A Phase 1b, multicenter, randomized, placebo-controlled, observer-blinded, dose-escalation study to evaluate the safety, tolerability, and immunogenicity of the rSm-p80 + GLA-SE (SchistoShield®) candidate vaccine in healthy adults in Burkina Faso and Madagascar.

Protocol Number:	**IVI VASA 001**
Principal Investigator: (Burkina Faso)	Prof. Sodiomon Bienvenu Sirima Groupe de Recherche Action en Santé (GRAS) 06 BP 10248 Ouagadougou 06, Burkina Faso
Principal Investigator: (Madagascar)	Prof. Raphaël Rakotozandrindrainy University of Antananarivo established in Campus d'Ankatso, Antananarivo 101, Madagascar,
Sponsor:	International Vaccine Institute SNU Research Park, 1 Gwanak-ro, Gwanak-gu, Seoul, 08826 Republic of Korea
Funding Agency:	European Union, Horizon 2020
Version Number and Date:	Version 4.0 and 06NOV2024

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List of Abbreviations

AAHI	Access to Advanced Health Institute
Ab	Antibody
ABZ	Albendazole
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AT	Ambient Temperature
BMI	Body Mass Index
BSA	Bovine Serum Albumin
°C	Degree Celsius
CBC	Complete Blood Count
CEPI	Coalition for Epidemic Preparedness Innovations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CMP	Clinical Monitoring Plan
CRF	Case Report Form
eCRF	Electronic Case Report Form
DSMB	Independent Data Safety Monitoring Board
EDC	Electronic Data Capture
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Expanded Program on Immunizations
FAS	Full Analysis Set
GCP	Good Clinical Practices
GCLP	Good Clinical Laboratory Practice
GLA-SE	Glucopyranosyl lipid A Stable Emulsion
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonization
IND	Investigational New Drug
IEC	Independent Ethics Committee
IFNγ	Interferon Gamma

IgG	Immunoglobulin G
IL	Interleukin
IM	Intermuscular
IP	Investigational Product
IRB	Institutional Review Board
ITT	Intention-to-treat
IVI	International Vaccine Institute
MOP	Manual of Procedures
NRA	National Regulatory Authority
µg	Microgram
N	Number
nm	Nanometer
NRA	National Regulatory Agency
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PP	Per Protocol
PIMMC	Potential Immune-Mediated Medical Conditions
PHI	Protected Health Information
PPS	Probability proportional sampling
PZQ	Praziquantel
QA	Quality assurance
QC	Quality Control
S.	Schistosoma
SAF	Safety analysis set
SAP	Statistical Analysis Plan
SBA	Serum Bactericidal Antibody
Site PI	Site Principal Investigator
SMC	Safety Monitoring Committee
SOE	Schedule of Events
SI	Sub-investigator
SUSAR	Suspected Unexpected Serious Adverse Reaction
TH1	T-Helper Type 1
UPT	Urine Pregnancy test
USP	United States Pharmacopeia
WBC	White blood cells
WFI	Water for injection
WHO	World Health Organization

LIST OF TABLES

Statement of Compliance

The study will be conducted according to the protocol and in compliance with International Council for Harmonization (ICH) E6 (R2) Good Clinical Practice (GCP), Belmont Principles, CIOMS guidelines, Declaration of Helsinki and other applicable regulations as required by National Regulatory Agency and sponsor requirement. The Principal Investigator(s) will ensure that no deviation from, or changes to the protocol will take place without prior agreement from the Sponsor and documented approval from the Institutional Review Board//Independent Ethics Committee (IRB/IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB/IEC for review and approval. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guideline and clinic site SOPs. Roles and responsibilities of study staff are presented in the Manual of Procedures.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Site Principal Investigator (Burkina Faso) _____

Print/Type Name

Signed: _____ Date: _____

Signature

Statement of Compliance

The study will be conducted according to the protocol and in compliance with International Council for Harmonization (ICH) E6 (R2) Good Clinical Practice (GCP), Belmont Principles, CIOMS guidelines, Declaration of Helsinki and other applicable regulations as required by National Regulatory Agency and sponsor requirement. The Principal Investigator(s) will ensure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board//Independent Ethics Committee (IRB/IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB/IEC for review and approval. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guideline and clinic site SOPs. Roles and responsibilities of study staff are presented in the Manual of Procedures.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Site Principal Investigator (Madagascar) _____

Print/Type Name

Signed: _____ Date: _____

Signature

Statement of Compliance

The study will be conducted according to the protocol and in compliance with International Council for Harmonization (ICH) E6 (R2) Good Clinical Practice (GCP), Belmont Principles, CIOMS guidelines, Declaration of Helsinki and other applicable regulations as required by National Regulatory Agency and sponsor requirement. The Principal Investigator(s) will ensure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board//Independent Ethics Committee (IRB/IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB/IEC for review and approval. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guideline and clinic site SOPs. Roles and responsibilities of study staff are presented in the Manual of Procedures.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Chief Investigator
(IVI) _____
Print/Type Name

Signed: _____ Date: _____
Signature

Statement of Compliance

The study will be conducted according to the protocol and in compliance with International Council for Harmonization (ICH) E6 (R2) Good Clinical Practice (GCP), Belmont Principles, CIOMS guidelines, Declaration of Helsinki and other applicable regulations as required by National Regulatory Agency and sponsor requirement. The Principal Investigator(s) will ensure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board//Independent Ethics Committee (IRB/IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB/IEC for review and approval. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guideline and clinic site SOPs. Roles and responsibilities of study staff are presented in the Manual of Procedures.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Medical Monitor
(IVI) _____
Print/Type Name

Signed: _____ Date: _____
Signature

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Sponsor: International Vaccine Institute (IVI)
Name of Investigational Product: SchistoShield®
Name of Active Ingredients: rSm-p80 (antigen) with GLA-SE (adjuvant)
Title of Study: A Phase 1b, multicenter, randomized, placebo-controlled, observer-blinded, dose - escalation study to evaluate the safety, tolerability, and immunogenicity of the rSm-p80 + GLA-SE (SchistoShield®) candidate vaccine in healthy adults in Burkina Faso and Madagascar.
Protocol Number: IVI VASA 001
Study Center(s): 1. Groupe de Recherche Action en Santé (GRAS) 06 BP 10248 Ouagadougou 06 Burkina Faso 2. University of Antananarivo established in Campus d'Ankatso, Antananarivo 101, Madagascar
Site Principal Investigators 1. Prof. Sodiomon Bienvenu Sirima. Groupe de Recherche Action en Santé (GRAS) 2. Prof. Raphaël Rakotozandrindrainy University of Antananarivo

Study Period (years/months)	**Phase of development:**
Estimated date of first participant enrollment: November 2023 Estimated date of last participant enrollment: October 2024 Estimated date of first participant's last visit: July 2024 Estimated date of last participant's last visit: June 2025 Estimated duration of the trial for participant: 32 weeks Estimated duration of the trial: 20 months	1b

Study Hypothesis

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

Objectives

Primary:

- To evaluate the safety and tolerability of 3 different dose formulations (low dose, medium dose, and high dose) of SchistoShield® vaccine given intramuscularly on D0, D28 and D56 to healthy participants 20 to 59 years of age in Burkina Faso and Madagascar.

Secondary:

- To evaluate the immunogenicity of 3 different dose formulations (low dose, medium dose, and high dose) of SchistoShield® vaccine 28 days post-vaccination on D28, D56, and D84 as compared with the baseline and with those who received placebo.

Exploratory:

- To describe the antigen-specific B- and T-cell responses, memory responses, and innate and adaptive immune signatures from samples collected at specified timepoints.

Study Design & Methodology

This is a phase 1b, multicenter, randomized, placebo-controlled, observer-blinded, dose-escalation study, assessing the safety, tolerability, and immunogenicity of a three-dose regimen, spaced four weeks apart, given intramuscularly in healthy adults (20-59 years old). Three different dose formulations of the study product with varying antigen contents will be investigated. A total of 120 eligible participants will be recruited in 3 sequential cohorts (A, B, and C) in Burkina Faso (N=60) and in Madagascar (N=60), as shown in the **Table 1**, below. Cohort A will receive the low-dose antigen formulation (10 µg) or placebo, Cohort B will receive the medium-dose antigen formulation (30 µg) or placebo, and Cohort C will receive the high-dose antigen formulation (100 µg) or placebo; all antigens with 5 µg adjuvant (GLA-SE). In each cohort, volunteers will be randomized in a blinded manner into one of two arms, candidate vaccine or placebo, by a 3:1 ratio. A subset of five out of 20 subjects in each cohort will be sampled by

convenience to enable us to further characterize the immune response using the peripheral blood mononuclear cells (PBMC).

To ensure that the study participants at enrollment do not have any active schistosomiasis or helminth infection and are schistosomiasis egg-negative, pre-screening activities including schistosomiasis treatment will be carried out in potential study participants prior to enrollment. Potential study participants will be identified in the catchment population and will be offered anti-helminth treatment using praziquantel (PZQ) and Albendazole (ABZ) as per local guidelines at study site. The pre-screening visit will be conducted 6-8 weeks before the screening visit. The last dose of PZQ/ABZ will be administered at least 5 weeks prior to the first dose of study product.

Table 1: VASA phase 1b study plan in Burkina Faso and Madagascar

Country	Age group	Cohort	Arm	Treatment	Number of volunteers
Burkina Faso	20 - 59 years	Cohort A	Arm A1	SchistoShield® LD	15
			Arm A2	Placebo	5
		Cohort B	Arm B1	SchistoShield® MD	15
			Arm B2	Placebo	5
		Cohort C	Arm C1	SchistoShield® HD	15
			Arm C2	Placebo	5
Sample size					60
Madagascar	20 - 59 years	Cohort A	Arm A1	SchistoShield® LD	15
			Arm A2	Placebo	5
		Cohort B	Arm B1	SchistoShield® MD	15
			Arm B2	Placebo	5
		Cohort C	Arm C1	SchistoShield® HD	15
			Arm C2	Placebo	5
Sample size					60
Total Sample Size					120

LD: Low dose (10µg Ag/5µg GLA-SE Adjuvant); MD: Medium Dose (30µg Ag/5µg GLA-SE Adjuvant); HD: High Dose (100µg Ag/5µg GLA-SE Adjuvant)

Dose escalation scheme:

All participants will receive three injections of the assigned study product, given 28 days apart at Days 0, 28, and 56. As this is the first-in-human study of SchistoShield® in schistosomiasis endemic countries, Cohort A will be enrolled first and will receive the low antigen adjuvanted product or placebo (Table 1). To progress to enrollment of Cohort B, the Safety Monitoring Committee (SMC) and site PIs will review blinded safety data of Cohort A participants collected through seven days following the second dose of study product injection and assess the data according to pre-specified criteria for halting dose escalation (section 10.4.3). If halting criteria are not met, Cohort B participants will be enrolled. Dose escalation to Cohort C will follow the same procedures requiring SMC and Site PIs review. These safety data reviews and decision to progress to the next dose level will occur for each site separately.

If dose escalation halting criteria are met at any point, the matter will be escalated to the independent Data Safety Monitoring Board (DSMB), which will review summarized safety data in an unblinded manner and will provide their recommendations to the sponsor for dose escalation before study product injection of the next cohort is initiated. The DSMB review is not required for dose escalation into the next cohort unless the halting criteria are met or the SMC requests for DSMB review.

All participants will be followed up for safety and immunogenicity at specific time points. Each participant will be in the study for approximately 32 weeks after enrollment. Eligible participants enrolled into the study will be vaccinated and observed at the study site for a minimum of 60 minutes after vaccination for immediate safety assessment. Solicited adverse events will be recorded on a diary card for 7 days following each study product injection. Unsolicited adverse events will be recorded for 28 days following each study product injection. Serious adverse events and adverse events of special interest (AESI) will be reported during the entire study period. Apart from designated study site personnel responsible for vaccine administration, study investigators, study nurses, those assessing clinical outcomes, and laboratory analysts will be blinded to vaccine allocation until database lock for the final analysis.

A total of 7 blood samples will be collected for safety assessment on Days -28 to -1, 7, 28, 35, 56, 63, and 84. A total of 8 blood samples will be collected for immunogenicity assessments at Days 0, 7, 28, 35, 56, 63, 84, and 224.

Enrollment/Vaccination rules:

The following rules will be applied for the randomization/administration of the investigational product to the participants within the first study cohort (Cohort A):

- First two participants will be enrolled to receive rSm-p80 + GLA-SE (or placebo).
- If within at least 24 hours, there is no serious adverse reaction following immunization, the second two participants will receive **r**Sm-p80 + GLA-SE (or placebo).
- If within at least a week after the first vaccination, there is no serious adverse reaction following immunization the vaccine can be administered to the rest of the cohort.

The same rules will be applied to all the study cohorts. A detailed description of the vaccination rules is described in the manual of procedures (MOP).

Estimated Number of participants to Enroll

A total of 120 healthy adults will be enrolled: 60 participants in Burkina Faso and 60 participants in Madagascar.

