers were detected in the vaccine group. These titers reached end-point titers of 1:1,638,400 at weeks 10 and 12 (Figure 2). These results demonstrate that the Sm-p80 antigen produced is highly antigenic when given with GLA-SE.

The SchistoShield® vaccine was able to significantly reduce the number of worms found in experimental animals. The levels of protection (62.69%) recorded are high for a murine model. The SchistoShield® vaccine was also able to significantly reduce eggs in both liver and intestine. Eggs in the liver and intestine were reduced by 92.57% and 87.46%, respectively, compared to control animals. Also significant, the vaccine was again able to preferentially kill female worms. Specifically, 89% of female worms were killed in the vaccine group (Figure 3).

Figure 2: Sm-p80-specific Total IgG Titers in Mice

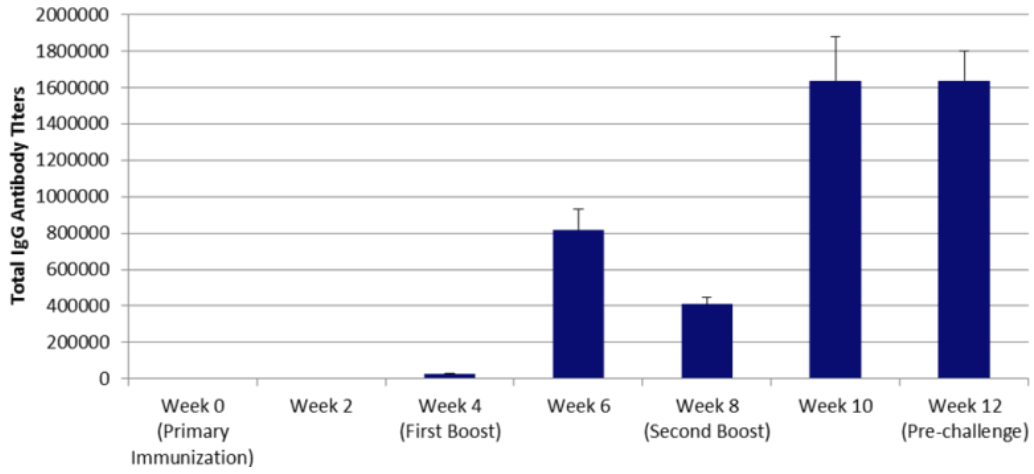
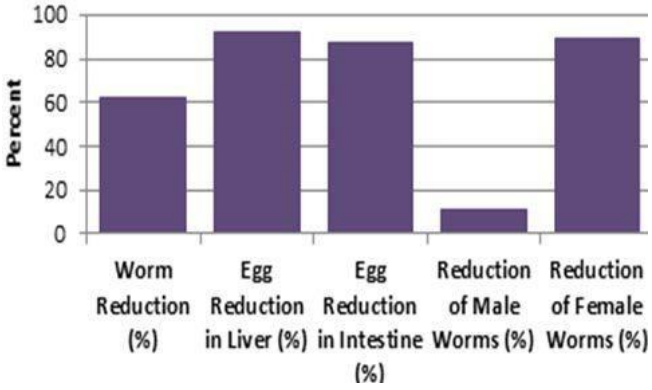


Figure 3: Sm-p80 + GLA-SE Vaccine-Mediated Efficacy in Mice.



2.1.3.2 Safety and Immunogenicity of Sm-p80 + GLA-SE in Non-Human Primates

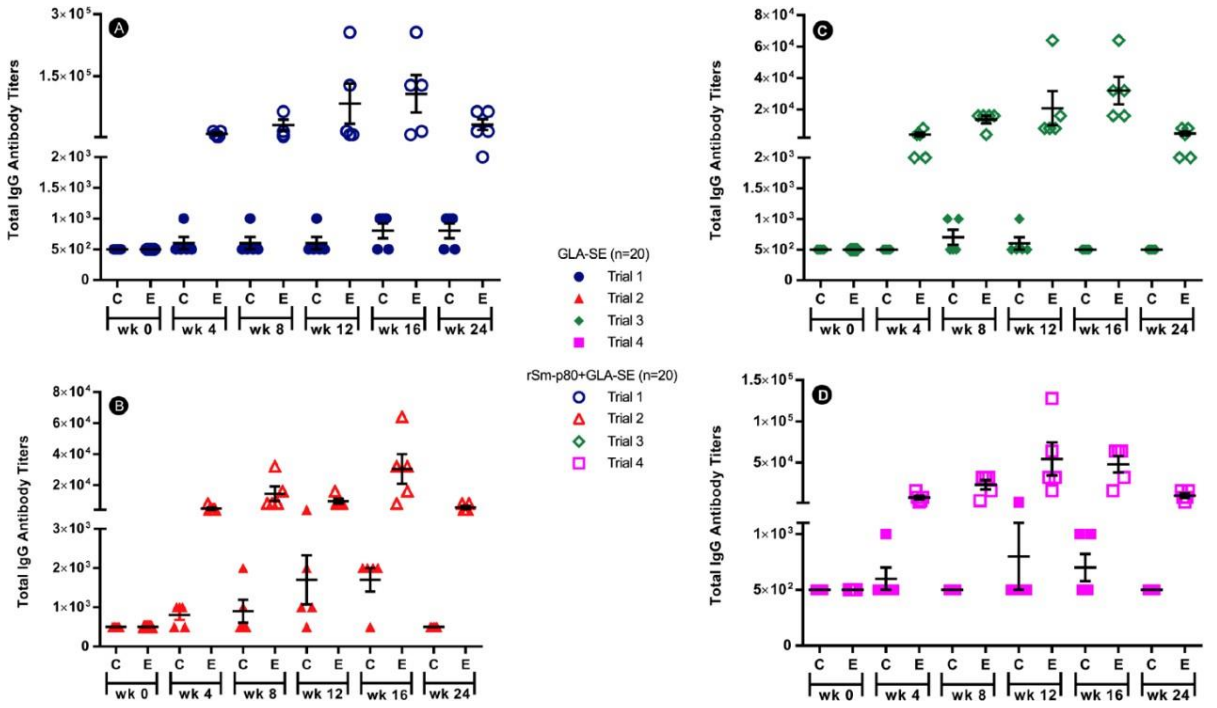
There were four independent double-blinded trials, with two groups of baboons (control and experimental) in each trial. A total of 10 baboons were randomly and equally divided into either control (n=5) or experimental (n=5) groups for each trial. Each baboon in the experimental group received 250 μg rSm-p80 formulated in 50 μg GLA-SE while baboons in the control group were immunized with 50 μg GLA-SE only. The baboon dose of vaccine was higher than that envisioned for the human clinical trial since baboons tend to be hyporesponsive to TLR4 agonists. Humans respond well to the proposed 5 microgram dose of GLA-SE. All animals received prime immunization followed by three boosts at four-week intervals. Four weeks following the last immunization, each baboon was percutaneously challenged with 1000 *S. mansoni* cercariae. To allow for disease progression, all baboons remained under observation for 8 weeks.

In the baboon trials, no notable changes in blood chemistry were identified. Blood chemistry evaluations included a complete blood count (including white blood cell [WBC] count, red blood cell count, hemoglobin, hematocrit, and platelet count) and chemistries (including alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase, creatine kinase, gamma-glutamyl transferase, amylase, lipase, albumin, total protein, globulin, total and fractionated bilirubin, blood urea nitrogen, creatinine, cholesterol, glucose, calcium, phosphorus, total carbon dioxide, chloride, potassium, sodium, albumin/globulin ratio, anion gap).

There were also no notable vaccine-related clinical observations. Specifically, the baboons did not show any problems in eating, drinking, urinating, or defecating, they were alert and responsive and no abnormal animal behaviors were noted. No neurological/motor problems were observed and no signs of swelling, hyperemia, or induration at or around the injection site were identified.

Sm-p80-specific total IgG levels were evaluated in sera of individual animals for all groups of vaccinated baboons at baseline and subsequently post-prime (Week 4), post-boosts (Weeks 8, 12, and 16), and finally post-challenge (Week 24) by ELISA (Figure 4). None of the animals had any antibody response against Sm-p80 at baseline (Week 0). In animals immunized with adjuvant alone no significant antibody responses were detected. In contrast, groups immunized with SchistoShield® mounted antibody responses following the first vaccination (Week 4) and strong antibody titers were observed in all animals post-boosting and remained high after challenge.

Figure 4: Kinetics of total Sm-p80-specific antibody in immunized baboons.



Panels depict the production of Sm-p80-specific total IgG antibodies in control animals (C, GLA-SE) and experimental animals (E, rSm-p80 + GLA-SE) in Trials 1, 2, 3, and 4, respectively. Endpoint titers were determined for Weeks 0, 4, 8, 12, 16, and 24.

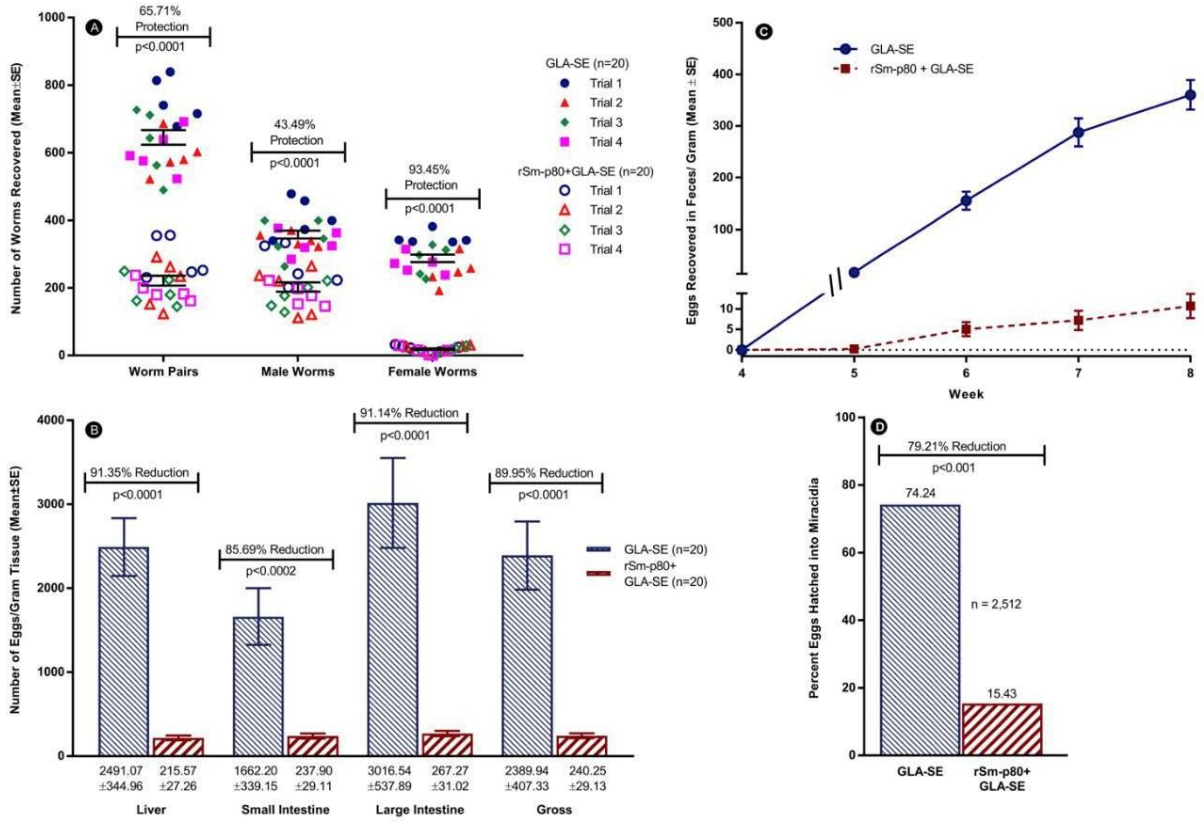
The role of anti-Sm-p80 antibodies and complement activation in *S. mansoni* juvenile worm killing was examined *in vitro*. Data from the cytotoxicity assay showed that pooled sera from baboons immunized with Sm-p80/GLA-SE promoted significant in vitro killing of schistosomula (43.75%). Addition of active guinea pig complement to the Sm-p80 sera significantly increased the percentage of dead schistosomula to 87.72% ($p < 0.001$).

2.1.3.3 Efficacy of Sm-p80 + GLA-SE in Non-Human Primates

The cumulative 40-baboon study yielded a statistically significant reduction of 65.71% ($p<0.0001$) in total worm pairs in animals vaccinated with Sm-p80/GLA-SE (Figure 5A); the protection was consistent across four independent experiments (Protection: Trial 1, 61.96%; Trial 2, 64.16%; Trial 3, 69.35%; Trial 4, 68.17%). Uniquely, the Sm-p80/GLA-SE vaccine can eliminate 93.45% ($p<0.0001$) of female parasites, and this vaccine-mediated sex-preferential killing was similar amongst all the experiments (female worm killing: Trial 1, 93.27%; Trial 2, 91.16%; Trial 3, 94.01%%; Trial 4, 95.19%). Less than 7% of the egg-producing females survived the vaccine effect, resulting in minimal pathology in host tissues/organs, as ascertained by highly significant ($p<0.0001$) reduction in egg retention in liver (91.35%), small intestine (85.69%), and large intestine (91.14%) in vaccinated animals (Figure 5B). Additionally, egg expulsion in feces was reduced by 40-fold in vaccinated animals; 5 eggs per gram in feces was the average output in vaccinated animals, compared to 204 eggs per gram release in control animals (Figure 5C). Those eggs that were excreted were impaired in their ability to hatch into miracidia, the parasite stage that infects snails to continue the life cycle with a 79.21% inhibition of hatching for eggs obtained from vaccinated animals (Figure 5D).

In summary, the four trials demonstrated that the vaccine is highly efficacious in the baboon model and leads to profound killing of pathogenic female worms.

Figure 5: Prophylactic efficacy of the Sm-p80 vaccine in baboons.



2.1.4 Safety of GLA-SE

The 5 µg dose of GLA-SE to be used in this trial is comfortably in the mid-range of tested doses of the adjuvant and has been shown to be safe and well tolerated in hundreds of human subjects.

Preclinical Safety

As reported in the GLA-SE Investigator Brochure (v5, March 2016) many preclinical and nonclinical studies in mice, guinea pigs, rats, rabbits, and non-human primates have demonstrated the immunostimulatory properties of GLA-SE with no significant safety signals.

Thousands of mice have been immunized with candidate antigens for infectious disease vaccines (e.g., leishmaniasis, tuberculosis, leprosy, malaria, influenza) formulated in GLA-SE. No safety issues have been observed when the following parameters were evaluated: clinical observations including general health and mortality; injection site reactions; body weights; food consumption; and, gross pathology. In addition, the potential for auto-antibody generation following GLA

administration was evaluated through extensive immunohistochemistry assays for immune complexes and/or autoimmune pathology, and no signs of detrimental autoimmune effects were found. Studies have also assayed for anti-nuclear antibody and have not found abnormal levels in GLA-immunized mice.