Criteria for Inclusion/Exclusion

Inclusion Criteria

To be eligible to participate in this study, any individual must meet the following criteria:

1. Healthy male or female participants aged 20 to 59 years at the time of consent.
2. Participant who has completed the deworming using praziquantel (PZQ) and albendazole (ABZ) according to local guidelines, with the last dose of PZQ/ABZ administered at least 5 weeks prior to first dose of study product.
3. Participant who, after the nature of the study has been explained, has voluntarily given informed consent, according to the local regulatory requirements, prior to study entry.
4. Participant who can comply with the study procedures and available for the entire duration of the study (32 weeks).
5. Individuals in good health as determined by the outcome of medical history, physical examination, hematology and biochemistry tests at the time of screening and the clinical judgment of the investigator.
6. Women of childbearing potential* with negative urinary test result on a human chorionic gonadotropin pregnancy test on the day of randomization, before receiving any study product.
7. Males or females of childbearing potential who are using an effective birth control method recommended by the national health system for at least four (4) weeks before the first vaccination (for female participants only) and up to four (4) weeks after the third vaccination (i.e., for at least 4 months).

* Participant who confirm state of menopause, hysterectomy, or tubal ligation during the pregnancy counselling process are considered not of childbearing potential.

Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Participant with major congenital abnormalities which in the opinion of investigator may affect the subject's participation in the study.
2. Participant concomitantly enrolled or scheduled to be enrolled in another trial.
3. Positive rapid test for HIV 1-2 confirmed by a positive blood test for human immunodeficiency virus (positive antibodies to HIV 1/2).
4. Participant seropositive for hepatitis B virus surface antigen (HBsAg).
5. Participant seropositive for hepatitis C virus (Antibodies to HCV).
6. Participant with active or chronic Schistosomiasis infection defined by a positive result for microscopy (Urine filtration, Kato-Katz (KK)) and/or point-of-care – circulating cathodic antigen (POC–CCA) and/or real-time PCR.
7. Participant with soiled transmitted helminths infections (STH) as diagnosed by microscopy (KK) and/or real-time PCR.
8. Participant with malaria infection/malaria as diagnosed by the blood smear (If an individual tested blood smear positive for malaria at the screening visit, received treatment, and subsequently tests negative on a blood smear within the designated screening window, they will be eligible for inclusion in the study).
9. Any other confirmed or suspected immunosuppressive or immunodeficient state such as asplenia, recurrent severe infections.
10. Body mass index (BMI) ≥ 35 kg/m^2
11. Chronic use of systemic steroids (>2 mg/kg/day or >20 mg/day prednisolone equivalent for periods exceeding 10 days), cytotoxic or other immunosuppressive drugs.
12. Receipt of blood or blood-derived products in the past 3 months.

13. Participant who has received other vaccines 4 weeks prior to test vaccination or plans to receive any vaccine within 4 weeks of last dose of study vaccine, exception made for COVID-19 vaccines.
14. Known history of allergy to study vaccine components and/or excipients or other medications, or any other allergies deemed by the investigator to increase the risk of an adverse event if they were to participate in the trial.
15. Individuals with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time resulting in contraindication for IM injections/blood extractions.
16. Any abnormality or chronic disease which in the opinion of the investigator might be detrimental for the safety of the participant and interfere with the assessment of the study objectives and compromise the health of the volunteers.
17. Any female participant who is lactating*, pregnant or planning for pregnancy** during the course of study period.
18. Individuals with behavioral or cognitive impairment or psychiatric disease or neural disorders that, in the opinion of the investigator, could interfere with the individual's ability to participate in the trial.
19. Any clinically significant abnormal finding on serum chemistry or hematology or urinalysis at the screening visit as per US FDA toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (any biological finding grade 4 constitutes an exclusion criteria).
20. Individuals who were research staff involved with the clinical study or family/household members of research staff.
21. As per Investigator's medical judgement individual could be excluded from the study despite meeting all inclusion/exclusion criteria mentioned above.

Temporary exclusion criteria (delay of vaccination) ***

1. Individuals with acute illness (moderate or severe) at the time of vaccination. Acute illness is defined as the presence of a moderate or severe disease with or without fever. Fever is defined by axillary temperature ≥37.5°C or tympanic temperature ≥38°C at the time of vaccination.

Vaccines can be administered to subject with a minor illness without fever at the discretion of the investigators.

2. Individuals with an active SARS-CoV-2 infection, as determined by a rapid antigen diagnostic test.
3. Individuals tested positive by malaria rapid diagnostic tests on the day of vaccine administration.

*Lactation: This IP has not been specifically studied in pregnant and lactating women. No data on lactating women are available. There is no information about harm to an unborn child or a child who is breastfeeding. Breastfeeding women will not be enrolled. Should a female participant decide to breastfeed during the vaccination period, she will be excluded from further vaccination but will be followed for safety until end of the study.

** Serum pregnancy test at screening and Urine Pregnancy Test (UPT) before vaccination is necessary for all female participants of childbearing age.

*** If any of these events occur at the scheduled time for the vaccination, randomization at a later date within the window period is permitted at the discretion of the investigator. If randomization cannot occur within the window period, rescreening is required. If a febrile 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 participant may receive study product injection.

Investigational Product, Dosage, and Mode of Administration

Study Product Description

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

rSm-p80 [antigen] 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, rSm-p80 for injection. The final 758 amino acid protein antigen has a predicted mass of approximately 87kDa. rSm-p80 is released by PAI Life Sciences Inc (Seattle, WA).

GLA-SE [adjuvant formulation] is a synthetic Monophosphoryl Lipid A-like molecule which is a Toll-like receptor 4 agonist formulated in a SE to produce GLA-SE. GLA-SE is formulated in a squalene emulsion and is manufactured and released by the Access to Advanced Health Institute (AAHI) (Seattle, WA).

rSm-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.

Formulation and Product Storage

rSm-p80 Antigen:

- Formulated as a lyophilized cake in glass vials and appears white to off-white cake.
- Supplied as single use glass vials.
- Each 3 mL glass vial contains 125 µg of rSm-p80.
- Reconstitution of rSm-p80 with WFI results in a clear, colorless solution
- Storage Conditions: between 2°C to 8°C, excursions up to 25°C are permitted for 4 hours.

GLA-SE Adjuvant:

- Formulated as 20 µg/mL GLA in a 4% SE and appears as a milky-white liquid.
- Supplied as single use glass vials.
- Each 2mL vial contains a fill volume of 0.4 mL
- Storage Conditions: between 2°C to 8°C, excursions up to 25°C are permitted for 24 hours but product should never be frozen.

rSm-p80 + GLA-SE for Injection

- Vaccine product (antigen + adjuvant) is only produced upon admixture prior to administration.
- Three adjuvanted formulations with varying antigen content of 10 µg, 30 µg, and 100 µg of rSm-p80 antigen
- Dissolved rSm-p80 antigen and the GLA-SE emulsion appears as a translucent milky white liquid.
- Storage Conditions: admixture is stable up for at least 4 hours at up to 25°C

Sterile Water for Injection

- The sterile WFI is nonpyrogenic and contains no bacteriostatic, antimicrobial agent or added buffer.
- This product will be used to dilute the vaccine (rSm-p80) and will be supplied as a single-dose vial.
- Storage Conditions: as per manufacturer's instructions provided in package insert.

Placebo

- Dose formulation: Sterile 0.9% sodium chloride

Storage Conditions: as per manufacturer's instructions provided in the package insert.

Doses and route of vaccination:

- Each study cohort will receive a three dose-regimen administered 28 days apart (D0, D28 and D56)
- Each dose of study vaccine or placebo will be administered as a 0.5 mL intramuscular (IM) injection

Criteria for Evaluation

Primary Endpoints

- Proportion of participants with any Serious Adverse Events (SAEs)/ adverse events of special interest (AESI) from the time of the first study vaccination through the final study visit.
- Proportion of participants with immediate adverse events (reactogenicity events) within 60 minutes from the time of each study vaccination.
- Proportion of participants with solicited local and solicited systemic AEs as measured for 7 days (inclusive) following immunization with the three different dose formulations.
- Proportion of participants with unsolicited AEs from the time of vaccination until 28 days post immunization with the three different dose formulations.
- Proportion of participants with clinical safety laboratory adverse events measured at 7 days and 28 days after each study vaccination.

Secondary Endpoints

- For Sm-p80 IgG antibodies, seroconversion rate at approximately 4 weeks (28 days) after each dose of study vaccination as compared to baseline.

  [Seroconversion defined as 4-fold rise in Sm-p80-specific total IgG antibodies after investigational product administration compared to baseline (D0)]
- For Sm-p80 IgG antibodies, seroconversion rate at approximately 24 weeks after third dose of study vaccination as compared to baseline.
- Geometric Mean Titers (GMTs) of serum Sm-p80 IgG antibodies at approximately 4 weeks after each dose of study vaccination.
- Geometric Mean Titers (GMTs) of serum Sm-p80 IgG antibodies at approximately 24 weeks after third dose of study vaccination.

Exploratory Endpoints

- For Sm-p80-specific cellular responses, enumeration of cytokine-secreting cells by IFNγ ELISpot (numbers of spot-forming cells) at specified time points.
- For Sm-p80 IgE antibodies, seroconversion rate at 4 weeks (28 days) after each dose of study vaccination as compared to baseline.

- Innate and adaptive immune signatures depicting the changes in a subset of immunized participants using PBMC's collected at specified time points as measured by RNA seq and quantification of cytokines.
- T-cell cytokine and chemokine responses from whole blood and PBMC samples collected at specified time points in a subset of participants.
- Enumeration of antibody-secreting and memory B cells from PBMC samples collected at specified time points in a subset of participants.
- Enumeration of the ability of Sm-p80-specific antibodies from human participants to kill schistosome larvae in vitro (schistosomula-killing assay).
- Identification of immune signature markers identified by RNA Seq that are related to protective efficacy in animal passive transfer studies to identify markers that can be used for future down-selection of this other schistosome vaccine products.
- Determination of the changes in the circulating anodic antigen (CAA) levels before vaccination compared with the levels 4 weeks after the 3rd dose of vaccination.

Statistical Considerations

Sample Size Calculation

A total of 120 participants will be enrolled in the study. The sample sizes in each study site are distributed by study cohorts and treatment arms. In each cohort, randomization will be performed in 3:1 allocation with 15 subjects in the rSm-p80 + GLA-SE arm and 5 subjects in the placebo arm.

No formal power analysis is applicable to this study, as descriptive statistics will be used to summarize the data. A sample size of 30 participants who received a low, medium, or high antigen-dose formulation of the study product will provide 95% confidence that the true incidence of SAEs is <12%, if no SAE are observed in those dose cohorts. A sample size of 90 participants who received the candidate vaccine, irrespective of antigen-dose formulation, among all cohorts will provide 95% confidence that the true incidence of SAEs is <5%, if no SAEs are observed.

Primary Endpoints Analysis

The primary endpoint analyses are safety analyses of treatment emergent adverse events (TEAEs). TEAEs are defined for this study as any following AEs/AESIs/SAEs that occur from the time of the first

study vaccination of rSm-p80+GLA-SE or placebo:

- SAEs and AESIs from the time of the first study vaccination through the final study visit.
- Immediate AEs (Reactogenicity Events) within 60 minutes from the time of each study vaccination.
- Solicited AEs (injection site and systemic reactogenicity events) from the time of each study vaccination through 7 days (inclusive) after each study vaccination.
- Unsolicited AEs from the time of each study vaccination through 28 days after each study vaccination.
- Clinical safety laboratory AEs (significant laboratory abnormality) at 1 and 4 weeks after each study vaccination.

All TEAEs will be summarized by frequency, percentage and associated 95% confidence interval by study cohort and study arm. The frequencies will also be presented separately by dose regimen and will be depicted by system organ class and preferred term. Additional frequencies will be presented with respect to maximum severity and relationship to study product. All these primary endpoint analyses will be conducted on the participants in the Safety Analysis Set.

Secondary Endpoint Analysis

The secondary endpoint analyses are immunogenicity analyses of anti-Sm-p80 IgG antibody levels measured at 4 weeks after each dose of study vaccination and at 24 weeks after the third dose of study vaccination. These analyses will be summarized descriptively by study cohort and treatment arm using seroconversion rates and associated 95% confidence interval. The GMT and 95% confidence interval of Sm-p80 IgG response at 4 weeks after each dose of study vaccination and at 24 weeks after the third dose of study vaccination will be summarized descriptively by study cohort and treatment arm. Secondary endpoint analysis will be conducted on the participants in the Full Analysis Set and Per Protocol Set.

Exploratory Endpoint Analysis

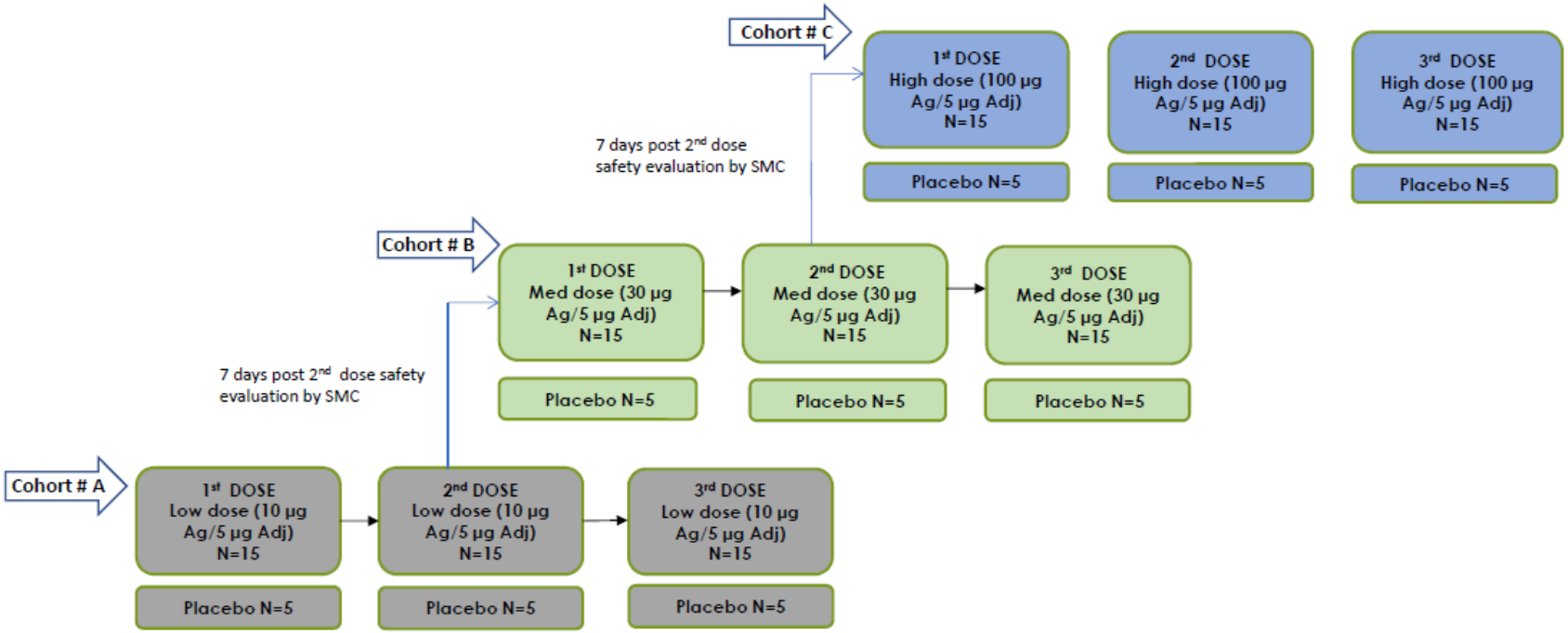
Antigen specific cellular immune responses by IFN-γ ELISpot, innate and adaptive immune signatures, and T and B cell response will be analyzed descriptively by study cohort and treatment arm using the geometric mean and associated 95% confidence intervals.

1.2 STUDY SCHEME

This is a phase 1b trial to evaluate the safety, tolerability, and immunological profile of the rSm-p80 + GLA-SE candidate schistosomiasis vaccine (SchistoShield®) in healthy adults living in an endemic area. A total of 60 participants aged 20 59 will be recruited in both Burkina Faso and Madagascar, with 120 participants in total. Participants will be evaluated in three cohorts in each study site: Cohort A) 10 µg rSm-p80 + 5 µg GLA-SE, Cohort B) 30 µg rSm-p80 + 5 µg GLA-SE and Cohort C) 100 µg rSm-p80 + 5 µg GLA-SE (Table 1). Each study cohort will include 20 participants randomized to receive either rSm-p80 product or placebo in a 3:1 ratio. All participants will receive three IM injections of 0.5 mL of the designated study product / placebo, on Days 0, 28, and 56 (28 days apart) in alternating arms.

Cohort A will receive the low-dose antigen formulation or placebo, Cohort B will receive the medium-dose antigen formulation or placebo, and Cohort C will receive the high-dose antigen formulation or placebo. The safety profile of any one formulation does not necessarily predict that of the others, therefore participants will be enrolled in a sequential dose-escalation manner, with safety review between each cohort's enrollment. After enrollment of participants in Cohort A, the Safety Monitoring Committee (SMC) and site PIs will review blinded safety data of Cohort A collected through seven days after the second dose of study product injection and assess this data according to pre-specified criteria for halting dose escalation (Section 10.4.3). If halting criteria are not met, Cohort B participants will be enrolled. Dose escalation to Cohort C will follow the same procedures requiring SMC and site PIs review. These safety data reviews and decision to progress to the next dose level will occur for each site separately. If dose escalation halting criteria *are* met at any point, the matter will be escalated to the DSMB, which will review the summarized safety data in an unblinded manner. The DSMB must provide their recommendations to the sponsor for dose escalation before study product injection of the next cohort is initiated (Figure 1). The DSMB review is not required for dose escalation into the next cohort unless the halting criteria are met or the SMC requests for DSMB review.

Figure 1. Study Schema



SMC reviews will happen for each site separately.

1.3 TABLE OF STUDY PROCEDURE/SCHEDULE OF EVENTS

Table 2: Schedule of Study Procedures / Schedule of Events (SOE)

Visit Number	V0[&]	V1[π]	V2	V3	V4	V5	V6	V7	V8	V9	UV[a]
Visit Description	Pre-screening	Screening	Vac 1	Vac 1+7 days	Vac 2	Vac 2 +7 days	Vac 3	Vac 3+7	One month follow-up visit, post V6	Six months follow-up visit, post visit V6	Unscheduled Visit
Visit Day (D) ± Window	¥	D -28 to -1	D0	D7+3	D28+3	D35+3	D56+3	D63+3	D84±7	D224±14	-
Visit Week	[§]	-4 ~ -1	0	1	4	5	8	9	12	32	-
Pre-screening informed consent	X										
Deworming with Praziquantel & Albendazole	X										
Informed Consent		X									
Inclusion & Exclusion Criteria		X									
Temporary Exclusion Criteria Review			X		X		X				
Medical History		X	X								
Full Physical Examination		X	X	X	X	X	X	X	X	X	X
Demographic, Height, Weight		X									
Vital Signs		X	X	X	X	X	X	X	X	X	X
Serology (HCV, HBsAg, HIV)		X									
Urinalysis [b]		X		X	X	X	X	X	X		
Pregnancy test (PT)[%]		X	X		X		X				
Malaria diagnosis [β]		X	X		X		X				
Blood sample for CAA[c]			X		X		X		X		
Stool sample for Schistosoma eggs and STH detection [d]		X									
Urine sample for POC-CCA and Schistosoma eggs detection.		X									
SARS-CoV-2 Rapid Antigen test		X	X [¶]		X [¶]		X [¶]				X [¶]
Use of effective contraceptive		X	X	X	X	X	X	X	X		
Enrollment & Randomization			X								
Safety Blood Samples (SBS) for Hematology [e] and Serum Chemistry [f]		SBS1		SBS2	SBS3	SBS4	SBS5	SBS6	SBS7		
Immunogenicity Blood Samples (IBS)			IBS1	IBS2	IBS3	IBS4	IBS5	IBS6	IBS7	IBS8	
Study Product Administration			X		X		X				

Visit Number	V0[&]	V1[π]	V2	V3	V4	V5	V6	V7	V8	V9	UV[a]
Visit Description	**Pre-screening**	**Screening**	**Vac 1**	**Vac 1+7 days**	**Vac 2**	**Vac 2 +7 days**	**Vac 3**	**Vac 3+7**	**One month follow-up visit, post V6**	**Six months follow-up visit, post visit V6**	**Unscheduled Visit**
Visit Day (D) ± Window	¥	**D -28 to -1**	**D0**	**D7+3**	**D28+3**	**D35+3**	**D56+3**	**D63+3**	**D84±7**	**D224±14**	-
Visit Week	§	**-4 ~ -1**	**0**	**1**	**4**	**5**	**8**	**9**	**12**	**32**	-
Post Vaccination 60 min observation			**X**		**X**		**X**				
Solicited adverse events			**X**	**X**	**X**	**X**	**X**	**X**			
Unsolicited adverse events within 28 days			**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**
Serious Adverse Event (SAE)			To be monitored throughout study duration following first dose.								
Adverse event of special interest (AESI)			To be monitored throughout study duration following first dose.								
Participant Diary Card (DC) distribution			**DC1**		**DC2**		**DC3**				
Participant Diary Card (DC) collection and verification				**DC1**		**DC2**		**DC3**			
Review of concomitant medications		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Safety Blood Volume at screening											
EDTA Hematology		3 mL									
Serum Serum chemistry, Serology, Serum pregnancy test		3 mL									
Safety Blood Volume after enrollment											
EDTA Hematology				3 mL	3 mL	3 mL	3 mL	3 mL	3 mL		
Serum Serum chemistry				3 mL	3 mL	3 mL	3 mL	3 mL	3 mL		
Immunogenicity Blood Volume											
Serum Antibody assays			9 mL	9 mL	9 mL	9 mL	9 mL	9 mL	9 mL	5 mL	
NaHep [#] PBMC isolation for T and B cell assays, RNAseq and cytokine multiplex Assay			17 mL	17 mL	17 mL	17 mL	17 mL	17 mL	10 mL	10 mL	
Total volume per visit (Excluding PBMC subset)		6 mL	9 mL	15 mL	15 mL	15 mL	15 mL	15 mL	15 mL	5 mL	
	[&, ¥, §] **Visit 0:** Pre-screening activities (which should be conducted -56 to -42 days or -8 to -6 weeks before the screening visit) will follow local standard of care to de-worm potential study participants prior to formal study screening; further details in section 7.1.3.2..										

Visit Number	V0[&]	V1[π]	V2	V3	V4	V5	V6	V7	V8	V9	UV[a]
Visit Description	Pre-screening	Screening	Vac 1	Vac 1+7 days	Vac 2	Vac 2 +7 days	Vac 3	Vac 3+7	One month follow-up visit, post V6	Six months follow-up visit, post visit V6	Unscheduled Visit
Visit Day (D) ± Window	¥	D -28 to -1	D0	D7+3	D28+3	D35+3	D56+3	D63+3	D84±7	D224±14	-
Visit Week	[§]	-4 ~ -1	0	1	4	5	8	9	12	32	-

[π] Visit 1 (screening visit) should happen within 28 days prior to Visit 2 (first vaccination visit). In other words, the first dose should be given within 28 days from the day of screening.

[a] During unscheduled visit, blood sample and naso-pharyngeal swab will be collected for further investigation when deemed necessary by the study physician.

[b] Urinalysis: blood, glucose and protein by dipstick

[%] Pregnancy test will be performed on all female participants of childbearing age at screening and before each study vaccination. Serum pregnancy test will be carried out at screening and urine pregnancy test at vaccination visits.

[β] Malaria diagnosis will be done using blood smear at the screening visit and rapid diagnostic test before each vaccination.

[¶] SARS-CoV-2 rapid antigen test will be done in case of suspected symptoms/signs of COVID-19 before each vaccination and at unscheduled visit if deemed necessary by the study physician.

[c] Circulating Anodic Antigen (CAA) assay will be done retrospectively using the serum.

[d] Detection of Schistosoma mansoni infection will be done by Kato-Katz and POC-CCA, confirmed using the RT-PCR. Soiled transmitted helminths infections will be detected by Kato-Katz and confirmed by the RT-PCR.

[e] Hematology: hemoglobin, complete blood count with differential, and platelets

[f] Serum chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), creatinine (CREAT) and C-reactive protein (CRP).

[#] Blood collection for PBMC isolation, RNAseq and cytokine multiplex assay will only be conducted in a subset of participants.

Phone calls will be arranged after vaccine administration to ensure completion of the Participant's DC and to check their health status. No deviation will be considered if the phone call could not be completed on the exact dates following vaccination visit.

2 INTRODUCTIONS

2.1 BACKGROUND

Global disease burden

Schistosomiasis is the most prevalent tropical disease having been reported in 78 countries, spanning Africa, Asia and Latin America, affecting impoverished communities without access to safe drinking water and adequate sanitation programs [1]. Estimations show that schistosomiasis affects at least 230 million people worldwide, resulting in significant health and socio-economic burdens [2]. Schistosomiasis ranks second only to malaria as the most common parasitic disease and is the deadliest neglected tropical disease (NTD). In the Africa region alone, about 280,000 deaths annually are attributed to schistosomiasis; furthermore, an estimated 3.3 million disability-adjusted life years, a measure of overall disease burden expressed as the number of years lost due to ill-health, disability, or early death, are lost annually. In 2017, at least 220.8 million people required preventive treatment for schistosomiasis, 90% of which lived in Africa [3].

Etiological agent

Schistosomiasis is caused by a trematode worm of the *Schistosoma (S.)* genus, a parasitic blood fluke. During the infectious stages, they are released from snails, which are intermediate hosts, 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 [4].

Clinical presentation & Pathology

Schistosomiasis progresses in three distinct phases: acute, chronic, and advanced disease. A maculopapular eruption, comprising discrete erythematous raised lesions that vary in size from 1 cm to 3 cm, may arise at the site of percutaneous penetration by schistosome cercariae. Migrants or tourists infected for the first time may develop a skin reaction within a few hours to as late as one week after exposure. This is less severe than, although similar to, "swimmers itch", which is not a sequela of acute schistosomiasis but rather an immune reaction that develops in sensitized people when they are reinfected by species of schistosomes that do not colonize humans [5,6].