More than 300 guinea pigs have been immunized with candidate tuberculosis vaccine antigens formulated with GLA. Again, no safety issues have been observed when the following parameters were evaluated: clinical observations including general health and mortality; injection site reactions; body weights; food consumption; and, gross pathology. In addition, a safety study was conducted in guinea pigs in which various GLA adjuvant formulations (oil-in-water emulsions, aqueous suspensions, liposomes) were administered without antigen by three different injection routes (subcutaneous, intramuscular [IM], and intradermal). Administration of GLA-SE by all three routes appeared to be safe and well tolerated.

GLA-SE has been tested in several safety, immunogenicity, and/or efficacy studies in nonhuman primates. The safety parameters evaluated included clinical observations, injection site evaluations, body weights, body temperatures, hematology, coagulation, clinical chemistry, and immunogenicity. In each study the adjuvant was safe, well tolerated, and increased the immune response to the co-administered antigen. No treatment-related adverse effects were observed in the safety parameters evaluated except for mild injection site reactions and a mild, transient acute phase inflammatory response characterized by increases in C-reactive protein (CRP), fibrinogen, and neutrophils.

GLA-SE has been tested in several toxicity studies in rabbits and rats conducted under GLP guidelines. Parameters evaluated included the following: morbidity/mortality; clinical signs; injection site reactogenicity; body weights; body temperatures; food consumption; ophthalmologic evaluations; clinical chemistry; hematology; coagulation; gross necropsy observations; organ weights; histopathology; and, immunogenicity. In these studies, GLA-SE was found to be safe, well tolerated, and immunostimulatory. No treatment-related adverse effects were observed in the parameters evaluated except for reversible injection site reactions (erythema, edema, inflammation) and a mild, transient acute inflammatory response (changes in hematologic values, increased fibrinogen levels) in some animals receiving GLA adjuvant formulations.

Clinical Experience

GLA-SE has been evaluated in numerous completed or ongoing clinical trials. These trials have investigated GLA-SE formulated with antigens, including recombinant antigens, developed for vaccines against a wide variety of diseases, including leishmaniasis,[15] tuberculosis,[16,17] influenza,[18,19] schistosomiasis,[20] and malaria[21]. These clinical trials have evaluated

GLA-SE at 0.5, 1, 2, 2.5, 5, 10, or 20 µg dose levels. In these studies, over 1100 subjects have received at least one study injection containing GLA-SE. Safety data have not revealed any significant safety issues at any dose level tested. These studies have revealed a generally acceptable safety profile:

- Study injections are generally well tolerated;
- There have been no serious adverse events (SAEs) related to study injection;
- Injection site reactions are common and may include pain, tenderness, erythema, and induration;
- Systemic reactions may include headache, fatigue, anorexia, fever, chills, myalgia, and arthralgia;
- Transient elevations in CRP levels were noted in a study of GLA-SE administered with the Fluzone influenza vaccine; and
- Hematologic changes may occur (including decreases in hemoglobin, WBC, and neutrophils).

These reactions varied from study to study, were generally mild, resolved quickly, and are typical of vaccinations by the IM route. GLA-SE often increases the rate and severity of local and systemic reactogenicity compared to the antigen alone. This is consistent with the nonclinical animal experience and is to be expected of a potent immunostimulant.

2.1.5 GLP Toxicity Studies of SchistoShield®

This GLP toxicology study was to evaluate the systemic toxicity and local tolerance of the SchistoShield® vaccine candidate protein antigen Sm-p80, both with and without GLA-SE, when administered by IM injection to New Zealand White rabbits. Serum was also collected for immunogenicity evaluations. The study was conducted at Battelle - West Jefferson in accordance with FDA Good Laboratory Practice regulations (21 CFR Part 58) and the CPMP guidance documents (CPMP/ SWP/ 465/ 95 and CPMP/ ICH 302/ 95). Groups of 20 rabbits (10 female and 10 male; 80 total for the study) were immunized with one of four formulations including a high-dose (100 µg) of protein alone, a mid-dose of 25 µg with 10 µg adjuvant, a high-dose of protein plus 10 µg adjuvant, and a saline control. Each group received four injections given on Days 0, 14, 28, and 42. The active product dose levels were selected based on intended mid-dose and high-dose to be used in human Phase I trials.

In this GLP study there were no findings definitively attributed to systemic toxicity of Sm-p80 with or without GLA-SE. All study animals survived until scheduled humane termination. There were no changes in body weights, body temperatures, or food consumption attributed to Sm-

p80 with or without GLA-SE. Ophthalmic examinations were normal for all animals prior to the first dose administration and prior to each scheduled necropsy. Observed ear discolorations and skin abrasions were attributed to blood collection and the clipping of injection sites, respectively. With regard to local tolerance, injection site observations were generally similar throughout the study for male and female rabbits in groups receiving Sm-p80 with or without GLA-SE. There were sporadic grades of 1 (very slight) and 2 (slight) edema at the injection site for rabbits in all 3 active treatment groups, however all were graded 0 by 72 hour post-injection. The immune response was highest in rabbits of both sexes that received injections containing both the high dose of Sm-p80 (100 µg) with adjuvant (10 µg GLA-SE). Low doses of Sm-p80 plus adjuvant (10 µg GLA-SE) produced higher titers than high dose (100 µg) of Sm-p80 alone justifying the use of the adjuvant. There were no major histopathological findings in animals humanely terminated 2 days following the fourth (final) administration. Slight increases in fibrinogen and C-reactive protein were observed in treatments groups on Day 24.

This toxicity study indicated that the SchistoShield® vaccine is safe and well tolerated in rabbits when administered IM for up to four injections at the highest proposed human dose of the Sm-p80 antigen given with GLA-SE. No significant local or systematic toxicity was found. As previously reported, general safety assessment of the SchistoShield® vaccine in baboons also indicated that the vaccine is safe and well tolerated for up to the highest dose of 250 µg of Sm-p80 and 50 µg of GLA-SE, further supporting the observation from the GLP-toxicity study in rabbits. Based on the N+1 rule, the GLP-toxicity study in rabbits supports the use of the SchistoShield® vaccine in the first-in-human clinical trial for up to three injections at the high dose of 100 µg Sm-p80 .

2.2 Scientific Rationale

2.2.1 Purpose of Study

The promising results of the preclinical evaluations and the urgent need for an effective schistosomiasis vaccine support advancement of the SchistoShield® vaccine candidate to an initial Phase 1 human trial in healthy volunteers in the United States. The purpose of this trial is to determine the safety, reactogenicity, and immunogenicity in 45 healthy adult subjects of the candidate investigational schistosomiasis vaccine. The trial will evaluate four study product formulations and two administration schedules, as outlined in Table 1. Subjects will receive three IM injections of 0.5 mL of the designated study product formulation, on Days 1, 29, and 57 (28 days apart) or on a delayed booster dose schedule on Days 1, 29, and 180.

To determine the need for adjuvant, the study will evaluate one group of subjects who receive the Sm-p80 antigen without adjuvant (Group A). The 100 µg antigen dose in Group A reflects the highest antigen content proposed for administration with adjuvant in this trial (Group E).

Groups B, C, D and E will receive study product, at doses of 10 µg, 30 µg, and 100 µg Sm-p80, adjuvanted with 5 µg of the GLA-SE adjuvant. The GLA-SE formulation has been used as a vaccine adjuvant in 13 clinical trials (seasonal and pandemic influenza, leishmaniasis, schistosomiasis, malaria, tuberculosis). Approximately 1100 subjects have received at least one study injection containing GLA-SE, with GLA doses ranging from 0.5 to 20.0 µg. This vaccine adjuvant is well-tolerated with generally only mild local and systemic adverse events (AEs) and no significant AEs related to the product. The adjuvant dose of 5 µg is selected for this trial as this dose is in the mid-range of doses evaluated, has been shown to increase antibody levels and cellular immune responses, and has been evaluated in multiple clinical trials, including trials administering three vaccinations, demonstrating an acceptable safety profile.

The preclinical studies of SchistoShield® do not inform the selection of antigen dose for this study, as non-human primates require much higher doses of antigen and adjuvant than humans due to the tolerizing effects of their environments. In the baboon studies, the animals that received active study product were given three injections, four weeks apart, of a formulation of 250 µg Sm-p80 in 250 µg CpG-ODN, a Toll like receptor 9 agonist. In other vaccine trials in humans, GLA-SE adjuvant has been administered with antigen doses in the range of approximately 10 to 100 µg micrograms. The lower antigen doses appear to be best to elicit T cell responses while the higher doses tend to elicit better humoral responses. Since it appears that the primary mechanism of action of SchistoShield® is mediated by humoral responses, this study will evaluate a range of Sm-p80 doses from 10 to 100 µg.

Study Groups A, B, D, and E will receive study vaccinations on a Day 1, 29 and 57 schedule (28 days between vaccinations) while Study Group C will receive study vaccinations on a Day 1, 29 and 180 delayed booster schedule. The comparison of the same vaccine formulation, 30 µg Sm-p80 + 5 µg GLA-SE, given on different schedules to Groups C and D will allow assessment of possible differences in immune responses to a schedule with a 5 month versus a 28-day interval between the second and third vaccinations. Longer intervals prior to the boost vaccination have been associated with enhanced immunogenicity, for example with H5 influenza vaccines given as a two-dose series either 28 days or 180 days apart[22] and for recombinant hepatitis B vaccine given on a 0, 1, and 2 month versus a 0, 1, and 6 month schedule.[23]

2.2.2 Study Population

The study population will include 45 healthy adults 18 through 55 years of age, inclusive, who have no prior exposure to schistosomes and have no history of treatment for schistosomiasis. The study population will be drawn from the membership of the Kaiser Permanente Washington health plan and from the population of the greater Seattle, Washington area and will exclude women who are pregnant or who are breast feeding a child.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The potential risks of participating in this trial are those associated with having blood drawn, the IM injection and possible reactions to the Sm-p80 recombinant antigen, with or without GLA-SE adjuvant, and the possibility of a breach of confidentiality.

Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the subject lie down with legs elevated. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure to the blood draw site for a few minutes after the blood is taken. IM injection may also cause transient discomfort and fainting. Drawing blood and IM injection may cause infection. The use of aseptic (sterile) technique will make infection at the site where blood will be drawn or where the study vaccination will be given extremely unlikely.

There is a small amount of risk to subjects who report that they are in good health but who have an unknown health problem at the time of screening. This trial will screen by physical exam, medical history, vital signs, and clinical laboratory tests, including WBC, hemoglobin, platelets, and ALT, as well as screening for HIV, hepatitis B, and hepatitis C infections.

For this first-in-human trial of the SchistoShield® vaccine there is no human safety data with which to estimate the potential risks of the study product formulations to be evaluated in this trial. Preclinical evaluations, including evaluations in non-human primates, have not identified a safety signal associated with administration of the Sm-p80 recombinant protein antigen (Sections 2.1.3.2 and 2.1.5).

There is potential for AEs to occur more frequently in the adjuvanted vaccine groups [24] than in the non-adjuvanted group and there is potentially a higher risk for AEs to occur more frequently in the higher dose Sm-p80 antigen groups than in the lower dose antigen groups. GLA-SE adjuvant has been evaluated in humans with other vaccine antigens and has been found to have an acceptable safety profile, as described in Section 2.1.4.

Expected AEs associated with GLA-SE administration include injection site reactions that may include pain, tenderness, erythema and induration, systemic reactions that may include headache, fatigue, anorexia, fever, chills, myalgia, and arthralgia. Transient hematologic changes have been observed in preclinical evaluations and in human clinical trials, including decreases in hemoglobin, WBC, and neutrophils. Transient elevations of CRP have been noted in preclinical evaluations and in one human clinical trial, in which GLA-SE was formulated with the Fluzone influenza vaccine.

Acute and potentially life-threatening allergic reactions, such as anaphylaxis, are also possible. These reactions occur in about 1 in 4 million people given a vaccination. These reactions can manifest as skin rash (hives), swelling around the mouth, throat, or eyes (angioedema), difficulty breathing (bronchospasm), a fast pulse (tachycardia), or decrease in blood pressure (hypotension). If these reactions occur, they can usually be stopped by the administration of emergency medications by the study personnel. As with any vaccine or medication, there is a very small chance of a death, although researchers do not expect this to occur.

Subjects will be asked to provide protected health information (PHI). All attempts will be made to keep this PHI confidential within the limits of the law. However, there is a chance that unauthorized persons will see the subject's PHI. All study 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 trial will be allowed access to the PHI that is collected. Any publications from this trial will not use information that will identify subjects by name. Organizations that may inspect and/or copy research records maintained at the participating sites for quality assurance and data analysis include groups such as the local IRB, NIAID, and the FDA.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This web site will not include information that can identify subjects.

There may be other risks, discomforts, or side effects that are unknown at this time.

2.3.2 Potential Benefits

There is no anticipated direct benefit to subjects resulting from participation in this trial.

There is potential benefit to society by adding to knowledge of possible interventions that could decrease the global morbidity and mortality from schistosomiasis.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

This is a Phase 1, open-label, dose-escalation clinical trial to evaluate the safety, reactogenicity, and immunogenicity of the Sm-p80 + GLA-SE candidate schistosomiasis vaccine (SchistoShield®) in healthy adults 18 through 55 years of age. The Sm-p80 antigen is a recombinant protein produced in *E. coli* and GLA-SE is an immunological adjuvant.

Five treatment groups, each including nine subjects, will receive three intramuscular (IM) injections of 0.5 mL of the designated study product on either Days 1, 29, and 57 or on Days 1, 29, and 180 (Table 1). Group A (unadjuvanted comparator) will receive 100 µg Sm-p80 alone on Days 1, 29, and 57, Group B (low dose standard schedule) will receive 10 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29 and 57, Group C (mid dose delayed booster) will receive 30 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29 and 180, Group D (mid dose standard schedule) will receive 30 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29, and 57, and Group E (high dose standard schedule) will receive 100 µg Sm-p80 + 5 µg GLA-SE on Days 1, 29 and 57. Dose escalation will proceed as described in the Protocol Summary.

Reactogenicity will be measured by the occurrence of solicited injection site and systemic reactions from the time of each study product injection through seven days after each study product injection. Unsolicited AEs of all severities, and concomitant medications, will be collected from the time of each study product injection through the next 28 days. During periods of follow-up outside of the 28-day post-vaccination interval, only SAEs, medically attended adverse events (MAAEs), new onset chronic medical conditions (NOCMCs), and potentially immune-mediated medical conditions (PIMMCs) will be collected. Clinical laboratory evaluations for safety will be performed on venous blood collected prior to, and approximately seven days after, each study vaccination.

Immunogenicity testing will include measuring titers of total IgG against the Sm-p80 protein by ELISA. Gene expression will be evaluated using RNA Seq analysis from whole blood.

For additional details on study procedures and evaluations and study schedule by study visits/days, see Sections 6 and 7 and Table 8 and Table 9.