Acute schistosomiasis (Katayama syndrome)

The symptoms of acute schistosomiasis are mediated by the immune system and usually begin with the deposition of schistosome eggs into host tissues. Symptoms may include fever, malaise, myalgia, fatigue, non-productive cough, diarrhea (with or without the presence of blood), hematuria (*S haematobium*), and right upper quadrant pain. Acute schistosomiasis is seen in people who are infected for the first time when travelling to endemic areas. In the case of *S japonicum*, schistosomiasis infection is also associated with either a superinfection or a hypersensitivity reaction in previously infected people [7].

Chronic and advanced disease

Mature schistosome infections are associated with a chronic local inflammatory response to schistosome eggs trapped in host tissues, which may lead to inflammatory and obstructive disease in the urinary system (*S haematobium*) or intestinal disease, hepatosplenic inflammation, and liver fibrosis (*S. mansoni*, *S. intercalatum*, *S. japonicum* and *S. mekongi i*) [5,6]. Immunopathological studies have shown that schistosomiasis results from the host's immune response to schistosome eggs and the granulomatous reaction caused by the antigens they secrete. Granulomas, which develop at the sites of maximal accumulation of eggs, destroy the eggs but result in fibrosis in host tissues [8,9].

Eggs of *S. mansoni* and *S. japonicum* embolize to the liver, where the granulomatous inflammatory response induces presinusoidal inflammation and periportal or clay-pipe-stem fibrosis, which population studies have shown are associated with sustained heavy infection and can take many years to develop [5,6]. Hepatomegaly, secondary to granulomatous inflammation, occurs early in the evolution of chronic disease. Periportal collagen deposits lead to the progressive obstruction of blood flow, portal hypertension, and ultimately varices, variceal bleeding, splenomegaly, and hypersplenism [5,6].

Disease control and prevention

Clinical misclassification of schistosomiasis is common. The current gold standard diagnostic modality is microscopic examination of stool or urine for eggs, but this method 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 true disease burden [10,11]. Adjusting for diagnostic sensitivity, the global burden of schistosomiasis estimated in 2007 was 391-587 million cases [12]. The mainstay of schistosomiasis control is preventative and therapeutic administration of the anthelminthic praziquantel. However, repeated treatment is needed for curative parasite clearance. Furthermore, 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 [13]. 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 [14,15]. 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 [16-18].

3 Test Vaccine

The study product is rSm-p80 + GLA-SE an adjuvanted recombinant protein vaccine also known as SchistoShield®. The vaccine antigen is an *Escherichia 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, Glucopyranosyl Lipid Adjuvant (GLA) is a synthetic Lipid A – like molecule and Toll-like receptor 4 agonist formulated in a squalene emulsion (GLA-SE), manufactured, and released by the Access to Advanced Health Institute (AAHI) (Seattle, WA). Development of effective recombinant protein vaccines for *S. mansoni* is possible due to 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 TH1 responses are now available; the proposed GLA-SE adjuvant has been extensively tested in thousands of human participants.

3.1 PRECLINICAL DATA

rSm-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 Smp80-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 [17]; 3) post-exposure therapeutic efficacy by

elimination of established adult worms in chronic disease [19]; 4) cross species-protection against *S. haematobium* (urinary schistosomiasis) [20]; and *S. japonicum* (Asiatic/oriental disease) [21]; 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 [22]; and 6) maternal transfer of Sm-p80-specific antibodies in baboons [22]. Smp80- 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 rSm-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.

Potency of rSm-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, 4 weeks apart. At week twelve, 4 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 6-8 weeks after challenge. Protection (P) was 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 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. These results demonstrate that the rSm-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.

Safety and Immunogenicity of rSm-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 4-week intervals. Four weeks following the last immunization, each baboon was percutaneously challenged with 1,000 *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 cell blood 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. 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.

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 rSm-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=0.0003). We also observed significant schistosomula killing when inactivated sera from control baboons is combined with control sera alone suggesting a possible role for alternative complement activation in schistosomula killing. Using baboon sera from independent trials, we observed a significant decrease in viable schistosomula of 72.76%, 53.14%, 55.06%, and 46.64% for Trials 1-4, respectively.

Efficacy of rSm-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 rSm-p80/GLA-SE; 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 rSm-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. 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. Those eggs that were excreted were impaired in their ability to hatch into mirace, the parasite stage that infects snails to continue the life cycle with a 79.21% inhibition of hatching for eggs obtained from vaccinated animals. 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.

Safety of GLA-SE

The 5µg 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 participants (refer to section 3.2)

Preclinical Safety

As reported in the GLA-SE Investigator Brochure 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, 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.

GLP Toxicology

In the GLP study to evaluate the systemic toxicity and local tolerance of vaccine candidate rSm-p80 with and without GLA-SE, there were no findings definitively attributed to systemic toxicity of rSm-p80 with or without GLA-SE when administered by IM injection on Days 1, 8, 15, and 22 to New Zealand White (NZW) rabbits. All study animals survived until scheduled humane termination. There were no changes in body weights, body temperatures, or food consumption attributed to rSm-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 rSm-p80 with or without GLA-SE. There were sporadic grades of 1 (very slight) and 2 (slight) edemas at the injection site for rabbits in all 3 treatment groups, however all were graded 0 by 72 hr post-injection. The immune response was highest in rabbits of both sexes that received injections containing both the high dose of rSm-p80 (100 µg) with adjuvant (10 µg GLA-SE). Low doses of rSm-p80 plus adjuvant (10 µg GLA-SE) produced higher titers than high dose (100 µg) of rSm-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.

3.2 CLINICAL DATA

Clinical Experience

GLA-SE adjuvant

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, [23] tuberculosis, [24, 25] influenza, [26, 27] schistosomiasis, [28] and malaria [29]. 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 participants 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 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, arthralgia, and anorexia;
- 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.

rSm-p80 recombinant antigen, with and without GLA-SE adjuvant

rSm-p80 recombinant antigen, with and without GLA-SE adjuvant, has been tested tested in Phase 1 dose escalation clinical trial with healthy adults aged 18-55 years in Seattle, USA. Five treatment groups (A, B, C, D, E), each including nine subjects, received three 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. Group A (unadjuvanted comparator) received 100µg rSm-p80 alone on Days 1, 29, and 57, Group B (low dose standard schedule) received 10µg rSm-p80 + 5µg GLA-SE on Days 1, 29 and 57, Group C (mid dose delayed booster) received 30µg rSm-p80 + 5µg GLA-SE on Days 1, 29 and 180, Group D (mid dose standard schedule) received 30µg rSm-p80 + 5µg GLA-SE on Days 1, 29, and 57, and Group E (high dose standard schedule) received 100µg rSm-p80 + 5µg GLA-SE on Days 1, 29 and 57. The objectives were to evaluate the safety and reactogenicity following receipt of three doses of vaccines mentioned above. The interim safety and immunogenicity data from this trial are summarized as follows:

Safety data

Of the 45 enrolled subjects, 43 (96%) experienced at least one local (injection site) solicited AE, 37 (82%) experienced at least one systemic solicited AE, and 39 (87%) experienced at least one unsolicited adverse event. One new onset chronic medical condition (NOCMC) was reported in Group A. No serious adverse events (SAEs) medically attended adverse events (MAAEs), nor potentially immune mediated medical conditions (PIMMCs) were reported. The most common systemic symptom post any dose was fatigue (31/45 or 69%) and headache (26/45 or 58%). The most common injection site symptom post any dose was tenderness (43/45 or 96%) and pain (29/45 or 64%). Tenderness was the most common solicited symptom post dose 1 (36/45 or 80%), post dose 2 (35/42 or 83%), and post dose 3 (34/42 or 81%). Of the 45 subjects enrolled, 28 (62%) experienced at least one systemic symptom and 36 (80%) experienced at least one injection site symptom within 7 days after the first dose. Of the 42 subjects who received the second dose,

24 (57%) experienced at least one systemic and 35 (83%) experienced at least one injection site symptom within 7 days after the second dose. Of the 42 subjects who received the last dose, 21 (50%) experienced at least one systemic and 34 (81%) experienced at least one injection site symptom within 7 days after the third dose.

Ten (22%) subjects reported at least one related unsolicited AE. The most common related unsolicited AE was arthralgia (3/45 or 7%). Thirty-six (80%) subjects reported at least one unrelated unsolicited AE. The most common unrelated unsolicited AEs were upper respiratory tract infection (9/45 or 20%) and vessel puncture site hemorrhage (6/45 or 13%).

No serious adverse events have been reported. Twenty-four moderate adverse events and 1 severe adverse event were reported. Three of the 24 moderate adverse events were related to study treatment. One unrelated adverse event of mild severity, thyroid nodules, was reported and classified as a NOCMC.

Three female subjects from Group A (age 38), Group C (age 24), and Group D (age 31) experienced a mild increase in creatinine levels, two of which were related to study vaccination (Group A and Group D). Three subjects from Group A (male, age 25), Group C (male, age 43), and Group E (female, age 24) experienced a mild decrease in white blood cell count, two of which were related to the study vaccination (Group A and Group E). One female subject (age 39) and one male subject (age 31) from Group B experienced multiple mild increases in platelets, all of which were related to the study vaccination. Lastly, two female subjects (age 24 and 31) in Group C experienced a mild platelet increase unrelated and related, respectively, to study vaccination. Four subjects in Group B (male, age 52), Group D (female, age 31), and Group E (female, ages 28 and 24) experienced a decrease in hemoglobin; three of these events were related to the study vaccination except for the Group B subject, and all these events were mild except the 28-year-old female in Group E who experienced a moderate decrease.

Immunogenicity data

All subjects enrolled in Group A, Group B, Group C, and Group D satisfied the criteria for inclusion in the mITT immunogenicity population. All 9 subjects enrolled in Group A and in Group C, 8 subjects enrolled in Group B, and 7 subjects enrolled in Group D satisfied the inclusion criteria for PP population after excluding results from the subject who later met an exclusion criterion, those who discontinued treatment, and results of 3 blood samples that were collected substantially out of window.

Antibody levels were similar between Group A, Group B, Group C, and Group D at baseline (Day 1: Pre-Dose 1). Group A and B antibody levels remained similar on Day 8 and Day 29; from Day 36 to Day 85, the distributions of Group B are shifted to the right when compared to Group A indicating higher antibody levels in this group. Group D antibody levels were generally higher when compared to Group A and Group B from Day 29 to Day 64; Group B and Group D had similar antibody levels on Day 85. Generally, Group C had consistently higher antibody levels compared to the other treatment groups across timepoints.

Group A consistently had the lowest GMT result when compared to other treatment groups across time points; generally, Group C had the highest GMT result across time points. Subject VM2.05052 in Group C had notably higher results at baseline (3715.35) skewing the GMT results (74.704 (19.641, 284.138)). In general, this subject had high IgG titers throughout the study; these results were still within the laboratory's acceptable range. GMT results increased up to Day 85 (28 Days Post Dose 3) for Group A, Group B, and Group D. GMT results increased up to Day 57 (28 Days Post Dose 2), then decreased at Day 180 (Pre-Dose 3), then increased again up to Day 208 (28 Days Post Dose 3) for Group C. The difference between the Group A and Group C GMT results were significantly different on Day 36 (Group A: 120.194 (37.282, 387.497); Group C: 1079.771 (406.714, 2866.647)) and Day 57 (Group A: 228.496 (91.931, 567.934); Group C: 2691.534 (1022.427, 7085.447)) as indicated by non-overlapping 95% confidence intervals (Wilson-score without continuity correction). There were no significant differences between other treatment groups nor at any other time point based on the overlapping 95% confidence intervals.

No subject had a four-fold rise 7 days Post Dose 1 (Day 8). One subject from Group A (1/9 or 11%) and Group D (1/8 or 13%) and 2 subjects from Group C (2/9 or 22%) had a four-fold rise 28 days Post Dose 1 (Day 29). By Day 36 (7 Days Post Dose 2), 7 (88%) subjects in Group D had a four-fold rise. By Day 64 (7 Days Post Dose 3), 8 (89%) subjects in Group A, 9 (100%) subjects in Group B had a four-fold rise. The maximum GMFR was on Day 85 (28 Days Post Dose 3) for Group A (GMFR: 48.133), Group B (GMFR: 145.676), and Group D (GMFR: 118.972). Eight (89%) subjects in Group C had a four-fold rise on 7 Days and 28 Days Post Dose 2 (Day 36 and Day 57) and Post Dose 3 (Day 187 and Day 208). The maximum GMFR was on Day 208 (28 Days Post Dose 3) for Group C (GMFR: 218.734). The p-values for Group B, Group C, and Group D 28 days Post Dose 1 and Post Dose 3 were >0.999 for both the mITT population and PP population. The p-values for Group B, Group C, and Group D 28 days Post Dose 2 were 0.294, 0.294, and 0.620, respectively, for the mITT population and 0.294, 0.294, and 0.088, respectively, for the PP population.

3.3 POTENTIAL RISKS AND BENEFITS

3.3.1 Known Potential Risks

The potential risks of participating in this trial are those associated with having blood drawn, nasopharyngeal swab collection, the IM injection and possible reactions to the Sm-p80 recombinant antigen, with or without GLA-SE adjuvant. Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the participant lie down and elevate his/her legs. 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. Nasopharyngeal swab collection may cause slight discomfort or pain while a 5 to 6 inches long bendable, one-time use stick, which has a cotton swab fixed in one end will be inserted through the nasal tract and throat. Appropriate aseptic precautions will be maintained while collecting the sample. Localized discomfort can occur in the nostril during mucosal fluid sampling. Infrequently, this can result in a small amount of epistaxis, which can be controlled with pressure to the affected area. 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 participants 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 liver enzymes (ALT/AST/TB), as well as screening for HIV, hepatitis B, SARS-CoV-2 infection, and hepatitis C infections. Preclinical evaluations, including evaluations in non-human primates, have not identified a safety signal associated with administration of the rSm-p80 recombinant protein antigen (Section 3.1).

There is potentially a higher risk for AEs to occur more frequently in the higher dose rSm-p80-antigen groups than in the lower dose antigen groups. Three doses of Low, medium and high dose of rSm-p80 either adjuvanted with or without 5µg of GLA-SE were recently tested for the fist time in adults in the USA. The product was well tolerated with a good safety profile. There were no serious adverse event (SAE), medically attended adverse event (MAAE) and potentially immune mediated medical conditions (PIMMCs) related to the IP. The most common expected systemic symtoms post any dose experienced within the 7 days post vaccination were fatigue, arthralgia, myalgia and headache. The most common expected local symptoms and signs reported during the 7 days post vaccination included the pain, tenderness induration/swelling. The most common unsolicited adverse event reported with 28 days post vaccination and related to the IP

was arthralgia. Increases in serum creatinine related to the IP were reported in study participants. Changes in hematology parameters related to the IP were reported and included decrease in white blood cell count, increase in platelet count and decrease in hemoglobin. Most of these clinical laboratory abnormalities were mild and transient. 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 3.1. Expected adverse events 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, arthralgia and anorexia. 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 (i.e., 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.

Participants 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 participant's PHI. All study records will be kept in a locked file cabinet or maintained in a locked room at the participating sites. Electronic files will be password-protected. All information or samples will be labeled with a unique study identification number and have no personal identifying information (PII). 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 participants 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/IEC and NRA. A description of this clinical trial will be available on www.clinicaltrialsregister.eu.This web site will not include information that can identify participants.

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

3.3.2 Known Potential Benefits

There is no anticipated direct benefit to participants 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 *Schistosoma* infection.

All volunteers will undergo a medical examination. All volunteers, whether accepted for enrollment into the trial (participant) or not, will benefit from this free health check-up. The results of all tests will be communicated to all volunteers and they will be closely followed to ensure safety throughout the planned visits and unplanned visits, if needed. If findings are of medical concern or where illnesses are newly diagnosed, a referral to an appropriate health provider will be made for the volunteer. In addition, optimal care will be ensured for any side effects related to the study product and procedures covered by participant compensation insurance throughout the whole study period which will provide adequate coverage to the volunteers in case of any unforeseen expenses which they may have to incur due to participation in the study.

3.4 STUDY RATIONALE

The promising results of the preclinical evaluations and satisfactory safety data from the non-endemic population from the Phase 1a clinical trial have supported the advancement of the SchistoShield® vaccine candidate to a Phase 1b human trial of healthy adults in endemic populations. The purpose of this trial is to determine the safety, tolerability, and immunogenicity of the investigational schistosomiasis vaccine candidate in adult populations living in endemic areas.

4 OBJECTIVES

4.1 PRIMARY OBJECTIVE

To evaluate the safety and tolerability of 3 different dose formulations (low dose, medium dose, and high dose) of SchistoShield® vaccine given intramuscularly on D0, D28 and D56 to healthy participants 20 to 59 years of age in Burkina Faso and Madagascar.

4.2 SECONDARY OBJECTIVES

To evaluate the immunogenicity of 3 different dose formulations (low dose, medium dose, and high dose) of SchistoShield® vaccine, 28 days post-vaccination on D28, D56, and D84 as compared with the baseline and with those who received placebo.

4.3 EXPLORATORY OBJECTIVES

To describe the antigen-specific B- and T-cell responses, memory responses, and innate and adaptive immune signatures from samples collected at specified timepoints.

5 INVESTIGATORS AND TRIAL ORGANIZATION

This IVI VASA 001 clinical trial is a consortium-led project. The consortium consists of the Cambridge University, International Vaccine Institute (IVI), University of Gothenburg (UofG) in Sweden, University of Antananarivo (UofA)/Madagascar Institute for Vaccine Research (MIVR), in Madagascar, Groupe de Recherche Action en Santé (GRAS) in Burkina Faso, PAI Life Sciences in USA, Leiden University Medical Center (LUMC) in Netherlands, the Texas Tech University Health Sciences Center (TTUHSC) in USA and the Eberhard Karls Universitat Tubingen in Germany.

Both Burkina Faso and Madagascar are endemic schistosomiasis countries, and the health authorities in both countries are keen to find public health interventions against schistosomiasis. This phase 1b trial will be conducted in healthy adult volunteers aged 20 to 59 years of age by the GRAS Ouagadougou site in Burkina Faso and by the UofA/MIVR site in Madagascar. Capacity will be built at sites to perform immunogenicity tests as much as possible.

The IVI will be responsible for overall coordination, program management and trial sponsorship. The IVI has extensive experience designing and implementing clinical trials to support vaccine development, vaccine public health evaluation and epidemiological investigation of vaccine preventable diseases for over 20 years. Eberhard Karls Universitat Tubingen in Germany will support the capacity strengthening activities at clinical trial sites in terms on trainings, monitoring the clinical trial progress and liaising with the ministries in endemic countries to determine barriers for schistosomiasis vaccine deployment. The UofG will undertake the system biology/immune signatures works. The TTUHSC will support the laboratory capacity building and perform the activities related of the correlates of protection identification. The PAI Life Sciences in USA will ensure the provision of the clinical trial material and technology transfer of vaccine to manufacturers.

A SMC and sites PIs will be involved in the regular review of safety data as described in the dose escalation scheme. An independent DSMB will be involved in the regular review of safety data including assessment of severity when required. Additionally, any related SAE or death will be promptly reviewed by the DSMB.

6 INDEPENDENT ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD

Before the investigational product can be shipped to the investigational sites and the inclusion of the first participant, this protocol, the informed consent form (ICF), participant recruitment procedures, and any other written information to be provided to participants, must receive favorable opinion from the appropriate IRB/IEC and NRA in Burkina Faso, the IEC in Madagascar and IVI IRB.

In accordance with Good Clinical Practice (GCP) and local regulations, each site Principal Investigator and/or the Sponsor are responsible for obtaining this approval and/or favorable opinion before the start of the trial. If the protocol is subsequently amended, approval must be re-obtained for each amendment. Copies of these approvals, along with information on the type, version number, and date of document, and the date of approval, must be forwarded by the site Principal Investigator to the Sponsor together with the composition of the IRB/IEC.

The site Principal Investigator will submit written summaries of the status of the trial to the IRB/IEC and NRA annually, or more frequently if requested. All SAEs occurring during the trial will be reported by the Investigator to the relevant IRB/IEC and NRA, according to the IRB/IEC and NRA policy.

7 STUDY DESIGN AND CLINICAL PROCEDURES

7.1 DESCRIPTION OF THE OVERALL TRIAL DESIGN AND PLAN

7.1.1 Trial Design

This is a phase 1b, multicenter, randomized, placebo-controlled, observer-blinded, dose-escalation study, assessing the safety, tolerability, and immunogenicity of a three-dose regimen, spaced four weeks apart, given intramuscularly in healthy adults (20-59 years old). Three different dose formulations of the study product with varying antigen contents will be investigated. A total of 120 eligible participants will be recruited in 3 sequential cohorts (A, B, and C) in Burkina Faso (N=60) and in Madagascar (N=60), as shown in the **Table 1**, below. Cohort A will receive the low-dose antigen formulation (10µg) or placebo, Cohort B will receive the medium-dose antigen formulation (30µg) or placebo, and Cohort C will receive the high-dose antigen formulation (100µg) or placebo; all antigens with 5µg adjuvant (GLA-SE). In each cohort, volunteers will be randomized in a blinded manner into one of two arms, candidate vaccine or placebo, by a 3:1 ratio. A subset of five out of 20 subjects in each cohort will be sampled by convenience to enable us to further characterize the immune response using the peripheral blood mononuclear cells (PBMC).

To ensure that the enrolled study participants are not having any active schistosomiasis or helminth infection and are schistosomiasis egg-negative, pre-screening activities including schistosomiasis treatment will be carried out in potential study participants prior to enrollment. Potential study participants will be identified in the catchment population and will be offered anti-helminth treatment using praziquantel (PZQ) and Albendazole (ABZ) as per local guidelines at study site. The pre-screening visit will be conducted 6-8 weeks before the Screening Visit. The last dose of PZQ/ABZ will be administered at least 5 weeks prior to the first dose of study product.

Table 1: VASA Phase Ib Trial Design In Burkina Faso and Madagascar

Country	Age group	Cohort	Arm	Treatment	Number of volunteers
Burkina Faso	20 - 59 years	Cohort A	Arm A1	SchistoShield® LD	15
			Arm A2	Placebo	5
		Cohort B	Arm B1	SchistoShield® MD	15
			Arm B2	Placebo	5
		Cohort C	Arm C1	SchistoShield® HD	15
			Arm C2	Placebo	5
Sample size					60
Madagascar	20 - 59 years	Cohort A	Arm A1	SchistoShield® LD	15
			Arm A2	Placebo	5
		Cohort B	Arm B1	SchistoShield® MD	15
			Arm B2	Placebo	5
		Cohort C	Arm C1	SchistoShield® HD	15
			Arm C2	Placebo	5
Sample size					60
Total Sample Size					120

LD: Low dose (10µg Ag/5µg Adjuvant); MD: Medium Dose (30µg Ag/5µg Adjuvant);
HD: High Dose (100µg Ag/5µg Adjuvant)

Dose escalation scheme:

All participants will receive three injections of the assigned study product, given 28 days apart at Days 0, 28, and 56. As this is the first-in-human study of SchistoShield® in schistosomiasis endemic countries, Cohort A will be enrolled first and will receive the low antigen adjuvanted product or placebo (Table 1). To progress to enrollment of Cohort B, the Safety Monitoring Committee (SMC) and sites PIs will review blinded safety data of Cohort A participants collected through seven days following the second dose of study product injection and assess this data according to pre-specified criteria for halting dose escalation (section 10.4.3). If halting criteria are not met, Cohort B participants will be enrolled. Dose escalation to Cohort C will follow the same procedures requiring SMC and Site PIs review. These safety data reviews and decision to progress to the next dose level will occur for each site separately.

If dose escalation halting criteria *are* met at any point, the matter will be escalated to the DSMB, which will review summarized safety data in an unblinded manner and will provide their recommendation to the sponsor for dose escalation before study product injection of the next cohort is initiated. The DSMB review

is not required for dose escalation into the next cohort unless the halting criteria are met or the SMC requests for DSMB review.

All participants will be followed up for safety and immunogenicity at specific time points. Each participant will be in study for approximately 32 weeks after enrollment. Eligible participants enrolled into the study will be vaccinated and observed at the study site for a minimum of 60 minutes after vaccination for immediate safety assessment. Solicited adverse events will be recorded on a diary card for 7 days following each study product injection. Unsolicited adverse events will be recorded for 28 days following each study product injection. Serious adverse events and adverse events of special interest (AESI) will be recorded during the entire study period. Except for designated study site personnel responsible for vaccine administration, study investigators, study nurses, those assessing clinical outcomes, and laboratory analysts will be blinded to vaccine allocation until database lock for the final analysis.

A total of 7 blood samples will be collected for safety assessment at Days -28 to -1, 7, 28, 35, 56, 63, and 84. A total of 8 blood samples will be collected for immunogenicity assessments at Days 0, 7, 28, 35, 56, 63, 84, and 224.

Enrollment/Vaccination rules:
The following rules will be applied for the randomization/administration of the investigational product to the participants within the first study cohort (Cohort A):

- First two participants will be enrolled to receive rSm-p80 + GLA-SE (or placebo).
- If within at least 24 hours, there is no serious adverse reaction identified following immunization, the second two participants will receive **r**Sm-p80 + GLA-SE (or placebo).
- If within at least a week after the first vaccination, there is no serious adverse reaction identified following immunization the vaccine can be administered to the rest of the cohort.

The same rules will be applied to all the study cohorts. A detailed description of the vaccination rules is described in the manual of procedures (MOP).

7.1.2 Justification of the Trial Design

The rSm-p80 + GLA-SE test vaccine has been studied in adult humans in non-endemic population. The ultimate objective of this candidate vaccine is to protect populations from Schistosomiasis infection in endemic countries, and the proposed study will be the first to generate safety and immunogenicity data of the vaccine in adults living in schistosomiasis-endemic settings. This study also features three different

study product formulations of adjuvanted low, middle, and high antigen dose content. The results of the study will support decision-making on the final antigen dose selection for the next phase of the investigational product clinical development. Burkina Faso and Madagascar are part of VASA clinical trial consortium where the trial will be conducted with high to moderate burden of schistosomiasis. Health authorities in both countries are keen to find public health interventions against schistosomiasis.

7.1.3 Trial Plan

A schedule of assessments and study vaccinations is provided in the Table 2, Section 1.3.