3.2 Study Objectives

3.2.1 Primary

- To assess the safety and reactogenicity following receipt of three doses of 1) 100 µg Sm-p80 (unadjuvanted), 2) 10 µg Sm-p80 + 5 µg GLA-SE, 3) 30 µg Sm-p80 + 5 µg GLA-SE, and 4) 100 µg Sm-p80 + 5 µg GLA-SE administered intramuscularly on Days 1, 29, and 57 and 5) 30 µg Sm-p80 + 5 µg GLA-SE administered on Days 1, 29, and 180.

3.2.2 Secondary

- To assess anti- Sm-p80 IgG antibody responses from samples collected at specified time points.

3.2.3 Exploratory

- To assess innate and adaptive immune signatures from samples collected at specified time points.

3.3 Study Endpoints or Outcome Measures

3.3.1 Primary

- Occurrence of study vaccine-related SAEs from the time of the first study vaccination through approximately 12 months after the last study vaccination.
- Occurrence of solicited injection site and systemic reactogenicity events from the time of each study vaccination through 7 days after each study vaccination.
- Occurrence of clinical safety laboratory AEs from the time of each study vaccination through approximately 28 days after each study vaccination.
- Occurrence of all unsolicited AEs, regardless of the assessment of seriousness or relatedness, from the time of each study vaccination through 28 days after each study vaccination.
- Occurrence of SAEs, MAAEs, NOCMCs, and PIMMCs from the time of the first study vaccination through approximately 12 months after the last study vaccination.

3.3.2 Secondary

- For Sm-p80 IgG antibodies, number of subjects achieving seroconversion, defined as a fourfold rise from baseline, at approximately 28 days after the first, second, and third study vaccinations.

- Geometric mean titers of serum Sm-p80 IgG antibodies from samples collected at 7 and 28 days after each vaccination, and at 124 days after the last vaccination.

3.3.3 Exploratory

- Identification and characterization of gene expression changes as measured using RNA-seq analysis from whole blood relative to last vaccination.
    - Days 8 and 29 vs. Day 1, and Day 36 vs. Day 29 for all study groups
    - Day 57 vs. Day 29, Day 64 vs. Day 57 for Groups A, B, D, and E
    - Day 187 vs. Day 180 for Group C
- Identification and characterization of gene expression changes as measured using RNA-Seq relative to last vaccination that correlate with peak Sm-p80 IgG antibody levels.
    - Days 8 and 29 vs. Day 1, and Day 36 vs. Day 29 for all study groups
    - Day 57 vs. Day 29, Day 64 vs. Day 57 for Groups A, B, D, and E
    - Day 187 vs. Day 180 for Group C

4 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

4.1 Study Product Description

The vaccine product is a two-component system comprised of Sm-p80 antigen and GLA-SE adjuvant.

Sm-p80 for injection

Sm-p80 is a recombinant protein produced in *E. coli* bacteria. The protein antigen is the large subunit of the *S. mansoni* calcium-activated neutral protease, calpain. The Sm-p80 protein is formulated and lyophilized to yield the vaccine antigen, Sm-p80 for Injection. The final 758 amino acid protein antigen has a predicted mass of approximately 87kDa.

GLA-SE Adjuvant

GLA-SE is a synthetic Monophosphoryl Lipid A-like molecule which is a Toll-like receptor 4 agonist formulated in a SE to produce GLA-SE.

Sm-p80 + GLA-SE

Sm-p80 + GLA-SE contains the antigen and adjuvant. After reconstitution of the antigen with water-for-injection (WFI) and mixing with the liquid adjuvant, it is ready for administration to the study participant.

4.1.1 Formulation, Packaging, and Labeling

Sm-p80 for Injection

Sm-p80 for Injection is formulated as a lyophilized cake in glass vials and appears white to off-white cake. Each 3 mL glass vial contains 125 µg of Sm-p80. Reconstitution of Sm-p80 with WFI results in a clear, colorless solution. Vials are for single-use only.

GLA-SE Adjuvant

GLA-SE Adjuvant is formulated as 20 µg/mL GLA in a 4% SE and is supplied as single use glass vials. GLA-SE appears as a milky-white liquid. Each 2 mL vial contains a fill volume of 0.4 mL.

Sm-p80 + GLA-SE for Injection

Sm-p80 for Injection and GLA-SE adjuvant are added to each other in a 1:1 ratio and mixed by inverting the vial or gentle pipetting up and down. This yields a 2% oil emulsion with GLA at 10 µg/mL and Sm-p80 at the desired concentration for the various dose levels of the antigen. After mixing, the dissolved Sm-p80 antigen and the GLA-SE emulsion appears as a translucent milky white liquid.

Sterile Water for Injection, USP

The sterile WFI is nonpyrogenic and contains no bacteriostatic, antimicrobial agent or added buffer. This product will be used to dilute the vaccine (Sm-p80) and will be supplied as a single-dose vial.

Each study product (Sm-p80 and GLA-SE) will be labeled according to manufacturer and regulatory specifications and include the statement: "Caution: New Drug-Limited by Federal Law to Investigational Use."

4.1.2 Product Storage and Stability

Sm-p80 for Injection

Sm-p80 for Injection must be stored between 2°C to 8°C; excursions up to 25°C are permitted for 24 hours.

GLA-SE Adjuvant

GLA-SE adjuvant is provided as a SE and must be stored between 2°C to 8°C; excursions up to 25°C are permitted, but the product should never be frozen. cGMP adjuvant lots have been on a stability program for over 5 years and are stable for this period when properly stored at 2°C to 8°C.

Sm-p80 + GLA-SE for Injection

The vaccine product (antigen + adjuvant) is only produced upon admixture prior to administration. A syringe stability study was completed to confirm stability of the admixture for at least 4 hours at up to 25°C.

Sterile Water for Injection, USP

The sterile WFI vials are stored at 20°C to 25°C [See USP Controlled Room Temperature].

4.2 Acquisition/Distribution

Sm-p80 for Injection

Sm-p80 for Injection will be provided by PAI Life Sciences Inc and distributed through the DMID Clinical Material Services (CMS), Fisher BioServices.

GLA-SE Adjuvant

The GLA-SE adjuvant is purchased from a commercial supplier (IDRI) and distributed through the DMID Clinical Material Services (CMS), Fisher BioServices.

Sterile Water for Injection, USP

The sterile WFI, USP will be supplied by the DMID CMS, Fisher BioServices.

4.3 Dosage/Regimen, Preparation, Dispensing and Administration of Study Intervention/Investigational Product

Preparation

Study product preparation, including vaccine dilutions, and admixing for the various dosing groups, will be performed by the site pharmacist using aseptic technique under a biological safety cabinet or laminar flow hood on the same day of study vaccine administration. See the protocol-specific MOP for detailed information on the preparation, labeling, and storage of study product for each Study Group.

The research pharmacist will visually inspect the study products upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter or if there are any concerns regarding its integrity, the pharmacist will not use the affected study product(s). The affected study product(s) will be quarantined as per storage requirements and labeled as 'Do Not Use' (until further notice). The site PI or responsible person will immediately contact the DMID Product Support Team (see MOP for contact information) and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to

the DMID CMS or destroy it on site. If the study product is unusable, study personnel will use another vial from the study supply. Replacement vials may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

For Groups B, C, D, and E the Sm-p80 will be appropriately formulated and mixed with diluted GLA-SE adjuvant. Group A participants will receive Sm-p80 (diluted to the appropriate concentration) without adjuvant. Once diluted, the unadjuvanted Sm-p80 vaccine must be used within 24 hours. The Sm-p80 plus GLA-SE adjuvant admixtures must be used within 4 hours of preparation. The four-hour window begins when the final formulated preparation is mixed and drawn into a syringe for administration to a study participant. The time of preparation and time of administration should be noted in the source documentation. Both the diluted vaccine and vaccine plus adjuvant admixture will be held at 2°C to 25°C. Study product will be administered within 30 minutes after dispensing from temperature-controlled storage.

Administration

See the protocol-specific MOP for detailed information on the administration of study product for each group. Each dose of study vaccine will be administered as a single 0.5 mL IM injection in the deltoid muscle of the participant's arm.

Participants will be observed in the clinic for a minimum of 30 minutes following the study product injection. After 30 minutes, the injection site will be examined and the participant will be questioned about the presence of any localized or generalized reactogenicity symptoms. Any spontaneous AEs that occur will be assessed. Participants will be instructed to complete a symptom Memory Aid for seven days post vaccination to record symptoms and to inform investigators immediately if they experience symptoms that may require medical attention. Participants may be instructed to return to the clinic if they feel febrile on any individual day or if they develop any severe reactions following vaccination.

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

Once received, study products will be stored in and dispensed by the Investigational Pharmacy.

The Food and Drug Administration (FDA) requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product disposition is maintained, and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product

disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the subject number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records must be available for inspection by the DMID monitoring contractors and are subject to inspection by a regulatory agency (e.g., the FDA) at any time. An assigned Study Monitor will review the pharmacy records.

Unused reconstituted investigational product vials will be stored at 2°C to 25°C in the Investigational Pharmacy until clinical trial accountability is completed. At study termination, all unused investigational product will be disposed in accordance with the MOP following complete drug accountability and monitoring.

4.5 Modification of Study Intervention/Investigational Product for a Participant

The study product will be administered to enrolled participants according to the study schedule. There is no planned modification of a study product administration for participants, with the exception that participants who have received a study product injection and who become ineligible for further study product injections (Section 5.2.1) will be precluded from these study procedures. Every effort will be made to follow vaccinated participants for safety evaluations.

5 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL

The study population of 45 subjects will be enrolled from a population of healthy, *Schistosoma*-naïve subjects aged 18 through 55 years at a single study site in Seattle, Washington.

Subject Inclusion and Exclusion Criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

5.1 Eligibility Criteria

5.1.1 Subject Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment in this study.

1. Male or non-pregnant female 18 through 55 years of age, inclusive, at the time of consent.
2. Able and willing to participate for the duration of the study and able to understand and comply with planned study procedures.
3. Able and willing to provide written (not proxy) informed consent.
4. Is in good health, as judged by the investigator, and determined by medical history and physical examination*.

   * Existing medical diagnoses or conditions (except those in the Subject Exclusion Criteria) must be deemed as stable. A stable medical condition is defined as no change in prescription medication, dose, or frequency of medication in the last three months (90 days) and health outcomes of the specific disease are considered to be within acceptable limits in the last six months (180 days). Any change due to change of health care provider, insurance company, 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 site PI or appropriate sub-investigator, will not be considered a violation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site PI or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of solicited events and immunogenicity.

Topical, nasal, and inhaled medications (with the exception of some uses of corticosteroids as outlined in the Subject Exclusion Criteria), vitamins, and contraceptives are permitted.

5. Women of childbearing potential* must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to each study product injection.

   *Not sterilized via bilateral tubal ligation, bilateral oophorectomy, or hysterectomy, or, if menopausal, still menstruating or < 1 year of the last menses

6. Women of childbearing potential must have used an acceptable form of contraception* in the 30 days prior to their first study product injection.

   *Acceptable single forms of contraception include abstinence from sexual activity that could lead to pregnancy, monogamous relationship with vasectomized partner who has been vasectomized for six months or more prior to enrollment, successful Essure® placement (permanent, non-surgical, non-hormonal sterilization), intrauterine devices, and hormonal methods, including the birth control patch, shot (Depo-Provera), pills, the vaginal ring (NuvaRing), and the contraceptive implant (Nexplanon). Acceptable barrier methods include diaphragm or cervical cap with spermicide and the contraceptive sponge.

7. Women of childbearing potential must agree to continue use of an acceptable form of contraception through 30 days after their last study product injection.

8. Weight ≥50 kg and body mass index (BMI) < 35.0 kg/m^2

9. Vital signs (oral temperature, pulse, and blood pressure) are all within normal protocol-defined ranges.*

   * The normal protocol-defined ranges for vital signs include (a) oral temperature less than 38°C (100.4°F), (b) pulse no greater than 100 bpm, (c) systolic blood pressure 85 to 150 mmHg, inclusive, and (d) diastolic BP ≤ 100 mmHg.

10. Screening clinical lab values are all within normal protocol-defined reference ranges.

    *The normal protocol-defined ranges for laboratory tests include (a) ALT of < 47 IU/L, (b) creatinine less than or equal to the laboratory upper limit of normal, (c) WBC ≥3.80 $x10^3$/UL and ≤13.00 $x10^3$/UL, (d) hemoglobin 11.5 g/dL or greater for females or 12.6 g/dL or greater for males, (e) platelets between 131 $x10^3$/UL and 415 $x10^3$/UL, inclusive.

5.1.2 Subject Exclusion Criteria

Subjects must not meet any of the exclusion criteria at baseline to be eligible for enrollment in this study.

1. Has had known schistosomiasis infection or has traveled to an endemic area for schistosomiasis infection and, during that travel, was potentially exposed to a Schistosoma species.
2. Has been treated for schistosomiasis.
3. Has previous exposure to schistosome vaccines or experimental products containing GLA-SE.
4. Female subjects who are breastfeeding a child, or who plan to breastfeed a child from the first study product injection through 30 days after the last study product injection.
5. Asthma, other than mild, well-controlled asthma*

   *Cold or exercise-induced asthma controlled with inhaled medications other than inhaled corticosteroids is permissible. Subjects should be excluded if they require daily bronchodilator use, or have had an asthma exacerbation requiring oral/parenteral steroid use or have used theophylline or inhaled corticosteroids in the past year
6. Known atherosclerotic cardiovascular disease or history of myocardial infarction, pericarditis, or myocarditis.
7. Diabetes mellitus
8. History of a psychiatric condition that may make study compliance difficult, such as schizophrenia, or poorly controlled bipolar disorder*

   *Includes persons with psychoses or history of suicide attempt or gesture in the 3 years before study entry or an ongoing risk for suicide.
9. Chronic or active neurologic condition (including seizures* and migraine headaches**).

   *Seizure within the past 5 years

   **Four or more migraine headaches in the past 12 months that interfered with normal daily activity or any migraine headache in the past 5 years that required emergency or inpatient medical care.
10. Autoimmune disease*

*Autoimmune hypothyroidism with or without replacement therapy, and vitiligo or mild eczema or psoriasis not requiring chronic therapy, are permissible.

11. Known or suspected congenital or acquired immunodeficiency including anatomic or functional asplenia* or immunosuppression as a result of underlying illness or treatment.

    *Any splenectomy is exclusionary.

12. Use of alcohol or drugs that, in the opinion of the investigator, may interfere with ability to comply with the protocol or increase risk to subject's health during the study period.

13. Active neoplastic disease*

    *Subjects with a history of malignancy may be included if treated by surgical excision, or by chemotherapy or radiation therapy and has been observed for a period that in the investigator's estimation provides a reasonable assurance of sustained cure (not less than 36 months). Cervical neoplasia under surveillance and non-melanoma skin cancer are not exclusionary.

14. Chronic topical or systemic corticosteroid use*

    *Corticosteroid nasal sprays for allergic rhinitis are permissible. Persons using a topical corticosteroid for a limited duration for mild uncomplicated dermatitis such as poison ivy or contact dermatitis prior to enrollment may be enrolled the day after their therapy is completed. Oral or parenteral (intravenous, IM, subcutaneous) corticosteroids given for non-chronic conditions not expected to recur are permissible if, within the year prior to enrollment, the longest course of therapy was no more than 14 days and no oral or parenteral corticosteroids were given within 30 days prior to enrollment. Intraarticular, bursal, tendon, or epidural injections of corticosteroids are permissible if the most recent injection was at least 30 days prior to enrollment. Topical or systemic corticosteroid use for study related AEs is not exclusionary.

15. Known contraindication to repeated phlebotomy*

    *Such as minimal venous access or recent history of anemia

16. Receipt or planned receipt of inactivated vaccine or allergy desensitization injection within 14 days before or after a study product injection.

17. Receipt or planned receipt of live attenuated vaccine within 28 days before or after a study product injection.

18. Receipt of blood products or immunoglobulin within six months prior to, or donation of a unit of blood within two months prior to, the first study product injection.
19. Receipt of any experimental agent* within 30 days prior to screening or planned receipt prior to the last study visit**.

    *Vaccine, drug, biologic, device, blood product, or medication.

    **Receipt of experimental COVID-19 related products are not necessarily exclusionary and will be evaluated on a case-by-case basis.
20. Plan to undergo surgery (elective or otherwise) within six months after study enrollment.
21. Plans to enroll in another interventional clinical trial* at any time during the study period.

    *Includes trials evaluating interventions such as a drug, biologic, or device.
22. Positive confirmatory test for HIV infection.
23. Positive serologic test for hepatitis B surface antigen (HBsAg).
24. Positive confirmatory test for hepatitis C virus (HCV) infection.
25. Acute febrile illness (oral temperature ≥ 38°C) or other acute illness within three days prior to study product injection.*

    *Note for afebrile, acute illness only: If a subject is afebrile, his/her acute illness is nearly resolved with only minor residual symptoms remaining, and, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol, the subject may receive study product injection without further approval from the DMID Medical Officer.
26. Not willing to avoid donating blood during the study.
27. Has any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

5.2 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination

5.2.1 Withdrawal from the Study or Discontinuation of the Study Product

Subjects may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled.

An investigator may also withdraw a subject from receiving the study product for any reason. Follow-up safety evaluations will be conducted if the subject agrees. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the electronic case report forms (eCRFs).

The reasons, might include, but are not limited to the following:

- Subject meets individual halting criteria (specified in Section 8.6.2).
- Subject develops a medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses.
- The study is terminated.
- Presence of signs or symptoms that could confound or confuse assessment of study product reactogenicity.
- As deemed necessary by the site PI or appropriate sub-investigator for noncompliance or other reasons.
- Subject refuses further study product injections.
- Subject withdraws consent.
- Subject lost to follow-up.

The primary reason for withdrawal from this trial will be recorded on the Study Status eCRF. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 6.3.2.

Although subjects are free to withdraw at any time or may be withdrawn by the investigator at any time, those subjects who received one or more doses of study product will be encouraged to remain in this trial for follow-up safety assessments (which may be conducted by phone call

rather than in person) continuing through approximately 12 months after their last study vaccination. These subjects will also be encouraged to provide venous blood samples for humoral immunogenicity assays and clinical safety laboratory testing if within 28 days after the last study vaccination. See the protocol-specific MOP for alternate follow-up requirements.

The investigator should be explicit regarding study follow-up (e.g. safety follow-up) that might be carried out despite the fact the subject will not receive further study product. If the subject consents, every attempt will be made to follow all AEs through resolution. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent form (ICF) or the investigator may seek subsequent informed consent using an IRB-approved ICF with the revised procedures.

In the case of subjects who fail to appear for a follow-up safety assessment, efforts (i.e., three documented contact attempts via phone calls made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject's study records.

The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study.

5.2.2 Subject Replacement

Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after administration of the study product will not be replaced. Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before administration of the study product may be replaced.

5.2.3 Study Termination

The NIAID 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, or due to natural disaster.

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB.

6 STUDY PROCEDURES

The Schedules of Events are included as Table 7, Table 8, and Table 9 in Appendix A. Refer also to Section 4 and Section 8.

Clinical assessment of subjects may be performed by any clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or a sub-investigator.

6.1 Screening, Visit 00 (Day -30 to Day -1)

All subjects will have a screening visit in the 30 days prior to their first study product injection visit. Screening laboratory evaluations with abnormal values may be repeated during this period only as specified in Section 7.2.1.

Subjects who are not enrolled within the 30-day window after the screening visit may be rescreened to refresh the window.

Written informed consent will be obtained prior to conducting any screening procedures. A single consent form will be used to document consent to screening and to participation in the study.

The following procedures will be performed at the initial screening visit (Table 7):

- Subjects will be provided with a description of this trial (purpose and study procedures) and asked to read and sign the ICF. The ICF will be signed prior to performing any study procedures, including any screening procedures.
- Demographic information will be obtained by interview of subjects.
- Eligibility criteria will be reviewed with subjects and eligibility assessed by review of the inclusion and exclusion criteria.
- A complete medical history will be obtained and all concomitant medications taken in the prior 60 days will be identified in order to determine the stability of chronic diseases and eligibility. Medications reported in the eCRF are limited to those taken within 30 days prior to the first study vaccination.
- Vital signs, including oral temperature, blood pressure, and pulse, will be obtained to ensure eligibility. Subjects must not eat or drink anything hot or cold, or smoke, within 10 minutes prior to oral temperature measurement.
- Height and weight will be obtained to calculate BMI.

- A physical examination that assesses general appearance and the following areas/systems: skin, lymph nodes, HEENT (head, ears, eyes, nose, and throat), neck, cardiovascular, pulmonary, abdomen, extremities, musculoskeletal, and neurological, will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.
- Venous blood will be collected for screening laboratory tests (WBC, hemoglobin, platelets, creatinine, ALT, HIV-1/HIV-2 testing, anti-HCV antibody, HBsAg, and pregnancy test for female subjects of childbearing potential) in volumes as specified in Table 7.
- HIV test counseling will be provided.
- For women of childbearing potential, contraceptive methods will be reviewed to ensure eligibility, recent menstrual history will be obtained, and pregnancy avoidance counseling will be provided.

The following procedures will be performed if the screening visit is repeated to re-screen the subject if they are not enrolled within the 30-day window:

- Eligibility criteria will be reviewed with subjects and eligibility assessed by review of the inclusion and exclusion criteria.
- A complete medical history will be obtained and all concomitant medications taken in the prior 60 days will be identified in order to determine the stability of chronic diseases and eligibility. Medications reported in the eCRF are limited to those taken within 30 days prior to the first study vaccination.
- Vital signs, including oral temperature, blood pressure, and pulse, will be obtained to ensure eligibility. Subjects must not eat or drink anything hot or cold, or smoke, within 10 minutes prior to oral temperature measurement.
- If indicated, a targeted physical examination will be performed.
- Venous blood will be collected for screening laboratory tests (WBC, hemoglobin, platelets, creatinine, ALT, and pregnancy test for female subjects of childbearing potential) in volumes as specified in Table 7. Testing for HIV-1/HIV-2, anti-HCV antibody, and HBsAg should be repeated only if the previous testing was more than six months earlier.
- For women of childbearing potential, contraceptive methods will be reviewed to ensure eligibility, recent menstrual history will be obtained, and pregnancy avoidance counseling will be provided.

6.2 Visit 01 Day 01, Enrollment and First Study Product Injection, Clinic Visit

Prior to the study product injection, the following procedures will be performed:

- Medical history and concomitant medications will be reviewed.
- A targeted physical examination will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator, if indicated by medical history.
- Vital signs, including oral temperature, blood pressure, and pulse, will be obtained to ensure eligibility and establish baseline. Subjects must not eat or drink anything hot or cold, or smoke, within 10 minutes prior to oral temperature measurement.
- Inclusion/exclusion criteria, including the results of all clinical screening laboratory evaluations, will be reviewed to verify eligibility
- For women of childbearing potential, contraceptive methods will be reviewed to ensure eligibility, recent menstrual history will be obtained, pregnancy avoidance counseling will be provided, and a urine pregnancy test will be performed. A test performed on a sample obtained within 24 hours prior to vaccination must be negative.
- A pre-administration reactogenicity assessment will be performed to establish baseline.
- Venous blood samples will be obtained for safety labs, PBMC isolation, and immunologic assays in volumes as specified in Table 8, for Groups A, B, D, and E, and Table 9 for Group C. The clinical safety laboratory results will not be available or reviewed prior to study vaccination.
- The assigned study product will be administered IM injection in the deltoid muscle of the preferred arm. The site of injection (right or left arm) and time of administration will be recorded on the appropriate eCRF.
- Subjects will be observed in the clinic for at least 30 minutes after the injection. The injection site will be examined, post-administration reactogenicity assessments will be performed, and any AE or SAE will be recorded on the appropriate eCRF prior to discharge from the clinic.
- Subjects will be provided with a web-based electronic Memory Aid (e-Memory Aid) or Paper Memory Aid and thermometer for recording daily maximum oral temperature, solicited injection site and systemic reactions, unsolicited AEs, and concomitant medications, beginning on the evening of Day 1 and continuing daily for the next 7 days. Subjects will be

encouraged to take their temperature around the same time each day and also if they feel feverish. Subjects will be instructed on how to use the memory aid and how to rate any AEs.

Subjects will be instructed to notify the study center if they develop any severe reactions and/or fever equal to or exceeding 38.0°C (100.4°F). If the investigator deems the reaction warrants further evaluation or intervention, the investigator will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

6.3 Planned Study Visits

The second study product injection will be administered at Visit 05, Day 29 (window ± 3 days).

The third study product injection will be administered at Visit 08, Day 57 (window ± 3 days) for subjects in Groups A, B, D, and E and will be administered at Visit 09, Day 180 for Group C.

At the Second and Third Vaccination Visits, prior to the study product injection:

- For a subject to receive the study product injection, Individual Halting Criteria will be reviewed (refer to Section 8.6.2).
- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, and symptoms suggestive of a MAAE/NOCMC/PIMMC, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.
- All concomitant medications will be recorded on the appropriate eCRF, if known and not previously collected.
- All newly identified AEs/SAEs/MAAEs/NOCMCs/PIMMCs will be recorded on the appropriate eCRF.
- Vital signs, including oral temperature, pulse, and blood pressure, will be obtained prior to the study product injection. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.
- A targeted physical examination, including an assessment for signs suggestive of a MAAE/NOCMC/PIMMC, may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

- For women of childbearing potential, contraceptive methods will be reviewed to ensure eligibility, recent menstrual history will be obtained, pregnancy avoidance counseling will be provided, and a urine pregnancy test will be performed. A test performed on a sample obtained within 24 hours prior to vaccination must be negative.
- Pre-administration reactogenicity assessments will be performed to establish baseline.
- Venous blood samples will be obtained for clinical safety labs, PBMC isolation, and immunologic assays, in volumes as specified in Table 8, for Groups A, B, D, and E, and Table 9 for Group C. The clinical safety laboratory results will not be available or reviewed prior to study vaccination.

Subjects will then receive a single dose of study product via IM injection into the deltoid muscle of the preferred arm.

- The second or third study product injections may be given in the same arm as the previous study product injection as long as there is no interference with the reactogenicity assessment. The site of injection (right or left arm) and time of administration will be recorded on the appropriate eCRF. Subjects will be observed in the clinic for at least 30 minutes after the study vaccination. The injection site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate eCRF prior to discharge from the clinic.
- Subjects will be provided with an e-Memory Aid or Paper Memory Aid and thermometer for recording daily maximum oral temperature, solicited injection site and systemic reactions, unsolicited AEs, and concomitant medications, beginning on the evening of the day of study product injection and continuing daily for the next seven days. Subjects will be encouraged to take their temperature around the same time each day and also if they feel feverish. Subjects will be instructed on how to use the memory aid and how to rate any AEs.
- Subjects will be instructed to notify the study center if they develop any severe reactions and/or fever equal to or exceeding 38°C (100.4°F). If the investigator deems the reaction warrants further evaluation or intervention, the investigator will give further instructions on the proper course of action, including a return to the clinic for immediate evaluation if appropriate.

6.3.1 Follow-up

Follow up visits are scheduled as indicated in the Schedule of Events, Table 8 for Groups A, B, D, and E, and Table 9 for Group C. Subjects will be followed for one year after their last study vaccination.

6.3.2 Early Termination Visit

The following activities will be performed at the early termination visit on subjects who withdraw, or are withdrawn or terminated from this trial and who are willing to participate in the visit, clinical evaluations, and study procedures:

- Interim medical history, including an assessment for new medical conditions and symptoms suggestive of a MAAE/NOCMC/PIMMC, will be obtained by interview of subjects and any changes since the previous clinic visit or contact will be noted.
- Memory Aid information will be reviewed with subjects (if within 7 days after the last study vaccination).
- All concomitant medications will be recorded on the appropriate eCRF (if within 28 days after the last study product injection).
- AEs will be assessed and recorded as described in Section 3.1.
- Vital signs, including oral temperature, pulse, and blood pressure, may be obtained if clinically indicated.
- A targeted physical examination, including an assessment for signs suggestive of a MAAE/NOCMC/PIMMC, may be performed, if indicated based on review of interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.
- The study vaccination site will be examined (if within seven days after the last study product injection).
- Post-administration reactogenicity assessments will be performed (if within seven days after the last study product injection).
- Approximately 8.5 mL of venous blood will be collected for serum antibody assays (if within 28 days after the last study vaccination).
- Approximately 7 mL of venous blood will be collected for clinical safety labs (WBC, hemoglobin, platelets, ALT, and creatinine), and performed by the clinical laboratory if within 28 days after the last study vaccination.

6.4 Unscheduled Study Visits (if needed)

Participants may be asked to come in for additional clinic visits if, for example, the need arises for follow-up of local or systemic AE such as febrile illnesses, or for requests for repeat blood draws for scheduled laboratory testing from the clinical laboratory due to sample hemolysis or clotting.

A supplemental visit eCRF will be filled and signed by the appropriate personnel. All unscheduled visits will be entered into Emmes' Advantage eClinical® internet data entry system on the appropriate eCRFs. Please see the MOP for details.

6.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions should be developed by the site and implemented promptly. It is the responsibility of the site PI and other study personnel to use continuous vigilance to identify and report protocol deviations. All individual protocol deviations will be addressed in subject study records. All protocol deviations, either individual, product, or site-specific will be collected and the record stored in a sponsor-determined location. Protocol deviations must be sent to the IRB per its guidelines. The site PI and other study personnel are responsible for knowing and adhering to their IRB requirements.

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

Complete medical history will be obtained by interview of subjects at the screening visit. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits after the first study vaccination, an interim medical history will be obtained by interview of subjects noting any changes since the previous clinic visit or contact. The interim medical history should include an assessment for new medical conditions and symptoms suggestive of a MAAE/NOCMC/PIMMC.

Concomitant medications will be collected as described in Section 7.1.1.