7.1.3.1 Vaccinations

Study Product Description

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

rSm-p80 [antigen] 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, rSm-p80. The final 758 amino acid protein antigen has a predicted mass of approximately 87kDa. rSm-p80 is released by PAI Life Sciences Inc (Seattle, WA).

GLA-SE [adjuvant formulation] is a synthetic Monophosphoryl Lipid A-like molecule which is a Toll-like receptor 4 agonist formulated in a SE to produce GLA-SE. GLA-SE is formulated in a squalene emulsion and is manufactured and released by the Access to Advanced Health Institute (AAHI) (Seattle, WA).

rSm-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.

Formulation and Product Storage

rSm-p80 Antigen

- Formulated as a lyophilized cake in glass vials and appears white to off-white cake.
- Supplied as single use glass vials.
- Each 3 mL glass vial contains 125 µg of rSm-p80.
- Reconstitution of rSm-p80 with WFI results in a clear, colorless solution.
- Storage Conditions: between 2°C to 8°C, excursions up to 25°C are permitted for 4 hours

GLA-SE Adjuvant

- Formulated as 20 µg/mL GLA in a 4% SE and appears as a milky-white liquid.

- Supplied as single use glass vials.
- Each 2mL vial contains a fill volume of 0.4 mL
- Storage Conditions: between 2°C to 8°C, excursions up to 25°C are permitted for 24 hours but product should never be frozen.

rSm-p80 + GLA-SE for Injection

- Vaccine product (antigen + adjuvant) is only produced upon admixture prior to administration.
- Three adjuvanted formulations with varying antigen content of 10 µg, 30 µg, and 100 µg of rSm-p80 antigen
- Dissolved rSm-p80 antigen and the GLA-SE emulsion appears as a translucent milky white liquid.
- Storage Conditions: admixture is stable up for at least 4 hours at up to 25°C.

Sterile Water for Injection

- The sterile WFI is nonpyrogenic and contains no bacteriostatic, antimicrobial agent or added buffer.
- This product will be used to dilute the vaccine (rSm-p80) and will be supplied as a single-dose vial.
- Storage Conditions: as per manufacturer's instructions provided in the package insert.

Placebo

- Dose formulation: Sterile 0.9% sodium chloride.
- Storage Conditions: as per manufacturer's instructions provided in the package insert.

Doses and route of vaccination:

- Each study cohort will receive a three dose-regimen administered 28 days apart (D0, D28, and D56)
- Each dose of study vaccine or placebo will be administered as a 0.5 mL IM injection.

7.1.3.2 Visits and Contacts

Pre-screening Visit (Visit 0: D-56 to D-42)

To enroll study participants who are free of any ongoing active schistosomiasis or helminth infection and are schistosomiasis egg-negative, pre-screening activities including schistosomiasis treatment will be carried out in potential study participants prior to enrollment. Potential study participants will be identified in the catchment population and will either be invited to the study site or engaged by study staff and community health care workers in the field.

Study staff will go through a Participant Information Sheet which describes that the pre-screening treatment is to ensure an individual's eligibility into a future vaccine clinical trial study. The anti-helminth treatments offered will follow the national and local standard-of-care guidelines using medications that are licensed and available in local pharmacies. The Participant Information Sheet will clearly state that agreeing to this

pre-screening treatment is neither a guarantee of future study enrollment nor a binding agreement to be in a future study. General information regarding the future vaccine clinical trial such as trial purpose, trial start date, and trial duration will be provided in the Participant Informed Consent Form. If interested, participants may also receive the clinical trial Informed Consent Form to review in their own time.

Potential study participants who agree to receive the pre-screening treatment will sign the pre-screening informed consent form to indicate their consent to receive a standard-of-care treatment and to give their basic contact information.

The following procedures will be performed at the pre-screening visit:

- Potential participant will be provided with a description of the pre-screening treatment and the future clinical trial that this treatment will make them possibly eligible for participation.
- Potential participant will be asked to read and sign the informed consent form if they agree to receive the standard-of-care treatment for schistosomiasis and other helminths.
- The treatment against Schistosomiasis and soil-transmitted helminths using PZQ and Albendazole will be given according to the local guidelines. Dosing will be supervised and documented. More details will be provided in the study MOP.
- Potential participant will provide basic contact information for future communication about the upcoming vaccine clinical trial they will be eligible to screen for enrollment.

The pre-screening visit will be conducted 6-8 weeks before the Screening Visit. The last dose of PZQ/ABZ will be self-administered at least 5 weeks before the first expected study product injection at Visit 2.

Screening (Visit 1: D-28 to -1)

The following procedures will be performed at the initial screening visit:

- Participants will be provided with a description of this vaccine 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.
- Eligibility criteria will be reviewed with participants and eligibility assessed by review of the inclusion and exclusion criteria.
- A complete medical history will be obtained, and all prior concomitant medications taken in the prior 60 days will be identified to determine the stability of chronic diseases and eligibility.
- Vital signs, including body temperature, respiratory rate, blood pressure, and pulse, will be taken.

- Demographic information will be obtained by interview of the participant.
- Height and weight will be obtained to calculate BMI.
- A physical examination that assesses general appearance and the following areas/systems: skin, lymph nodes, Ear Nose Throat, neck, cardiovascular, pulmonary, abdomen, extremities, musculoskeletal, and neurological, will be performed by a study clinician.
- HIV test counseling will be provided.
- Venous blood will be collected for screening laboratory tests (RBC, WBC, hemoglobin, platelets, creatinine, ALT/AST, TB, CRP, HIV-1/HIV-2 testing, anti-HCV antibody, HBsAg, serum pregnancy test for all female participants of childbearing age).
- A urine sample will be obtained for urine dipstick screening tests (blood, glucose, and protein.
- Examination of urine and stool for ova to exclude schistosomiasis will be done using urine filtration, POC-CCA, Kato-Katz confirmed by RT-PCR. A urine and stool collection kit will be given to participant to collect urine and stool samples with instructions on how to contact study staff for collection. Details of urine and stool collection procedures will be described in MOP.

- Blood smear for Malaria diagnostic.
- Stool examination for soil-transmitted Helminths using Kato-Katz and confirmed by the RT-PCR.
- A SARS-CoV-2 rapid antigen test will be conducted.
- 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 investigator will determine whether the subject is eligible to participate in the trial after confirming that participants fulfill all of the eligibility criteria.

Enrollment and First Study Product Injection, Clinic Visit (Visit 2: D0)

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

- Temporary exclusion criteria will be reviewed.
- Prior and concomitant therapy will be reviewed.
- A full physical examination will be performed by a study clinician.
- Vital signs, including body temperature, blood pressure, respiratory rate and pulse, will be taken.
- 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 urine sample obtained within 24 hours prior to vaccination must be negative.

- A SARS-CoV-2 rapid antigen test will be conducted, in case of suspected symptoms/signs.
- Venous blood samples will be obtained for the malaria rapid diagnostic test, the CAA assay, and the immunogenicity assessments at baseline.
- Randomization (Section 8.4), to receive test vaccine (rSm-p80 with GLA-SE), or placebo, will be performed.
- The assigned study product formulation or placebo will be administered by IM injection in the deltoid muscle of the non-dominant arm. The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form.

After the study product injection, the following procedures will be performed:

- Participants will be observed in the clinic for at least 60 minutes after the injection. The injection site will be examined, vital signs will be obtained, post-administration reactogenicity assessments will be performed, and any AE/SAEs/AESI will be recorded on the appropriate data collection form prior to discharge from the trial site.
- Participants will be provided with a Diary Card 1, measuring tape and thermometer for recording daily maximum body 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. Participants will be encouraged to take their temperature around the same time each day and any time they feel feverish. In addition, field workers (Study nurses) will be paying a daily home visit to the participants to perform the required assessments and complete the Diary card 1.
- In case participants are required to complete the Diary Card, he/she will be instructed on how to fill in the Diary Card and how to grade any AEs.
- Participants will be instructed to notify the study center if they develop any severe reactions and/or any fever. If the investigator deems the reaction warrants further evaluation or intervention, the investigator will give further instructions on the proper course of action, including visit to the study site for immediate evaluation if appropriate.
- Instruct the participant to contact the site staff or investigator immediately if a medically attended event (e.g., doctor's visit, emergency room visit) or hospitalization occurs.

- Scheduling an appointment for the participant to return for the next study visit and reminding him/her to bring the diary card (when needed), other health records and any medical prescription.
- For illiterate subjects, participant diary card will be completed by the site staff after collecting adverse event information through daily telephonic contact/home visit to subject.
- The investigator or an authorized designee completes the case report forms (CRFs) and any sources documents and an unblinded study agent dispenser/administrator updates the study intervention accountability records.

Study Product Injection Visits (Visit 4: D28+3 and Visit 6: D56+3)

The second and third study product injection will be administered at Visit 4, Day 28 (window + 3 days) and Visit 6, Day 56 (window + 3 days) respectively. Procedure to be followed at these visits are:

- All concomitant medications will be reviewed and recorded on the appropriate data collection form.
- All newly identified AEs/SAEs/AESI will be recorded.
- A full physical examination will be performed by a study clinician.
- Vital signs, including body temperature, respiratory rate, pulse, and blood pressure, will be obtained prior to the study product injection.
- Temporary exclusion criteria will be reviewed.
- Contraindications for vaccination will be reviewed.
- 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 SARS-CoV-2 rapid antigen test will be conducted, in case of suspected symptoms/signs.
- Venous blood samples will be obtained for lab safety assessments, malaria rapid diagnostic test, CAA assay and immunogenicity assays, in volumes as specified in Table 2: Schedule of Events and Study Procedures. The clinical safety laboratory results must be available and reviewed prior to study vaccination.
- A urine sample will be obtained for urine dipstick screening tests (Blood, glucose, and protein.
- Participants will then receive a single dose of study product or placebo via IM injection into the deltoid muscle.
- The site of injection (right or left arm) and time of administration will be recorded on the appropriate data collection form. Participants will be observed in the clinic for at least 60 minutes

after the study vaccination. The injection site will be examined, vital signs will be obtained, post-administration reactogenicity assessments will be performed, and any AE/SAEs/AESI will be recorded on the appropriate data collection form prior to discharge from the clinic.

- Participants will be provided with Dairy Card 2 at Visit 4 and Diary Card 3 at Visit 6 for recording daily maximum body 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. Participants will be encouraged to take their body temperature around the same time each day and also if they feel feverish. Participants will be instructed on how to use the Diary Card and how to rate any AE. In addition, field workers (Study nurses) will be paying a daily home visit to the participants to perform the required assessments and complete the Diary card 2, and 3.
- Participants will be instructed to notify the study center if they develop any severe reactions and/or fever. 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.
- Instruct the participant to contact the site staff or investigator immediately if a medically attended event (e.g., doctor's visit, emergency room visit) or hospitalization occurs.
- Scheduling an appointment for the participant to return for the next study visit and reminding him/her to bring the diary card (when needed), his/her health record and any medical prescription.
- For illiterate subject, participant diary card will be completed by the site staff after collecting adverse event information through daily telephonic contact/home visit to subject.

The investigator or an authorized designee completes the CRFs and any source documents and an unblinded study agent dispenser/administrator updates the study intervention accountability records.

Follow-up Visits (Visit 3, Visit 5, Visit 7, Visit 8)

Follow-up visit number, study day, and windows are shown below.

- Visit 03, Day 7, Window +3 days
- Visit 05, Day 35, Window +3 day
- Visit 07, Day 63, Window +3 day
- Visit 08, Day 84, Window ±7 day

For visit 3, 5, 7 and 8 clinical evaluations and procedures to be performed are:

- Reviewed and collect all new or ongoing adverse events.
- Record current concomitant medications/treatments.
- Perform full physical examination.
- Record vital signs.
- Review contraceptive measure with female participants of childbearing potential.
- Collect blood for lab safety assessments (Hematology, serum chemistry and CRP).
- A urine sample will be obtained for urine dipstick screening tests (Blood, glucose and protein.
- Collect and verify diary cards at visits 3, 5, and 7.
- Collect whole blood and serum for immunology assessment at visit 3, 5, 7 and 8.
- Collect serum for CAA assay at visit 8 only.
- Instruct the participant to contact the site staff or investigator immediately if a medically attended event (e.g., doctor's visit, emergency room visit) or hospitalization occurs.
- Schedule an appointment for the participant to return for the next study visit and reminding him/her to bring his/her health record and any medical prescription.
- The investigator or an authorized designee completes the CRFs and any source documents.

Last Study Visit (Visit 9)

For the end-of-study visit, Visit 09 (Day 224 $\pm$ 14) the following evaluations will be performed:

- Review and record all SAEs/AESIs.
- Review and record current concomitant medications/treatments, if any.
- Review and record non-study vaccinations, if any.
- Perform full physical examination.
- Collect whole blood and serum for immunology assessment.
- Inform the participant of the end of the study.
- The investigator or an authorized designee completes the CRFs and any sources documents.

Unscheduled Visit (any time after Visit 2)

Participants may be asked for unscheduled visit at discretion of PI or participant can visit site anytime during the study duration due to any safety concerns:

- Review and collect all new or ongoing adverse events.