At the screening visit a physical examination will be performed on all subjects as described in Section 6.1. At subsequent clinic visits, a targeted physical examination may be performed, if indicated based on the subject's interim medical history, by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator. Targeted physical examinations should also include an assessment for signs suggestive of a MAAE/NOCMC/PIMMC.

Vital signs (oral temperature, pulse, and blood pressure) will be collected at the screening visit, at vaccination visits, and at clinic visits scheduled within 7 days after a vaccination. At other visits, vital signs will be assessed if clinically indicated. Vital signs assessed on Day 1 prior to the first study product injection will be considered as baseline. Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. Height and weight will be collected at the screening visit for the calculation of BMI.

Reactogenicity assessments will include an assessment of solicited AEs occurring from the time of each study product injection through seven days after each study product injection, which includes an assessment of injection site reactions including pruritus, erythema, induration/ swelling, pain, and tenderness as well as systemic reactions including fever, chills, fatigue, malaise, myalgia, arthralgia, headache, and nausea or vomiting. Pre-administration

reactogenicity assessments will be performed to establish baseline, then the study product injection will be given.

Subjects will be observed in the clinic for at least 30 minutes after each study vaccination. The injection site will be examined, post-administration reactogenicity assessments will be performed, and any AE/SAEs will be recorded on the appropriate eCRF prior to discharge from the clinic. The injection site will also be examined approximately 7 days after each study product injection.

All subjects will complete a Memory Aid from the time of each study product injection through 7 days after each study product injection. Subject Memory Aids will be reviewed with the subjects for AEs (solicited injection site and systemic reactions and unsolicited AEs) during each post-injection clinic or phone visit through 7 days after the injection.

7.1.1 Assessment of Concomitant Medications/Treatments other than Study Product

Administration of any medications, therapies, or vaccines will be recorded on the appropriate eCRF. Concomitant medications will include all current medications and medications taken in the 60 days prior to the first vaccination and those taken from the time of vaccination through approximately 28 days after each vaccination, as described in Section 3.1. Medications reported in the electronic case report form (eCRF) are limited to those taken within 30 days prior to the first study product injection and those taken in the 28-day post-vaccination interval. Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements. In addition, receipt of non-study vaccines will be solicited from the time of each vaccination through approximately 28 days after each vaccination and reported in the eCRF. Use of a new medication should prompt evaluation for the occurrence of any MAAE, including a NOCMC.

Medications that might interfere with the evaluation of the investigational product(s) should not be used during the trial-reporting period (approximately 12 months after the last study vaccination) unless clinically indicated as part of the subject's health care. Medications in this category include the prohibited medications per the Subject Exclusion Criteria (see Section 5.1.2). In addition, the site PI or appropriate sub-investigator may identify other medications that should not be used due to a risk to subject safety or assessment of reactogenicity and immunogenicity.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

Subjects will be screened for HIV-1 and HIV-2 infection by a fourth-generation testing algorithm, for HCV infection, and for HBsAg. Screening labs also include WBC, hemoglobin, platelets, creatinine, and ALT.

Serum (screening visit) and urine (study product injection visits) pregnancy tests will be performed. Serum pregnancy tests will be performed by the Quest clinical diagnostic laboratory. Urine pregnancy CLIA-waved tests will be performed by research staff at the research site specimen processing laboratory. Pregnancy testing will be done at the screening visit and within 24 hours prior to each study product injection on all women of childbearing potential. Results must be negative and known prior to enrollment on Day 1 and administration of each study product injection.

Clinical safety laboratory parameters (WBC, hemoglobin, platelets, ALT, and creatinine) will be collected from each subject on the day of and prior to each study product injection, approximately 7 days after each study product injection, and approximately 28 days after each study product injection (which may also serve as the clinical safety laboratory samples for the second and/or third study product injection). These evaluations will be performed by the Quest laboratory. Venous blood samples will be collected for these safety labs. The results from the clinical safety laboratory parameters collected on the day of study product injections will not be available or reviewed prior to study product injection.

Laboratory values that are outside the range of eligibility but are thought to be due to an acute condition or due to laboratory error may be repeated once. 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.

The volume of venous blood to be collected for the clinical safety laboratory evaluations is presented in Table 8 and Table 9.

7.2.2 Research Assays

The research assays to be performed for the immunologic endpoints are specified below.

7.2.2.1 ELISA (anti Sm-p80 IgG)

Vaccine-specific IgG antibody responses will be assessed by ELISA using serum collected from subjects (8.5 ml of blood collected in a serum separator tube) at specific time points selected to evaluate the kinetics of the response after each administration, peak response, and durability, including prior to each vaccination on the day of vaccination, at 7 and 28 days after each vaccination, and at 124 days after the last vaccination. The 28-day post-vaccination sample also serves as the day of vaccination sample for a subsequent vaccination given at a 28-day interval.

This volume is sufficient to provide 1 primary and 1 backup sample for analysis. The goal will be to compare the serum IgG titers at each time point among participants who received an Sm-p80 study product across treatment groups. Endpoint titer ELISAs will be performed by SCRI as follows. Titers of total IgG will be measured against the Sm-p80 protein. Briefly, thawed serum samples are serially diluted (12 dilutions at 4-fold each) using robotic liquid handlers on duplicate 384-well plates. The serially diluted sera are transferred and incubated on ELISA plates coated with Sm-p80 protein. Appropriate standards (pooled participant sera) and controls will be included for plate quality control based on criteria established during prior optimizations. Following, secondary antibody (HRP-conjugated) incubation, plates will be developed and read at OD 450-570nm. Endpoint titer will be calculated for each curve and duplicates will be averaged if within established variance limits.

7.2.2.2 RNA-Seq

RNA Sequencing (RNA-Seq) will be performed by Novogene from the whole blood PAXgene tubes that will be shipped from TTUHSC. Novogene will perform total RNA extraction, RNA quantification and qualification, library preparation, Illumina whole-transcriptome sequencing, and raw data quality checking. Novogene will transfer the raw FASTQ files to Emmes. Emmes will perform RNA-Seq data analyses, including pre-alignment sequence quality control, reference alignment, post-alignment quality control, gene expression quantification, differential gene expression analyses, gene cluster and pathway analyses, as well as predictive modeling (see Section 10.5.4).

7.2.2.3 Laboratory Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are included in the laboratory manual and protocol-specific MOP as appropriate.

7.2.2.4 Laboratory Specimen Shipping

Specimens collected during the study will be shipped according to the study MOP. Specimen labeling requirements will also be included in the study MOP.

8 ASSESSMENT OF SAFETY

8.1 Assessing and Recording Safety Parameters

Safety will be assessed by the frequency and severity of:

1. SAEs occurring from the time of the first study product injection through the end of follow-up which is approximately 12 months after the last study product injection.

2. Solicited AEs – reactogenicity events occurring from the time of each study product injection through 7 days after each study product injection:

   a) Injection site reactions including pruritus, erythema, induration/swelling, pain, and tenderness.

   b) Systemic reactions including fever, chills, fatigue, malaise, myalgia, arthralgia, headache, nausea, and vomiting.

3. Clinical safety laboratory AEs assessed as described in Section 7.2.1. Parameters to be evaluated include WBC, hemoglobin, platelets, ALT, and creatinine.

4. Unsolicited AEs – non-serious AEs occurring from the time of each study product injection through 28 days after the study product injection.

5. MAAEs, NOCMCs, and PIMMCs occurring from the time of the first study product injection through the end of follow-up which is approximately 12 months after the last study product injection.

8.1.1 Adverse Events (AEs)

The International Conference on Harmonisation (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. The FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study

visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs, including solicited local (injection site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate eCRF. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

If an event meets both the criteria of a study endpoint and an AE, the event will be reported either as a study endpoint or as an AE (not both).

8.1.1.1 Adverse Events Grading

All AEs (laboratory and clinical symptoms) will be graded for severity and assessed for relationship to study product (see definitions). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate eCRF.

Severity of Event:

AEs will be assessed by the investigator using the protocol-defined grading system specified in Section 8.1.2 and Appendix C. For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

Relationship to Study Product: The assessment of the relationship of an AE to the administration of study product is made only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator based on all available information at the time of the completion of the eCRF. Whether the AE is related or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. The relationship to study product will be assessed for AEs using the terms related or not related:

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.1.2 Reactogenicity

Reactogenicity events are AEs that are known to occur with this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Table 3: Injection Site Reactogenicity Grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site (spontaneous discomfort)	Subject is aware of pain but it does not interfere with daily activity **and** no pain medication is taken	Subject is aware of pain; there is interference with daily activity **or** it requires use of pain medication	Subject is aware of pain **and** it prevents daily activity
Tenderness – hurts only when injection site is touched or the arm is moved	The area immediately surrounding the injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it prevents daily activity
Pruritus (itching)	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Swelling*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity

* Will be also measured in cm but size will not be used as halting criteria.

Table 4: Injection Site Reactogenicity Measurements

Local (Injection Site) Reaction	Small (Grade 1)	Medium (Grade 2)	Large (Grade 3)
Erythema (Redness)*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
Induration (Hardness)/Swelling*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm

* Will not be used as halting criteria.

Table 5: Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Chills	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia (Body Aches/Muscular Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (Joint Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Nausea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Vomiting	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

* Not at injection site

Oral temperature[#] will be graded as follows:

Table 6: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral[†]	38.0°C – 38.4°C 100.4°F – 101.1°F	38.5°C – 38.9°C 101.2°F – 102.0°F	>38.9°C >102.0°F

[#] Oral temperature assessed on Day 1 (Visit 01) before the first study product injection will be considered as baseline.

*Note: A fever can be considered not related to the study product if an alternative etiology can be documented.

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

8.1.3 Serious Adverse Events (SAEs)

An AE or suspected adverse reaction is considered an SAE if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening AE*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

*Life-threatening AE. An AE is considered "life-threatening" if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 or by the Institution as the site Principal Investigator or Sub-Investigator.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study physician (for Investigational New Drug Application [IND] studies, a physician listed on the Form FDA 1572 as the site PI or Sub-Investigator).
- Reviewed and evaluated by DMID, the DSMB or SMC (periodic review unless related), and the IRB/IEC.

8.2 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

8.2.1 Solicited Events

Solicited events are AEs that are common and known to occur following administration of study product.

8.2.2 Unsolicited Events

Unsolicited events are any other AEs that occur following administration of study product.

8.2.3 New-Onset Chronic Medical Conditions (NOCMCs)

NOCMCs are defined as any new ICD-10 diagnosis that is applied to the subject during the duration of the study, after receipt of the study agent, that is expected to continue for at least 3 months and requires continued health care intervention.

8.2.4 Medically-Attended Adverse Events (MAAEs)

Defined as a hospitalization, ER visit, or an otherwise unscheduled visit to or from medical personnel for any reason; and considered related to study product.

8.2.5 Potentially Immune-Mediated Medical Conditions (PIMMCs)

PIMMCs constitute a group of AEs that includes diseases which are clearly autoimmune in etiology and other inflammatory and/or neurologic disorders which may or may not have autoimmune etiologies. PIMMCs currently in effect are presented in Appendix B List of PIMMCs.

8.3 Reporting Procedures

8.3.1 Reporting Serious Adverse Events

SAEs 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.

Any AE (including MAAEs, NOCMC, and PIMMCs) that is considered an SAE must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)

6500 Rock Spring Dr. Suite 650

Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the DCC system. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

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 regulatory reporting and potential impact on study subject safety and protocol conduct.

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

8.3.2 Regulatory Reporting for Studies Conducted Under a DMID Sponsored IND

Following notification from the site PI or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected unexpected SAE only if there is evidence to suggest a causal relationship between the study intervention and the AE. DMID will submit an IND safety report to the FDA and will notify the site PI of potential serious risks from clinical studies or any other source, as soon as possible. DMID will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. If the event is not fatal or life-threatening the IND safety report will be submitted within 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available.

All SAEs designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

8.3.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via Advantage eClinical on the Pregnancy Report form. No further study vaccinations will be administered to pregnant subjects, but with the subject's permission all protocol-required venous blood samples will be obtained and the subject will continue to be followed for safety for the duration of this trial. Efforts will be made to follow all pregnancies reported during the course of this trial to pregnancy outcome pending the subject's permission.

The pregnancy will be documented on the Pregnancy Reporting Form provided by Emmes, and this form will be used for the pregnancy event data entry into Advantage eClinical. All study mandated blood samples will be obtained and the participant will continue in follow-up for safety events. Pregnancies will be followed until 30 days after delivery to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. No subsequent product injections will be administered to pregnant participants.

8.3.4 Disclosure of Study Related Information

Subjects can choose to receive written disclosure of their treatment assignment after the database has been locked.

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

AEs will be assessed and followed from initial recognition of the AE through end of the protocol defined follow-up period.

SAEs will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined follow-up period.

Resolution of an AE is defined as the return to pre-treatment 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.

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

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

Clinical safety laboratory results from specimens obtained after the first study product injection will be graded as indicated in the Toxicity Tables in Appendix C. Clinical laboratory evaluations assessed on the day of and prior to the first study product injection (Day 1) will be considered as baseline.

Systolic and diastolic blood pressures obtained following vaccination will be defined as AEs and graded as indicated in the Toxicity Tables in Appendix C below. However, baseline values prior to the first vaccination, including values obtained at the screening visit and at Visit 1 prior to vaccine administration, may meet the inclusion criteria of systolic blood pressure 85 to 150 mmHg, inclusive, but fall within values defined as Grade 1 severity in the Toxicity Table. Similarly, baseline values may meet the inclusion criteria of diastolic blood pressure ≤ 100 mmHg but fall within values defined as Grade 1 severity in the Toxicity Table. For those participants with baseline blood pressure values that are defined as Grade 1 in the Toxicity Table, subsequent, post-vaccination, values will not be defined as an AE unless the post-vaccination value meets the criteria for Grade 2 or 3 and therefore represents a change from baseline. Those values that represent a change from baseline will be defined as Grade 1 AEs for the Grade 2 values and Grade 2 AEs for the Grade 3 values as they represent a change of one two grades from baseline, respectively.

8.6 Halting Rules

8.6.1 Study Halting Criteria

Further enrollment and study product administrations for all study groups will be halted for SMC review and discussion of all appropriate safety data if any of the following halting criteria are met one or more subjects in any treatment group experience any of the following AEs from the time of first study product administration through 28 days after last study product administration.

- Any death occurring within 28 days after administration of a study product injection that was not the result of trauma or accident, regardless of relatedness to study product.
- Any subject experiences ulceration, abscess, or necrosis at the injection site considered related to study product administration.

- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 24 hours after administration of study product that is considered related to study product.
- Two or more subjects experience generalized urticaria within 72 hours after administration of study product that is considered related to study product.
- Any other SAE deemed to be related to study product based on close temporal relationship or other factors.
- Three or more subjects in the same dose/schedule study group discontinue study product injections due to AEs.
- Three or more subjects develop same Grade 3 clinical laboratory AE related to the study product.

The study will also be halted for SMC review/recommendation if, within 7 days after administration of study product, any of the following occurs:

- ・Two or more subjects experience the same Grade 3 study product-related injection site reaction (excluding the size of erythema and swelling/induration).
- ・Two or more subjects experience the same Grade 3 study product-related subjective systemic reaction, for which the severity (grade) is corroborated by study personnel.
- ・Two or more subjects experience the same Grade 3 study product-related quantitative systemic reaction.
- ・Two or more subjects experience the same Grade 3 study product-related laboratory abnormality.

Grading scales for solicited injection site and systemic (subjective and quantitative) reactions are included in Section 8.1.2.

Grading scales for clinical safety laboratory AEs are included in Appendix C.

If any of the study halting rules are met, the remaining enrollments and study product injections will be halted and will not be resumed without a review by and recommendation from the SMC to proceed.

DMID retains the authority to suspend additional enrollment and administration of study product during the entire trial, as applicable.

The DMID Medical Monitor is empowered to stop enrollment and study product injections if AEs that meet the halting criteria are reported. Similarly, the PI may, using discretion, ask for the study to be placed on hold and an SMC meeting to be held for any single event or combination of multiple events which, in the PI's professional opinion, jeopardize the safety of the subjects or the reliability of the data.

8.6.2 Individual Halting Criteria

The study product injections will be discontinued in an individual if:

- The subject has a systemic hypersensitivity reaction following a study product injection that is judged related to the study product
- The subject has ulceration, abscess, or necrosis at the injection site related to study product administration.
- 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 or equal to 37.8°C, on the day of a scheduled study product injection, the study product injection should be postponed/deferred until the signs, symptoms, or acute illness have resolved, or are improving as further specified below, and if within the acceptable protocol-specified window for that visit No exceptions to the protocol-specified window will be made.

  Note for afebrile, acute illness only: If a subject is afebrile, has an acute illness that is nearly resolved with only minor residual symptoms remaining, is still within the acceptable protocol-specified window for the study product injection and, in the opinion of the site PI or appropriate sub-investigator, the residual symptoms will not interfere with the ability to assess safety parameters as required by the protocol, the subject may receive the study product injection without further approval from the DMID Medical Officer. No exceptions to the protocol-specified window will be made.
- Any clinically significant medical condition that, in the opinion of the participating site PI or appropriate sub-investigator, poses an additional risk to the subject if they continue to participate in the study.
- Grade 3 clinical safety laboratory value (according to the Toxicity Tables Appendix C) that does not decrease to Grade 2 or less prior to the subsequent study product injection. Any clinical safety laboratory parameter may be re-evaluated only once prior to the subsequent study product injection. If the clinical safety laboratory value decreases to Grade 2 or less, the subject may receive the subsequent study product injection. The subsequent study

product injection should be scheduled to occur within the acceptable protocol-specific window for that visit. No exceptions to the protocol-specified window will be made.

- Severe or sustained reaction or disability related to prior administration of study product.

8.6.3 Dose Escalation Halting Criteria

Subjects are scheduled to receive three injections of the assigned study product, given on Days 1, 29, and 57 or on Days 1, 29, and 180. Dose escalation will proceed as described in the Protocol Summary. If one or more dose escalation stopping rules are met the SMC will be convened to review the safety information and determine whether dose escalation should proceed.

- Any subject develops a Grade 3 solicited or unsolicited systemic AE or a Grade 3 clinical laboratory AE that is judged related to the study product.
- Three or more subjects in the most recently enrolled study group develop the same Grade 3 solicited injection site AE related to the study product.
- Three or more subjects develop the same Grade 2 or higher solicited or unsolicited systemic AE or the same Grade 2 or higher clinical laboratory AE that is judged related to study product.
- Any subject has an event meeting an individual halting criterion.

8.7 Safety Oversight

This clinical study will utilize an SMC, which is an independent group of experts that advises the DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to the DMID and comprises at least three voting members. The SMC will consist of members with appropriate phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in a SMC charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study and the principal investigator may request an ad hoc SMC meeting through DMID.

The SMC will review cumulative safety data at time points that may include but not limited to: if dose escalation criteria are not met, if halting criteria are met, on an ad hoc basis if a potential safety concern is identified, at least annually for safety data review, or for final safety data review after completion of the study safety follow-up. These activities will be delineated in the SMC charter.

9 HUMAN SUBJECTS PROTECTION

9.1 Institutional Review Board/Independent Ethics Committee

The site principal investigator for this single-site trial will obtain IRB approval for this protocol to be conducted at the research site and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB must be registered with Office of Human Research Protection (OHRP) as applicable to the research. DMID must receive the documentation that verifies IRB approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB before they are implemented. IRB review and approval will occur at least annually throughout the enrollment and follow-up of subjects and may cease if annual review is no longer required by applicable regulations and the IRB. The investigator will notify the IRB of deviations from the protocol and reportable SAEs, as applicable to the IRB policy.

Each institution engaged in this research will hold a current FWA issued by the OHRP for federally funded research.

9.2 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of the trial that are experimental, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, alternative procedures that may be available and the important potential benefits and risks of these available alternative procedures.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written ICF, the subject is authorizing such access.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved, and subjects will be asked to read and review the consent form. Subjects must sign the ICF prior to starting any study procedures being done specifically for this trial.

Once signed, a copy of the ICF will be given to the subject(s) for their records. The subject(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subject(s) will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study personnel may employ recruitment efforts prior to obtaining study consent if the IRB has agreed that persons identified from a patient database (of Kaiser Permanente Washington) may be sent recruitment materials, and - if interested - receive further information about the study by phone and provide oral consent to respond to questions to determine presumptive eligibility by phone. If the IRB has agreed, persons interested in participating in the study who are presumptively eligible by the phone screen could provide oral consent to allow review of their Kaiser Washington electronic medical record. If that review does not identify exclusionary information, a screening clinic visit would be scheduled. At that visit, research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all ICFs that they sign.

9.2.1 Requirements for Permission by Parents/Guardians and Assent by Children (in case of a minor)

Not applicable.

9.2.2 Other Informed Consent Procedures

Not applicable.

9.3 Consent for Secondary Research Use of Stored Specimens and Data

9.3.1 Secondary Use of Stored Specimens and Data

Secondary Human Subject Research is the re-use of identifiable data or identifiable biospecimens that were collected from some other ‘‘primary’’ or ‘‘initial’’ activity, such as the data and samples collected in this protocol. This section will detail the samples and data

available for secondary research. Any use of the secondary sample or data, however, will be presented in a separate protocol and require separate IRB approval.

9.3.2 Samples for Secondary Research

Residual samples/specimens are those that are left over after protocol-specified testing and this study has been completed. As a condition of study participation, subjects agree that residual samples will be stored coded indefinitely at the DMID CMS. On completion of the trial, residual specimens may be shared with investigators at the participating site and with other investigators at other institutions. The recipients of specimens will be informed that these specimens have a NIH certificate of confidentiality. The information provided to the recipient will not contain direct identifiable information.

Samples will be stored indefinitely at a DMID-designated storage facility. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality. Secondary research with coded samples and data may occur, however, subject confidentiality will be maintained as described for this protocol. An IRB review of the secondary research using coded specimens is required.

Residual/Repository Research Samples, upon written request and approval from DMID and any approvals required by the site or network, may be shared for secondary research with investigators at the participating site, with researchers at other Infectious Disease Clinical Research Consortium (IDCRC) sites or other institutions, or company-designated research laboratories. The samples will not be sold or used directly for production of any commercial product. DMID will authorize shipment from the DMID CMS.

TTUHSC may receive serum samples and PBMC before the final database lock. The serum samples can be aliquoted and stored for secondary research. The PBMC may be received and stored for secondary research. TTUHSC will not evaluate the samples until after the final database lock and approval received from DMID and the IDCRC per the IDCRC secondary research policy.

Reports from secondary research will not be kept in the subjects' health records or shared with subjects, unless required by law. Reports will not be sent to the specimen repository.

The subject's decision can be changed at any time by notifying the study doctors or nurses in writing. To participate in this study, subjects must consent for storage of samples for secondary use. If the subject subsequently changes his/her decision, the samples will be destroyed if the samples have not been used for research or released for a specific research project.

9.3.3 Data Sharing for Secondary Research

Data from this study may be used for secondary research. All of the individual subject data collected during this study will be made available after de-identification. The SAP and Analytic Code will also be made available. Data will be available immediately following publication, with no end date. Upon written request, with provision of a methodologically sound proposal, and approval from DMID and any approvals required by the site or network, data may be shared for secondary research with investigators/researchers. The data will be available for only the purpose outlined in the approved proposal.

For access to genomic data in the NIH designated controlled access database, an investigator (or data requestor) must submit a Data Access Request which certifies adherence to the NIH Security Best Practices for Controlled-Access data subject to the NIH Genomic Data Sharing (GDS) Policy. The participating site PI may request removal of data on individual study subjects from NIH data repositories in the event that a research subject withdraws or changes his or her consent. However, some data that have been distributed for approved research use cannot be retrieved.

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

All healthy adults 18 through 55 years of age, who meet all of the inclusion and none of the exclusion criteria (see Section 5.1), regardless of religion, sex, or ethnic background, will be included in the study. Due to the nature of the study, and the fact that no benefit of study participation exists, children will not be enrolled at this time. Women who are pregnant or plan to become pregnant up to 30 days after the last study product injection and persons <18 years of age are excluded from the study.

9.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences. The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or

hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

9.6 Certificate of Confidentiality

As this research is funded by the NIH, it is covered by NIH policy which effectively issues the research a Certificate of Confidentiality (COC). By this policy, researchers cannot be forced to disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the FDA.

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, or for the purposes of other research that is in compliance with applicable Federal regulations governing the protection of human subjects in research.

9.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the subject, subject's

insurance or third party. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, for any injury suffered due to participation in this trial.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

This Phase 1, first-in-human study is not designed to test a formal null hypothesis. Rather, it is intended to obtain exploratory, initial data on the study product safety and effect of antigen dose and inclusion of the GLA-SE adjuvant on humoral immune responses to guide future dose selection.

10.2 Sample Size Considerations

This Phase 1 study is not designed for confirmatory testing but rather to detect AEs related to the study product. If an AE has a 20% chance of occurring after receiving one of the product formulations being tested, then there is an 87% probability that the event would be observed in one or more of the 9 subjects in that treatment group in this study. If the event has a 20% chance or higher of occurring in **any** of the product formulations being tested, then there is at >99% probability that the event would be observed in one or more of the 45 subjects in any treatment group in the study. The below table shows analogous probabilities for events with other frequencies.

Probability of event after receiving study product	Probability of observing event among 9 subjects	Probability of observing event among 45 subjects
0.01	0.086	0.364
0.05	0.370	0.901
0.10	0.613	0.991
0.15	0.768	0.999
0.20	0.866	>0.99
0.25	0.925	>0.99

10.3 Treatment Assignment Procedures

10.3.1 Randomization Procedures

This is a non-randomized trial. Per ICH guideline E6: GCP, screening records will be kept at each participating VTEU site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical Data Coordinating Center's (SDCC) Advantage eClinical Electronic Data Capture System.

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the subject will be enrolled. All subjects will receive three study product injections; the same study product formulation will be given to the subject for each study product injection. In the unlikely event that a subject withdraws prior to receiving the first injection, the subject will be replaced with a new subject who will be given the same treatment assignment.

10.3.2 Masking Procedures

This is an open-label clinical trial.

Subjects, investigators, study personnel performing any study-related assessments following study vaccine administration are not blinded to study treatment. Laboratory personnel performing immunologic assays will receive samples blinded to subject ID number and sample visit number.

The SMC may receive data in aggregate and presented by Study Group.

10.4 Planned Interim Analyses

Interim analyses will be used to terminate this trial in the event that unanticipated safety events deemed to be of sufficient concern require such action by the sponsor. These assessments will not be made on the basis of testing a formal statistical hypothesis; therefore, p-value adjustment will not be made to any analyses. A SMC will be convened by DMID to review study progress and participant, clinical, safety, reactogenicity and immunogenicity data as described in Section 8.7

To guide protocol development of future studies of the candidate vaccine, interim analyses of safety data and immunogenicity assays, including the IgG ELISA assay, will be performed at the discretion of the study team prior to completion of follow-up of all subjects. The interim immunogenicity analyses report(s) will include between-Group comparisons of those who received different Sm-p80 study product formulations and different schedules. The information from the report may be used in abstracts and scientific presentations and reports.

10.4.1 Interim Safety Review

An interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, dosing compliance, 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 DMID. The SMC may receive data in aggregate and presented by treatment arm. The SMC will meet and review this data at scheduled time points or ad hoc as needed during this trial as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding

with study vaccinations (as applicable), and to continue, modify, or terminate this trial.

Additionally, this trial will be monitored to determine if any of the halting rules described in Section 8.6 are met.

10.4.2 Interim Immunogenicity or Efficacy Review

Interim immunogenicity analyses of, for example, IgG ELISA responses will be performed for each Study Group, as the data is available for each study group. Depending on the timing of data availability, a report may cover one or more Study Groups. Immunogenicity reports will be provided by the SDCC to the DMID Scientific Lead and CPM, the IDCRC LOU representative(s), the study PI and investigators, and the SMC. Reports will include data summarized by Study Group. The data will be cleaned but not locked. These reports along with interim safety results may be presented in publications, or public forums and used by the product developers for internal decision-making.

10.5 Final Analysis Plan

Analyses for this Phase 1 study will be descriptive and are exploratory rather than confirmatory in nature. Every attempt will be made to collect all planned measurements. Statistical adjustments for missing data are not planned, but sensitivity analyses may be performed if warranted.

An interim CSR will be completed when all primary and secondary endpoint data are available, received by the SDCC, cleaned, and locked. Exploratory endpoint data will be included in an addendum to the CSR once available, received by the SDCC, cleaned, and locked. Endpoint data may be included in publication of manuscript(s) or other reports.

Separate SAP documents (interim and addendum) will be generated that will contain the details of the final analyses that will be performed following database lock. This section outlines the major components of the final analyses.

10.5.1 Analysis of Demographics

Descriptive summaries of demographic variables, including age, sex, race, and ethnicity, will be summarized by study group. Numeric variables will be summarized by mean and standard deviation; categorical variables will be tabulated.

10.5.2 Analysis of Safety Data

All participants who received at least one vaccination will be included in safety summaries. Solicited and unsolicited events will be summarized graphically and numerically. Numbers and proportions of participants experiencing solicited systemic events will be summarized by

symptom, study group and maximum severity. Furthermore, proportions of participants experiencing systemic events will be summarized by study day, study group, and maximum severity. Solicited injection site events will be summarized in the same way as systemic solicited events.

Numbers and proportions of participants experiencing unsolicited AEs will be summarized by MedDRA System Organ Class and Preferred Term, study group, and maximum severity. Unsolicited AEs will also be summarized by relationship to study product, study group, and maximum severity.

Detailed listings of MAAEs, NOCMCs, PIMMCs, and all SAEs, will be presented, including the most recent dose number, number of days since most recent dose, relationship to study product, severity, outcome, MedDRA System Organ Class and Preferred Term, and study group.