- Review and record current concomitant medications/treatments, if any.
- Perform full physical examination.
- Record vital signs.
- Review Dairy Cards if applicable.
- During this visit, other than study-related activities, other appropriate workups/laboratory testing at the discretion of PI's best medical judgement and other national guidelines may be offered as part of routine medical service. including SARS-CoV-2 rapid antigen test.
- The investigator or an authorized designee completes the CRFs and any source documents.

7.1.3.3 Blood Sampling

At screening and at visits specified in the SOE, blood samples will be collected for immunogenicity and safety assessments and serum pregnancy test in female participants of childbearing potential. Blood volume per visit is specified in the SOE (Table 2). Additional blood samples will be collected for any unscheduled visits if deemed necessary by the investigator as specified in the SOE. Participants may be asked to come back for additional blood draws, if collected blood sample is lost or unevaluable for any reason.

7.1.3.4 Total Duration

Visit dates and windows must be calculated from Day 0. All participants will be followed for 24 weeks following the last dose of the study product. Each participant will be part of the study for approximately 32 weeks after enrollment (Visit 2).

7.1.4 Visit Procedures

Visit procedure as per section visits and contacts 7.1.3.2.

7.1.5 Planned Trial Calendar

The actual dates may differ as, for example, the trial will not start until all the appropriate regulatory and ethical approvals have been obtained. The following dates are approximate.

Estimated date of first participant enrollment: November 2023.

Estimated date of last participant enrollment: October 2024.

Estimated date of first participant's last visit: July 2024.

Estimated date of last participant's last visit: June 2025.

7.2 ENROLMENT AND RETENTION OF STUDY POPULATION.

7.2.1 RECRUITMENT PROCEDURES

Details of visit procedures during enrollment are described below and in the MOP.

Initial meetings will be held with local stakeholders (e.g., community chiefs) to inform about the purpose of the trial and to ask for their engagement/contribution for the success of the trial.

In Burkina Faso, participants will be recruited from Banfora Health district catchment area. In Madagascar, participants will be recruited in the Antananarivo city. In both countries, participants will be recruited within the general community, as well as from the health facilities such as youth clinics, teaching institutions (high school students 20 years and above, universities, etc.). The trial teams will hold local meetings at these places to explain the trial to potential eligible research subjects. During these meetings, the investigators will explain the following: the need for the schistosomiasis vaccine, the purpose of the trial, the trial screening and informed consent procedures, potential benefit, and risk of the vaccination. After these meetings, a list will be compiled of potential subjects who are interested in participating in the study and the potential subjects will be invited to the trial sites for the screening visits. In order to enhance the community referral to the trial recruitment sites, IRB/IEC approved advertising materials such as leaflets could be used through social media and through the research institution website. These leaflets include the following information: schistosomiasis and vaccination, the purpose of the research, a summary of eligibility criteria, potential benefits (if any) and risks for taking part of this study, a general description of the time commitment and number of participants needed for the trial, the name and address of the participant recruitment site and who to contact for more information. Study staff will be available at the recruitment centers to answer questions and make appointments for actual recruitment assessment and enrolment at designated timeslots. Informed consent will be obtained from all participants before enrolment. During the course of the recruitment, repeated evaluation of enrolment status, adherence to SOPs and encountered challenges will be conducted.

7.2.2 INFORMED CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a continuing process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Information about the risks and possible benefits of participation will be provided to the participants through extensive discussion. Consent forms will be IRB/IEC-approved prior to their use and the participants will be asked to read the document for the understanding of the study.

The investigator or designated study team member will explain the study to the participants and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. The participants will have the opportunity to carefully go through the written consent form and ask questions prior to signing. Participants may wish to discuss the study with family or friends before making any decision as to whether to participate in the study and come back later to inform the site Investigator or designee of his/her decision. For those individuals who express interest in continuing with the consent process, the site investigator or designee will review the consent form privately in detail with the participants and answer any questions.

Before signing the consent, participants will be asked to undergo an informed consent process validation to ensure that they fully understand the purpose of the study, procedures, potential risks and their rights in this study.

If the participant is illiterate (i.e., not able to read and sign the ICF), then it must be signed and dated by an impartial witness who is independent of the Investigator. A witness who signs and dates the consent form is certifying that the information in this form and any other written information had been accurately explained to and understood by the participant.

The participant will sign the informed consent documents prior to any procedures being done specifically for the study. The participant may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be handed over to the participants for their records. The rights, safety, and wellbeing of the participant will be protected by emphasizing that the quality of their medical care will not be adversely affected even if they decline to participate in this study.

7.2.3 COMPENSATION FOR PARTICIPATION

Compensation for time and inconvenience of study participation will be provided to participants in accordance with the site-specific IRB/IEC approved plan. This includes scheduled visits. Expenses that are directly related to the participant's participation in the trial (for example cost of transportation for attending visits) will be reimbursed. More details about the amount are provided by the country site-specific ICFs.

7.2.4 PREGNANCY PREVENTION COUNSELING ON FEMALE PARTICIPANTS OF CHILDBEARING POTENTIAL

Clinical staff must perform pregnancy prevention counseling on female participants of childbearing potential and/or male participants with partner with childbearing potential to evaluate if participants are able to meet the birth control eligibility criteria. Pregnancy test results must also be confirmed as negative for these participants during screening. This procedure must be carried out to evaluate if participants are able to meet the birth control eligibility criteria. The documentation of this procedure is explained in detail in the MOP. Additionally, female participants of childbearing potential will receive pregnancy prevention counseling throughout the vaccination period.

7.2.5 ELIGIBILITY CRITERIA

7.2.5.1 INCLUSION CRITERIA

To be eligible to participate in this study, any individual must meet the following criteria:

1. Healthy male or female participants 20 to 59 years of age at the time of consent.
2. Participant who has completed the deworming using praziquantel (PZQ) and albendazole (ABZ) treatment according to local guidelines, with the last dose of PZQ/ABZ administered at least 5 weeks prior to receiving the first dose of study product
3. Participants who, after the nature of the study has been explained, have voluntarily given informed consent, according to the local regulatory requirements, prior to study entry.
4. Participants who can comply with the study procedures and available for the entire duration of the study (32 weeks).

5. Individuals in good health as determined by the outcome of medical history, physical examination, hematology, and biochemistry tests, at the time of screening and the clinical judgment of the investigator.
6. Women of childbearing potential* with negative urinary test result on a human chorionic gonadotropin pregnancy test on the day of randomization before receiving any study product.
7. Males or Females of childbearing potential who are using an effective birth control method recommended by the national health system for at least four (4) weeks before the first vaccination (for female participants only) and up to four (4) weeks after the third vaccination (i.e., for at least 4 months).

*Participant who confirm state of menopause, hysterectomy, or tubal ligation during the pregnancy counselling process, are considered not of childbearing potential

Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Participant with major congenital abnormality which in the opinion of investigator may affect the subject's participation in the study.
2. Participants concomitantly enrolled or scheduled to be enrolled in another trial.
3. Positive rapid test for HIV 1-2 confirmed by a positive blood test for human immunodeficiency virus (positive antibodies to HIV 1/2).
4. Participant seropositive for hepatitis B virus surface antigen (HBsAg).
5. Participant seropositive for hepatitis C virus (Antibodies to HCV).
6. Participant with active or chronic Schistosomiasis infection defined by a positive result for microscopy (Urine filtration, Kato-Katz (KK)) and/or point-of-care – circulating cathodic antigen (POC –CCA) and/or real-time PCR.
7. Participant with Soil-transmitted helminths infections (STH) as diagnosed by microscopy (KK) and/or real-time PCR.
8. Participant with malaria infection/malaria as diagnosed by the blood smear (If an individual tested blood smear positive for malaria at the screening visit, received treatment, and subsequently tests negative on a blood smear within the designated screening window, they will be eligible for inclusion in the study).

9. Any other confirmed or suspected immunosuppressive or immunodeficient state such as asplenia, recurrent severe infections.
10. Body mass index (BMI)≥ 35 kg/m^2
11. Chronic use of systemic steroids (>2 mg/kg/day or >20 mg/day prednisolone equivalent for periods exceeding 10 days), cytotoxic or other immunosuppressive drugs.
12. Receipt of blood or blood-derived products in the past 3 months.
13. Participant who has received other vaccines 4 weeks prior to test vaccination or plans to receive any vaccine within 4 weeks of last dose of study vaccine, exception made for COVID-19 vaccines.
14. Known history or allergy to study vaccine components and/ or excipients or other medications, or any other allergies deemed by the investigator to increase the risk of an adverse event if they were to participate in the trial.
15. Individuals with a known bleeding diathesis, or any condition that may be associated with a prolong bleeding time resulting in a contraindication with IM injections/blood extractions.
16. Any abnormality or chronic disease which in the opinion of the investigator might be detrimental for the safety of the participant and interfere with the assessment of the study objectives and compromise the health of the volunteers.
17. Any female participant who is lactating*, pregnant or planning for pregnancy** during the course of study period.
18. Individuals with behavioral or cognitive impairment or psychiatric disease or neural disorders that, in the opinion of the investigator, could interfere with the individual's ability to participate in the trial.
19. Any clinically significant abnormal finding on serum chemistry or hematology or urinalysis at the screening visit as per US FDA toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (any biological finding grade 4 constitutes an exclusion criteria).
20. Individual who are research staff involved with the clinical study or family/household members of research staff
21. As per Investigator's medical judgement individual could be excluded from the study despite meeting all inclusion/exclusion criteria mentioned above.

Temporary exclusion criteria (delay of vaccination)***:

1. Individuals with acute illness (moderate or severe) at the time of vaccination. Acute illness is defined as the presence of a moderate or severe disease with or without fever. Fever is defined by axillary temperature ≥37.5°C or tympanic temperature ≥38°C at the time of vaccination. Vaccines can be administered to subject with a minor illness without fever at the discretion of the investigators.
2. Individuals with an active SARS-CoV-2 infection, as determined by a rapid antigen diagnostic test.
3. Individuals tested positive by malaria rapid diagnostic tests on the day of vaccine administration.

* Lactation: This IP has not been specifically studied in pregnant and lactating women. No data on lactating women are available. There is no information about harm to an unborn child or a child who is breastfeeding. Breastfeeding women will not be enrolled. Should a female participant decide to breastfeed during the vaccination period, she will be excluded from further vaccination but will be followed for safety until end of the study

**Serum pregnancy test at screening and UPT before vaccination is necessary for all female participants of childbearing age.

*** If any of these events occur at the scheduled time for the vaccination, randomization at a later date within the window period is permitted at the discretion of the investigator. If randomization cannot occur within the window period, rescreening is required. If an afebrile 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 participant may receive study product injection.

7.2.6 MEDICAL HISTORY

Medical history will be obtained by the Investigator or qualified designee at the time of screening. Medical history will include all active conditions, and any other past conditions, as well as surgical procedures, that are considered to be clinically significant by the Investigator.

Illnesses first occurring or detected after first dosing, and/or worsening of an existing illness in severity, frequency or nature after first dosing are to be documented as AEs on the eCRF. Prior treatments, defined as administered up to 60 days prior to the time of informed consent and stopped prior to dosing on Day 0, should be recorded in the eCRF as prior medications. Concomitant treatments, defined as continuing or new treatments taken at or after first dosing, should be recorded in the eCRF as concomitant medications.

7.2.7 CONTRAINDICATIONS FOR SUBSEQUENT VACCINATIONS

7.2.7.1 DEFINITIVE CONTRAINDICATIONS

The following conditions/situations listed below, are definitive contraindications and the site investigator must discontinue participants from vaccination:

1) Pregnancy, as indicated by a positive urine test.
2) An anaphylactic or other significant allergic reaction to the initial dose of vaccine.
3) Participant deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized without his/her consent.
4) Self-reported or suspected congenital or acquired immunodeficiency, or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months, or long-term systemic corticosteroids therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months).
5) Clinically significant AE or biological abnormality related to previous vaccination and, in the Investigator's opinion, contraindicating further vaccination.
6) SAE related to the study vaccine following the previous trial vaccination.

Participants who have provided Informed Consent and have received at least 1 dose of the study vaccine but are discontinued from vaccination or the study must be encouraged to continue study participation for safety follow up, see section 7.2.14.

7.2.8 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request, without justification and without prejudice. The Principal Investigator may also decide to discontinue participation of participant from study interventions in the following cases:

1) An acute reaction (allergy, hypersensitivity reaction, etc.) to the investigational product.

2) Occurrence of an illness or serious adverse event or adverse event that in the judgment of the investigator may be detrimental for the participant's safety.

3) A study participants' withdrawal of informed consent or dropout. The reason for a withdrawal or dropout should be clearly documented in the source documents and on the eCRF.

4) A study participant's medical condition or use of medication that in the judgment of the investigator may compromise the participant's safety and/or the scientific integrity of the study.

5) Violation of the inclusion/exclusion criteria by the participant.

6) Significant non-compliance with the protocol, based on the Investigator's judgment and lost to follow-up.

7) Any other reason of study discontinuation as per the judgment of the Principal Investigator.

Withdrawn participants will not be replaced. Any unsolicited AE, SAE and concomitant medications will be recorded in eCRF

7.2.9 HANDLING OF PARTICIPANT DISCONTINUATION OR TERMINATION

Discontinuation from study vaccination does not mean discontinuation from the study, and remaining study procedures will be completed as indicated by the study protocol. If a clinically significant finding is identified after enrollment, the PI will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event.

Study team will encourage participant to continue in the study for safety follow-up. If she/he declines, this will end the participant's interaction with the study team for this protocol. The study team will engage in no further communication with the volunteer except as directed by an IRB/IEC about participant safety information. Protocol-specified safety follow-up procedures will be discussed with the participant to capture AEs, serious adverse events (SAEs), and unanticipated problems (UPs). The reason for participant discontinuation or withdrawal from the study will be recorded on the study follow-up electronic Case Report Form (eCRF). Only data and samples already collected will be analyzed according to protocol. Based on the conditions of the consent and withdrawal, the study team will not utilize samples or data from this volunteer for any future use and will discard residual samples when the study is completed. Counseling

about any issue will be provided if he/she decides to discontinue participation in the study. Medical advice will also be provided in the best interest of the participant.

7.2.10 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for any of scheduled visits and remains unreachable to study site staff.

The following actions will be taken if a participant fails to return to the clinic for a required study visit:

- The site staff will attempt to contact the participant and counsel on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant. These contact attempts should be documented in the participant's medical record or study file. In the case of participants who fail to return for a follow-up examination, documented reasonable effort (i.e., documented telephone calls and certified mail) should be undertaken to locate or recall them at least 3 times, to determine their health status while fully respecting their rights. These efforts should be documented in the eCRF and in the source documents.

Should the participant continue to be unreachable, he/she will be considered as lost to follow-up.

7.2.11 Discontinuation from Vaccination Phase in Case Of Pregnancy

Pregnancy is an exclusion criterion for enrolment in this study, but a participant could potentially become pregnant during her participation. All pregnancy cases should be reported as described in the section Follow-up and reporting of Pregnancy, 7.2.14. If a female participant who has already received at least one injection becomes pregnant during the trial, she will continue to be followed for safety assessments including surveillance for the disease that the vaccine aims to prevent, and she will not be discontinued from the trial; however, no additional vaccinations will be administered.
Study staff must then maintain contact with the participant to obtain information about the outcome of the pregnancy that begins during this study following the procedures of section 7.2.14.

7.2.12 CLASSIFICATION OF PARTICIPANTS WHO DID NOT COMPLETE THE TRIAL OR THE VACCINATION PHASE

Participants who receive at least one product administration are expected to continue with planned follow-up visits until the end of the study. However, for any participant who discontinues the trial prior to completion, the reason for early termination will be noted in the eCRF, classified as one of the following:

Serious adverse event: To be used when a participant drops out of or is withdrawn from the study by the Investigator because of the occurrence of an SAE.

Other adverse event: To be used when a participant drops out of or is withdrawn from the study by the Investigator because of the occurrence of an AE other than an SAE.

Non-compliance with protocol: To be used when the Investigator withdraws a participant from the study because of failure to follow protocol guidelines. This termination category may also be used if it is retrospectively discovered that a participant did not fulfill the eligibility criteria. The Investigator will provide a comment as to the specific cause of non-compliance.

Lost to follow-up: To be used when the Investigator withdraws a participant from the study because of failure to establish contact. The Investigator will provide documentation that contact was attempted (i.e., number of calls, return of unsigned certified letter receipt).

Subject' decision: withdraw consent, discontinue study participation, personal reason.

Others: To be used when a participant drops out of the study for any reason other than those listed above (Section 7.2.8).

Pregnancy: To be used when a participant becomes pregnant during her participation and is discontinued from the trial.

7.2.13 FOLLOW-UP OF PARTICIPANTS WHO DID NOT COMPLETE THE TRIAL OR THE VACCINATION PHASE

For participants where the reason for early termination is voluntary withdrawal, the site will attempt to contact them to obtain further safety information.

For participants where the reason for early termination is pregnancy, follow-up of the participant will follow the procedures described in section 7.2.14.

7.2.14 FOLLOW-UP AND REPORTING OF PREGNANCIES

Female participants of childbearing potential will receive pregnancy prevention counseling throughout the vaccination period. Even though pregnancy is an exclusion criterion for enrolment in this study, a participant could potentially become pregnant during her participation. For this reason, women will be asked to inform the site immediately if they suspect or learn they are pregnant during the study. In case of pregnancy, participants will be discontinued from the product administration schedule. However, she will not be discontinued from the trial and can continue to be followed up until the end of the study for safety assessments including surveillance for disease the study vaccine aims to prevent. Participants can have additional blood draws at the discretion of the site PI and the sponsor. All pregnancy cases should be reported as protocol deviation if they occurred during this study. Study staff must then maintain contact with the participant to obtain information about the outcome of the pregnancy. This information should be provided to the Sponsor within one month of the end of the pregnancy. The site should follow the local law and regulatory requirements related to the pregnancy follow-up.

Pregnancy itself is not considered an AE, but any complications during pregnancy are to be considered as AEs, and in some cases could be considered SAEs. Spontaneous abortions, Blighted ovum, fetal death, stillbirth, an elective termination for medical rationale and congenital anomalies reported in the baby are always considered as SAEs, and the information should be provided using an SAE form.

7.2.15 PROTOCOL DEVIATIONS

The ICH E3 Q&A R1 defines a protocol deviation (PD) as “any change, divergence, or departure from the study design or procedures defined in the protocol.” In other words, protocol deviation is any noncompliance with the clinical trial protocol, ICH E6 (R2) GCP, E6 (R2), E11 (R1) or MOP requirements by the participant, the investigator, or the study site staff. It is the responsibility of the site PI to use continuous vigilance to identify and report all protocol deviations to the site IRB/IEC and to the sponsor. The PI is responsible for knowing and adhering to the site requirements. The Principal Investigator will

report all protocol deviations to IVI IRB and only major PD has to be reported to IVI IRB within 10 calendar days of their awareness.

The ICH guidelines also introduce a definition for "important" protocol deviations, defining them as "a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a participant's rights, safety, or well-being." Important, "major", "critical" and "significant" are synonyms when referring to important protocol deviations.

To align the sponsor terminology with that of the health authorities' i.e., Agency inspectors we are using the terminology major and minor instead of the classification of important and non-important protocol deviations.

Major deviations are defined as those that jeopardize the safety or rights of the participant or the scientific integrity of the study which is applicable to cases listed below.

- Violation of inclusion and exclusion criteria.
- Vaccination with wrong vaccine as defined in the protocol.
- Vaccination not following the vaccination schedule defined in protocol.
- Visit outside window for the immunogenicity assessment after discussion with the Study medical monitor.
- Missed samples for immunogenicity.
- Missing SAE report.
- Unblind-observer involved in the study subject evaluation.

Major protocol deviations thought to affect the scientific integrity of the study and/or the safety and rights of the participant will be reported and discussed with investigator, monitor, sponsor, and statistician for their exclusion from the per protocol analysis.

For minor protocol deviations considered not to affect the scientific integrity of the study, the extent of deviation or delay as well as reason will be accurately documented. The equivalent definition to minor protocol deviation would be, a non-important protocol deviation. However, there is no formal definition of a non-important protocol deviation in ICH. It follows that if a protocol deviation does not meet the criteria of important, it is non-important.

Protocol deviation documentation must include a description of the deviation (including at which visit the deviation occurred, if applicable), the cause, and the plan to correct and prevent such deviations from occurring in the future. The root cause identification analysis and the corrective and preventive actions are to be implemented promptly.

7.3 PROTOCOL AMENDMENTS

Any protocol amendments must be made only with the prior approval of the Sponsor and the amended version of the protocol will replace the earlier version. Agreement from the investigator must be obtained for all protocol amendments and amendments to the informed consent document. All amendments require IRB/IEC approval, and those amendments that affect the conduct of the trial or the safety of participants must also be forwarded to regulatory authorities according to local requirements. An administrative amendment to a protocol is one that modifies some administrative or logistical aspect of the trial but does not affect its design or objectives or have an impact on the participants' safety. Some Regulatory authorities need only be notified about administrative changes.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which IEC/IRB approval has already been given, are not initiated without IEC/IRB review and approval.

7.4 PREMATURE TERMINATION OR SUSPENSION OF STUDY

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. IVI and other regulatory authorities reserve the right to terminate the study. Each site PI will notify the respective site IRB/IEC of the study termination in writing and provide documentation to the IND Sponsor. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigator, the sponsor, the regulatory authorities, and IRB/IECs.
Circumstances that may warrant termination or suspension are:

- Determination of unexpected, significant, or unacceptable risk to participant as recommended by the PI
- Poor protocol compliance

Study may resume once concerns about safety, protocol compliance, data quality is addressed, resolved, and meeting the requirements from the sponsor, IRB/IECs and/or NRA.

7.5 END OF STUDY

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the SOE according to group allocation.

8. INVESTIGATIONAL PRODUCT AND CONTROL DESCRIPTION

8.1 IDENTITY OF THE INVESTIGATIONAL PRODUCT.

Code name: SchistoShield® (rSm-p80 [antigen] + GLA-SE [adjuvant formulation])

Manufacturer: rSm-p80 [antigen]released by PAI Life Sciences Inc. (Seattle, WA),
GLA-SE, manufactured and released by the Access to Advanced Health Institute (AAHI) (Seattle, WA)

Form: Liquid for IM injection.

Appearance: Appears as a translucent milky white liquid

Dose: Three adjuvanted formulations with varying antigen content (10µg, 30µg, or 100 µg rSm-p80, all with 5µg GLA-SE)

Batch number: To be determined (TBD)

Mode of Administration: Intramuscular

Storage Conditions: 2°C to 8°C

8.1.1 Composition

Table 3: Vial Contents for rSm-p80 for Injection

Content	Purpose	Concentration
rSm-p80 Protein	Vaccine Antigen	125 µg/vial
D-mannitol	Bulking Agent	5%
Sucrose	Stabilizer	0.4%
Polysorbate-80	Surfactant	0.1%
Tris	Buffer	50 mM, pH = 8.0

The lyophilization process cycle involves freezing at -40°C, followed by an annealing step; the primary drying step is at -20°C to sublime bulk water, followed by a secondary drying step at 25°C to remove bound

water. The final lyophilized product is referred to as "rSm-p80 for Injection." The antigen, rSm-p80 for Injection, will be formulated at 125 µg/vial with excipients as shown in Table 3 when reconstituted with 0.5mL of sterile WFI following lyophilization. It should form a clear, colorless liquid upon reconstitution.

Sterile Water for Injection

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

GLA-SE Adjuvant

GLA-SE Adjuvant is a stable oil-in-water emulsion containing glucopyranosyl lipid adjuvant A. The product should be milky-white liquid, and should show no signs of obvious contamination, foreign matter, damage, or other conditions that could affect quality. AAHI maintains a Type II Biologics Master File with the US FDA that contains specific information on the chemistry, manufacturing, and controls for GLA-SE adjuvant and emulsion formulations. cGMP adjuvant lots have been on a stability program for over five years and are stable for this period when properly stored at 2-8° C. Complete chemistry, manufacturing, and control information for the cGMP GLA-SE Adjuvant Formulation (AP10-201) is found in AAHI's Type II Master File BB-MF #13849. Glucopyranosyl Lipid Adjuvant (GLA) is a synthetic monophosphoryl lipid A (MPL)-like molecule, a TLR4 agonist.

MW: 1,762.311

MF: C96H184N3O22P

Type: Monophosphoryl lipid A-like

Form: Salt with ammonium counter ion

Table 4: Sample Composition of a Lot of GLA-SE Adjuvant

Component	Function	Quantity
Active		
Glucopyranosyl Lipid A	TLR4 Agonist	8 µg
Inactive		
Squalene	Oil	16 µL
Glycerin	Isotonicity	9.2 mg
D,L-α-Tocopherol (Vitamin E)	Anti-oxidant	0.08 mg
Egg phosphatidylcholine (L-α-lecithin)	Emulsifier/stabilizer	3.0 mg
Poloxamer 188	Emulsifier/stabilizer	0.14 mg
Ammonium phosphate, monobasic	Buffer	1.0 mg
Ammonium phosphate, dibasic	Buffer	0.068 mg
Sterile Water for Injection	Solvent	0.37 mL

8.1.2 Preparation and Administration

Refer to the investigator brochure (IB) and Pharmacy manual for information on study products preparation and use. Refer to the group assignment for the study participants.

Prior to administration, all study products must be inspected visually for cracks, broken seals, correct label content, and extraneous particulate matter and/or discoloration, whenever solution and container permit. If any of these conditions exists, the vaccine must not be administered. A replacement dose is to be used, and the event is to be reported to the Sponsor. If a vial or syringe is accidentally broken and the product spilled out, appropriate disinfection procedures must be used (refer to the MOP and/or site center's Procedures). Site staff should practice universal precautions and dispose of syringes in keeping with the site policy and practices.

A detailed operating manual will be developed to guide the preparation of the bedside vaccine mixtures for dose escalation and avoid errors. Training will be conducted with dummy product.

8.1.3 Dose Selection and Timing

This is a phase Ib trial to evaluate the safety, tolerability and immunological profile of the rSm-p80 + GLA-SE candidate schistosomiasis vaccine (SchistoShield®) in healthy adults.
A total of 60 participants aged 20-59 will be recruited in both Burkina Faso and Madagascar, with 120 participants in total. Participants will be evaluated