Time trends of laboratory parameter values will be visualized with background shading indicating normal and graded ranges for that parameter.

10.5.3 Analysis of Immunogenicity Data

Immunogenicity summaries will include all participants who received at least one study vaccination and contributed both pre- and at least one post-study vaccination blood sample for immunogenicity testing for which valid results were reported. Antibody levels will be summarized graphically and numerically. Individual time trends will be visualized as will reverse cumulative distribution plots. Numbers and proportions of participants with seroconversion, defined as a fourfold rise from baseline, will be presented along with 95% CIs by time point and study group. Statistical summaries will include geometric means, geometric mean fold-rise, mean, median, standard deviation, minimum, and maximum by time point and study group.

10.5.4 RNA-Seq Analysis

For the transcriptomics exploratory analysis, sequencing quality will be inspected using FASTQC. RNA-Seq data will be pre-processed by removing adapters and low-quality reads, trimming low quality ends, and mapping sequences to the latest human reference genome using splice-aware alignment software such as *HISAT2.[25]* Gene expression quantification will be carried out by using the *Subread* software [26]using the latest Ensembl[27]gene model annotations as a reference. Systematic differences in sequence coverage between samples will be accounted for using the TMM method [31]as implemented in the *edgeR* R package.[28] Principal component analysis, non-metric multidimensional scaling, and hierarchical clustering analysis will be used to identify potential global outliers and systematic batch effect. Negative binomial models as implemented in *edgeR* [28]will be used to identify genes for each dose group and post-

vaccination day that were differentially expressed compared to last vaccination: Days 8 and 29 vs. Day 1, and Day 36 vs. Day 29 for all study groups, Day 57 vs. Day 29, Day 64 vs. Day 57 for Groups A, B, D, and E, Day 187 vs. Day 180 for Group C. To control for testing multiple genes, the false-discovery rate based on the Benjamini-Hochberg procedure[32] as implemented in the *p.adjust* R function will be applied. The *pvclust* [33]R package will be used to identify co-expressed DE gene clusters. Mean cluster $\log_2$ fold change time trends and associated 95% bootstrap confidence intervals (CIs) will be presented by group across post-vaccination days. To functionally characterize differently expressed genes, pathway enrichment analysis based on the latest MSigDB [34]and KEGG databases[35] as well as Blood Transcription Modules will be carried out using the implementation provided by the *goseq* R package [36]which accounts for RNA-Seq gene length bias. Pathway maps color-coded by treatment effect ($\log_2$ fold change compared to pre-vaccination) will be visualized for significantly enriched KEGG pathways. Pathway enrichment trends by group across post-vaccination days will be visualized using bubble plots contrasting median pathway response with pathway enrichment as well as time trend plots of median pathway responses (based on median average $\log_2$ fold changes of all pathway members) and associated 95% bootstrap CIs. Regularized linear and/or logistic regression analysis as implemented in the *glmnet* R package [37]will be utilized to identify gene expression changes that are predictive of antibody levels that provide protective efficacy *ex vivo* as measured using passive transfer. Cross-validation in combination with bootstrap resampling will be used to select optimal models and to assess robustness of variable selections. Please see the separate document “Statistical Analysis Plan” for additional information on exploratory RNA-Seq analyses.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

This study uses direct data entry for the participating clinic site and the eCRFs serve as the source documents for data collected.

Subjects will use a web-based electronic Memory Aid (e-Memory aid), or paper Memory Aid if internet access is not available. Data entered by subjects into the e-Memory Aid are stored in the data system for clinic staff review during scheduled visits. The e-Memory Aid is not considered source data. After clinic staff review and save the data, the data will be entered into Advantage eClinical as source.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site(s) and its subcontractors are responsible for conducting routine quality assurance and quality control activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC 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 participating site(s) for clarification and resolution.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

The site principal investigator is responsible to ensure the accuracy, completeness, and timeliness of the data reported.

Data will be entered electronically over the Internet by site study staff into Advantage eClinical, developed and maintained by the SDCC. The eCRFs serve as the source documents for data collected. Paper case report forms derived from the eCRF are provided by the SDCC and are to be used only when Advantage eClinical is unavailable. Details on data handling procedures, procedures for data monitoring, and instructions for use of the system and completion of the eCRFs are provided in the study MOP, eCRF Instructions, and Advantage eClinical User's Guide.

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the eCRF.

13.2 Data Coordinating Center/Biostatistician Responsibilities

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

Emmes will serve as the SDCC for this study, and will be responsible for data management, quality review, analysis, and reporting of the study data.

13.3 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values) and reactogenicity will be entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by Emmes. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Site staff who are delegated the responsibility by the study PI will be the data originators for clinical data entered directly into the eCRF. A list of all authorized data originators, including site staff, will be included on the Study Personnel/Signature Responsibility List.

13.4 Types of Data

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

13.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study drug disposition records will be retained for two years after a marketing application is approved for the study product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the study product, until two years after the investigation is discontinued and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. ICFs for future use will be maintained as long as the sample/specimen exists.

No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the site principal investigator when these documents no longer need to be retained. The participating VTEU sites must contact DMID for authorization prior to the destruction of any study records.

14 CLINICAL MONITORING

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and will document site visit findings and discussions.

15 PUBLICATION POLICY

Following completion of the study, the lead PI is expected to publish the results of this research in a scientific journal. All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

16 LITERATURE REFERENCES

1. Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet*. Sep 23 2006;368(9541):1106-18. doi:10.1016/S0140-6736(06)69440-3
2. Savioli L, Mott KE. Urinary schistosomiasis on Pemba Island: low-cost diagnosis for control in a primary health care setting. *Parasitol Today*. Oct 1989;5(10):333-7.
3. De Vlas SJ, Engels D, Rabello AL, et al. Validation of a chart to estimate true Schistosoma mansoni prevalences from simple egg counts. *Parasitology*. Feb 1997;114 (Pt 2):113-21.
4. King CH. Parasites and poverty: the case of schistosomiasis. *Acta Trop*. Feb 2010;113(2):95-104. doi:10.1016/j.actatropica.2009.11.012
5. Hotez PJ, Strych U, Lustigman S, Bottazzi ME. Human anthelminthic vaccines: Rationale and challenges. *Vaccine*. Jun 24 2016;34(30):3549-55. doi:10.1016/j.vaccine.2016.03.112
6. Mo AX, Colley DG. Workshop report: Schistosomiasis vaccine clinical development and product characteristics. *Vaccine*. Feb 17 2016;34(8):995-1001. doi:10.1016/j.vaccine.2015.12.032
7. Stylianou A, Hadjichrysanthou C, Truscott JE, Anderson RM. Developing a mathematical model for the evaluation of the potential impact of a partially efficacious vaccine on the transmission dynamics of Schistosoma mansoni in human communities. *Parasit Vectors*. Jun 17 2017;10(1):294. doi:10.1186/s13071-017-2227-0
8. Ahmad G, Zhang W, Torben W, et al. Preclinical prophylactic efficacy testing of Sm-p80-based vaccine in a nonhuman primate model of Schistosoma mansoni infection and immunoglobulin G and E responses to Sm-p80 in human serum samples from an area where schistosomiasis is endemic. *J Infect Dis*. Nov 2011;204(9):1437-49. doi:10.1093/infdis/jir545
9. Zhang W, Molehin AJ, Rojo JU, et al. Sm-p80-based schistosomiasis vaccine: double-blind preclinical trial in baboons demonstrates comprehensive prophylactic and parasite transmission-blocking efficacy. *Ann N Y Acad Sci*. Aug 2018;1425(1):38-51. doi:10.1111/nyas.13942
10. Siddiqui AJ, Molehin AJ, Zhang W, et al. Sm-p80-based vaccine trial in baboons: efficacy when mimicking natural conditions of chronic disease, praziquantel therapy, immunization, and Schistosoma mansoni re-encounter. *Ann N Y Acad Sci*. Jun 11 2018;doi:10.1111/nyas.13866
11. Karmakar S, Zhang W, Ahmad G, et al. Use of an Sm-p80-based therapeutic vaccine to kill established adult schistosome parasites in chronically infected baboons. *J Infect Dis*. Jun 15 2014;209(12):1929-40. doi:10.1093/infdis/jiu031
12. Karmakar S, Zhang W, Ahmad G, et al. Cross-species protection: Schistosoma mansoni Sm-p80 vaccine confers protection against Schistosoma haematobium in hamsters and baboons. *Vaccine*. Mar 5 2014;32(11):1296-303. doi:10.1016/j.vaccine.2013.12.057

13. Molehin AJ, Sennoune SR, Zhang W, et al. Cross-species prophylactic efficacy of Sm-p80-based vaccine and intracellular localization of Sm-p80/Sm-p80 ortholog proteins during development in Schistosoma mansoni, Schistosoma japonicum, and Schistosoma haematobium. *Parasitol Res*. Nov 2017;116(11):3175-3188. doi:10.1007/s00436-017-5634-4
14. Zhang W, Ahmad G, Le L, et al. Longevity of Sm-p80-specific antibody responses following vaccination with Sm-p80 vaccine in mice and baboons and transplacental transfer of Sm-p80-specific antibodies in a baboon. *Parasitol Res*. Jun 2014;113(6):2239-50. doi:10.1007/s00436-014-3879-8
15. Coler RN, Duthie MS, Hofmeyer KA, et al. From mouse to man: safety, immunogenicity and efficacy of a candidate leishmaniasis vaccine LEISH-F3+GLA-SE. *Clin Transl Immunology*. Apr 2015;4(4):e35. doi:10.1038/cti.2015.6
16. Coler RN, Day TA, Ellis R, et al. The TLR-4 agonist adjuvant, GLA-SE, improves magnitude and quality of immune responses elicited by the ID93 tuberculosis vaccine: first-in-human trial. *NPJ Vaccines*. 2018;3:34. doi:10.1038/s41541-018-0057-5
17. Penn-Nicholson A, Tameris M, Smit E, et al. Safety and immunogenicity of the novel tuberculosis vaccine ID93 + GLA-SE in BCG-vaccinated healthy adults in South Africa: a randomised, double-blind, placebo-controlled phase 1 trial. *Lancet Respir Med*. Apr 2018;6(4):287-298. doi:10.1016/S2213-2600(18)30077-8
18. Pillet S, Aubin E, Trepanier S, et al. Humoral and cell-mediated immune responses to H5N1 plant-made virus-like particle vaccine are differentially impacted by alum and GLA-SE adjuvants in a Phase 2 clinical trial. *NPJ Vaccines*. 2018;3:3. doi:10.1038/s41541-017-0043-3
19. Treanor JJ, Essink B, Hull S, et al. Evaluation of safety and immunogenicity of recombinant influenza hemagglutinin (H5/Indonesia/05/2005) formulated with and without a stable oil-in-water emulsion containing glucopyranosyl-lipid A (SE+GLA) adjuvant. *Vaccine*. Nov 19 2013;31(48):5760-5. doi:10.1016/j.vaccine.2013.08.064
20. Santini-Oliveira M, Coler RN, Parra J, et al. Schistosomiasis vaccine candidate Sm14/GLA-SE: Phase 1 safety and immunogenicity clinical trial in healthy, male adults. *Vaccine*. Jan 20 2016;34(4):586-594. doi:10.1016/j.vaccine.2015.10.027
21. Sirima SB, Durier C, Kara L, et al. Safety and immunogenicity of a recombinant Plasmodium falciparum AMA1-DiCo malaria vaccine adjuvanted with GLA-SE or Alhydrogel(R) in European and African adults: A phase 1a/1b, randomized, double-blind multi-centre trial. *Vaccine*. Oct 27 2017;35(45):6218-6227. doi:10.1016/j.vaccine.2017.09.027
22. Belshe RB, Frey SE, Graham I, et al. Safety and immunogenicity of influenza A H5 subunit vaccines: effect of vaccine schedule and antigenic variant. *J Infect Dis*. Mar 1 2011;203(5):666-73. doi:10.1093/infdis/jiq093
23. Marsano LS, Greenberg RN, Kirkpatrick RB, et al. Comparison of a rapid hepatitis B immunization schedule to the standard schedule for adults. *Am J Gastroenterol*. Jan 1996;91(1):111-5.
24. Madan A, Segall N, Ferguson M, et al. Immunogenicity and Safety of an AS03-Adjuvanted H7N9 Pandemic Influenza Vaccine in a Randomized Trial in Healthy Adults. *J Infect Dis*. Dec 01 2016;214(11):1717-1727. doi:10.1093/infdis/jiw414

25. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods*. Apr 2015;12(4):357-60. doi:10.1038/nmeth.3317
26. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*. Apr 1 2014;30(7):923-30. doi:10.1093/bioinformatics/btt656
27. Aken BL, Achuthan P, Akanni W, et al. Ensembl 2017. *Nucleic Acids Res*. Jan 4 2017;45(D1):D635-D642. doi:10.1093/nar/gkw1104
28. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. Jan 1 2010;26(1):139-40. doi:10.1093/bioinformatics/btp616
29. Siddiqui AA, Pinkston JR, Quinlin ML, et al. Characterization of the immune response to DNA vaccination strategies for schistosomiasis candidate antigen, Sm-p80 in the baboon. *Vaccine*. Feb 10 2005;23(12):1451-6. doi:10.1016/j.vaccine.2004.09.018
30. Torben W, Ahmad G, Zhang W, et al. Role of antibody dependent cell mediated cytotoxicity (ADCC) in Sm-p80-mediated protection against Schistosoma mansoni. *Vaccine*. Nov 6 2012;30(48):6753-8. doi:10.1016/j.vaccine.2012.09.026
31. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol*. 2010;11(3):R25. doi:10.1186/gb-2010-11-3-r25
32. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the royal statistical society Series B (Methodological)*. 1995:289-300.
33. Suzuki R, Shimodaira H. Pvclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics*. Jun 15 2006;22(12):1540-2. doi:10.1093/bioinformatics/btl117
34. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdottir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics*. Jun 15 2011;27(12):1739-40. doi:10.1093/bioinformatics/btr260
35. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. Jan 1 2000;28(1):27-30. doi:10.1093/nar/28.1.27
36. Young MD, Wakefield MJ, Smyth GK, Oshlack A. Gene ontology analysis for RNA-seq: accounting for selection bias. *Genome Biol*. 2010;11(2):R14. doi:10.1186/gb-2010-11-2-r14
37. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *J Stat Softw*. 2010;33(1):1-22.

17 APPENDICES

Appendix A. Schedules of Events

Table 7: Screening Visit Evaluations and Procedures.

		Screen
Study day		-30 to -1
Study visit		00
Clinical Evaluations and Procedures	Tube	Screen
Sign consent form		X
Assessment of eligibility		X
Review medical history and con meds and collect demographic information		X
Complete physical exam and height and weight, calculate BMI		X
Vital signs (blood pressure, pulse, and oral temperature)		X
Provide HIV test counseling		X
Review of contraceptive/menstrual history; pregnancy avoidance counseling[1]		X
Pregnancy test (serum)[1], creatinine, ALT, HIV-1/HIV-2 testing, anti-HCV antibody, HBsAg (mL)	SST	25.5
WBC, hemoglobin, platelets (mL)	EDTA	4
Blood Volume (mL)		29.5

[1]For women of childbearing potential.

Table 8: Schedule of Events and Study Procedures, Study Groups A, B, D, and E

		Study Product Injection and Follow Up Visits												
Study day		1	2	4	8	29	32	36	57	60	64	85	181	422
Days after most recent vaccination		0	1	3	7	28	3	7	28	3	7	28	124	365
Visit Window (± number of days)		0	0	1	1	3	1	1	3	1	1	3	14	14
Study visit		01	02P	03	04	05	06	07	08	09	10	11	12	13P
Clinical Evaluations and Procedures	Tube													
Assessment of eligibility		X												
Assessment of individual halting criteria						X			X					
Review medical history and con meds[3]		X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs (blood pressure, pulse, and oral temperature[5])		X		X	X	X	X	X	X	X	X	X[4]	X[4]	
Enrollment		X												
Study Product Injection[1]		**I-1**				**I-2**			**I-3**					
Targeted physical exam		{X}		{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	
Pre-administration reactogenicity assessment		X				X			X					
Evaluate study product injection site		X		X	X	X	X	X	X	X	X			
Provide and explain use of memory aid and thermometer		X				X			X					
Review memory aid			X	X	X		X	X		X	X			
Collection of solicited AEs		X	X	X	X	X	X	X	X	X	X			

		Study Product Injection and Follow Up Visits												
Study day		1	2	4	8	29	32	36	57	60	64	85	181	422
Days after most recent vaccination		0	1	3	7	28	3	7	28	3	7	28	124	365
Visit Window (± number of days)		0	0	1	1	3	1	1	3	1	1	3	14	14
Study visit		01	02P	03	04	05	06	07	08	09	10	11	12	13P
Clinical Evaluations and Procedures	Tube													
Collection of unsolicited AEs and SAEs[3]		X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test (urine)[2]		X				X			X					
WBC, hemoglobin, platelets (mL)	EDTA	3			3	3		3	3		3	3		
Creatinine, ALT[6]	SST	10			10	10		10	10		10	10		
Serum collection for antibody assays and secondary research (mL)	SST	8.5			8.5	8.5		8.5	8.5		8.5	8.5	8.5	
PBMC isolation for secondary research (mL)	NaHep/ ACD[7]	60			60	60		60	60		60	34	60	
Whole blood collection for gene expression assays	PAXGene	2.5			2.5	2.5		2.5	2.5		2.5			
Total volume per visit (mL)		84			84	84		84	84		84	55.5	68.5	
Cumulative volume (prior 56 days)		113.5			197.5	281.5		365.5	420		420	391.5	68.5	

{ } required at this visit only if clinically indicated

P=phone assessment

[1]0.5 mL of the designated study product will be injected IM in the deltoid muscle. Prior to the injection, a pre-administration reactogenicity assessment will be performed to establish baseline. After the injection, subjects will be observed for 30 minutes. The injection site will be evaluated and vital signs obtained during that period and before the subject leaves the clinic. Subjects will be provided with a Memory Aid and thermometer for recording daily maximum oral temperature, solicited injection site and systemic reactions, unsolicited AEs, and concomitant medications, beginning on the evening of the day of study product injection and continuing daily for the next seven days. Subjects will be encouraged to take their temperature around the same time each day and also if they feel feverish. Subjects will be instructed on how to use the memory aid and how to rate any AEs. Subjects will also be instructed to notify the study center if they develop any severe reactions and/or fever ≥ 38°C (100.4°F).

[2]For women of childbearing potential. Must be confirmed as negative prior to injection of study product. The visit will also include review of contraceptive/menstrual history and pregnancy avoidance counseling.

[3]After Study Visit 11 (or Study Day 85 if Study Visit 11 is not attended), through the end of study follow-up (Day 422), only SAEs, MAAEs, NOCMCs, and PIMMCs will be collected. Other unsolicited AEs and concomitant medications will not be collected.

[4]Vital signs will be assessed only if clinically indicated.

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

[6] Blood collection volumes shown are the maximum that may potentially be drawn from the participant at each visit based on collection tube availability.

[7]ACD collection tubes can be used to collect blood that will be processed for PBMC if appropriate NaHep tubes are unavailable.

Table 9: Schedule of Events and Study Procedures, Study Group C.

Study day		1	2	4	8	29	32	36	57	180	183	187	208	304	545
Days after most recent vaccination		0	1	3	7	28	3	7	28	151	3	7	28	124	365
Visit Window (± number of days)		0	0	1	1	3	1	1	3	7	1	1	3	14	14
Study visit		01	02P	03	04	05	06	07	08	09	10	11	12	13	14P
Clinical Evaluations and Procedures	Tube														
Assessment of eligibility		X													
Assessment of individual halting criteria						X				X					
Review medical history and con meds[3]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs (blood pressure, pulse, and oral temperature[5])		X		X	X	X	X	X	X	X	X	X	X[4]	X[4]	
Enrollment		X													
Study Product Injection[1]		**I-1**				**I-2**				**I-3**					
Targeted physical exam		{X}		{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	{X}	
Pre-administration reactogenicity assessment		X				X				X					
Evaluate study product injection site		X		X	X	X	X	X		X	X	X			
Provide and explain use of memory aid and thermometer		X				X				X					
Review memory aid			X	X	X		X	X			X	X			
Collection of solicited AEs		X	X	X	X	X	X	X		X	X	X			

Study day		1	2	4	8	29	32	36	57	180	183	187	208	304	545
Days after most recent vaccination		0	1	3	7	28	3	7	28	151	3	7	28	124	365
Visit Window (± number of days)		0	0	1	1	3	1	1	3	7	1	1	3	14	14
Study visit		01	02P	03	04	05	06	07	08	09	10	11	12	13	14P
Clinical Evaluations and Procedures	Tube														
Collection of unsolicited AEs and SAEs[3]		X	X	X	X	X	X	X	X[3]	X	X	X	X	X	X
Pregnancy test (urine)[2]		X				X				X					
WBC, hemoglobin, platelets (mL)	EDTA	3			3	3		3	3	3		3	3		
Creatinine, ALT[6]	SST	10			10	10		10	10	10		10	10		
Serum collection for antibody assays and secondary research (mL)	SST	8.5			8.5	8.5		8.5	8.5	8.5		8.5	8.5	8.5	
PBMC isolation for secondary research (mL)	NaHep/ACD[7]	60			60	60		60	60	60		60	34	60	
Whole blood collection for gene expression assays	PAXGene	2.5			2.5	2.5		2.5		2.5		2.5			
Total volume per visit (mL)		84			84	84		84	84	84		84	55.5	68.5	
Cumulative volume (prior 56 days)		113.5			197.5	281.5		365.5	420	84		168	223.5	68.5	

{ } required at this visit only if clinically indicated

P=phone assessment

[1]0.5 mL of the designated study product will be injected IM in the deltoid muscle. Prior to the injection, a pre-administration reactogenicity assessment will be performed to establish baseline. After the injection, subjects will be observed for 30 minutes. The injection site will be evaluated and vital signs obtained during that period and before the subject leaves the clinic. Subjects will be provided with a Memory Aid and thermometer for recording daily maximum oral temperature, solicited injection site and systemic reactions, unsolicited AEs, and concomitant medications,

beginning on the evening of the day of study product injection and continuing daily for the next seven days. Subjects will be encouraged to take their temperature around the same time each day and also if they feel feverish. Subjects will be instructed on how to use the memory aid and how to rate any AEs. Subjects will also be instructed to notify the study center if they develop any severe reactions and/or fever ≥ 38°C (100.4°F).

[2]For women of childbearing potential. Must be confirmed as negative prior to injection of study product. The visit will also include review of contraceptive/menstrual history and pregnancy avoidance counseling.

[3]After Study Visit 8 (or Study Day 57 if Study Visit 8 is not attended), through the time of the third study vaccination at Study Visit 09, only SAEs, MAAEs, NOCMCs, and PIMMCs will be collected. Other unsolicited AEs and concomitant medications will not be collected during this period. Collection of unsolicited AEs and concomitant medications will then resume at the time of the third vaccination and continue through Study Visit 12 (or Study Day 208 if Study Visit 12 is not attended), after which only SAEs, MAAEs, NOCMCs, and PIMMCs will be collected.

[4]Vital signs will be assessed only if clinically indicated.

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

[6] Blood collection volumes shown are the maximum that may potentially be drawn from the participant at each visit based on collection tube availability.

[7]ACD collection tubes can be used to collect blood that will be processed for PBMC if appropriate NaHep tubes are unavailable.

Appendix B. List of Potentially Immune-Mediated Medical Conditions (PIMMCs)

Gastrointestinal disorders

- Celiac disease (gluten-sensitive enteropathy)
- Inflammatory bowel disease (Crohn's disease, ulcerative colitis or proctitis)

Liver disorders

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

Metabolic diseases

- Primary adrenal insufficiency (Addison's disease)
- Chronic lymphocytic thyroiditis (Hashimoto's disease) (Not exclusionary for enrollment)
- Diabetes mellitus type I
- Graves' or Basedow's disease

Musculoskeletal disorders

- Antisynthetase syndrome
- Dermatomyositis
- Juvenile chronic arthritis/juvenile idiopathic arthritis (including juvenile Still's disease [macrophage activating syndrome])
- Mixed connective tissue disorder
- Polymyalgia rheumatica
- Polymyositis
- Psoriatic arthritis
- Relapsing polychondritis
- Rheumatoid arthritis
- Scleroderma, including diffuse systemic form and CREST syndrome

- Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
- Systemic lupus erythematosus
- Systemic sclerosis

Neuroinflammatory disorders

- Acute disseminated encephalomyelitis, including site specific variants (e.g., non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis)
- Possibly immune-mediated cranial nerve disorders (e.g., Bell’s palsy)
- Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
- Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy
- Multiple sclerosis
- Optic neuritis
- Transverse myelitis
- Myasthenia gravis, including Lambert-Eaton myasthenic syndrome (LEMS)

Skin disorders

- Alopecia areata
- Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis
- Cutaneous lupus erythematosus
- Erythema nodosum
- Localized scleroderma (morphea)
- Lichen planus
- Psoriasis (May not be exclusionary for enrollment, see Section 5.1.2)
- Acute febrile neutrophilic dermatosis (Sweet’s syndrome)
- Vitiligo (May not be exclusionary for enrollment, see Section 5.1.2)

Vasculitides

- Large artery vasculitis: Takayasu’s arteritis and giant cell arteritis (temporal arteritis)
- Small and medium-sized artery vasculitis:

- Polyarteritis nodosa
- Mucocutaneous lymph node syndrome (Kawasaki disease)
- Microscopic polyangiitis
- Granulomatosis with polyangiitis (Wegener's granulomatosis)
- Eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome)
- Thromboangiitis obliterans (Buerger's disease)
- Necrotizing vasculitis (systemic necrotizing vasculitis)
- Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis
- IgA vasculitis (Henoch-Schonlein purpura)
- Behcet's syndrome
- Leukocytoclastic vasculitis (hypersensitivity vasculitis)

Others

- Antiphospholipid syndrome
- Autoimmune hemolytic anemia
- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
- Autoimmune myocarditis/cardiomyopathy
- Autoimmune thrombocytopenia
- Goodpasture syndrome
- Idiopathic pulmonary fibrosis
- Pernicious anemia
- Raynaud's phenomenon
- Sarcoidosis
- Sjögren's syndrome
- Stevens-Johnson syndrome
- Uveitis

Appendix C. Toxicity Tables

Parameters for solicited AEs are reported in Table 3, Table 4, Table 5, and Table 6.

Pulse and Blood Pressure

Physiologic Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Tachycardia - beats per minute	101 - 115	116 - 130	> 130
Hypotension (systolic) mm Hg[1]	85 – 89	80 – 84	<80
Hypertension (systolic) mm Hg[2]	141 – 150	151 – 155	> 155
Hypertension (diastolic) mm Hg[3]	96 – 100	101 – 105	>105

The inclusion criteria for systolic blood pressure includes values from 85 through 150, inclusive and for diastolic blood pressure includes values ≤ 100.

[1]Subjects with baseline (prior to first vaccination) Grade 1 values of 85-89 mm Hg are defined with a hypotension (systolic) AE only due to post-vaccination values ≤ 84 mm Hg and the AE is Defined as Grade 1 based on values of 80 through 84 and Grade 2 based on values less than 80.

[2]Subjects with baseline (prior to first vaccination) Grade 1 values of 141-150 mm Hg are defined with a hypertension (systolic) AE only due to post-vaccination values over 150 mm Hg and the AE is Defined as Grade 1 based on values of 151 through 155 mm Hg and Grade 2 based on values over 155.

[3]Subjects with baseline (prior to first vaccination) Grade 1 values of 96-100 mm Hg are defined with a hypertension (diastolic) AE only due to post-vaccination values ≥ 101 mm Hg and the AE is Defined as Grade 1 based on values of 101 through 105 mm Hg and Grade 2 based on values over 105.

Clinical Safety Laboratory Values

Hematology	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
WBC 10^3/UL (Decrease)	2.50 – 3.50	1.50 – 2.49	<1.50
WBC 10^3/UL (Increase)	13.01 – 15.00	15.01 – 20.00	>20.00
HgB g/dL (Decrease) (Female)	11.0 – 11.49	9.5 – 10.9	<9.5
HgB g/dL (Decrease) (Male)	12.0 – 12.5	10.0 – 11.9	<10.0
Platelets 10^3/UL (Decrease)	120 – 130	100 – 119	<100
Platelets 10^3/UL (Increase)	416 – 550	551– 750	>750
Chemistry			
ALT IU/L (Increase)	47 – 105	106 – 175	>175
Creatinine mg/dL (Increase)	> ULN – 1.7	1.8 – 2.0	>2.0

ULN = upper limit of normal range

Clinical Adverse Events			
CARDIOVASCULAR	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Arrhythmia		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required
Hemorrhage, Blood Loss	Estimated blood loss ≤ 100 mL	Estimated blood loss > 100 mL, no transfusion required	Transfusion required
RESPIRATORY	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Cough	Transient- no treatment	Persistent cough;	Interferes with daily activities
Bronchospasm, Acute	transient; no treatment; 71% - 80% FEV1 of peak flow	requires treatment; normalizes with bronchodilator; FEV1 60% - 70% (of peak flow)	no normalization with bronchodilator; FEV1 <60% of peak flow
Dyspnea	Does not interfere with usual and social activities	Interferes with usual and social activities, no treatment	Prevents daily and usual social activity or requires treatment
GASTROINTESTINAL	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity or requires IV hydration

Diarrhea	2 - 3 loose or watery stools or < 400 gms/24 hours	4 - 5 loose or watery stools or 400 - 800 gms/24 hours	6 or more loose or watery stools or > 800gms/24 hours or requires IV hydration
SYSTEMIC REACTIONS	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema or anaphylaxis
All Other conditions	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention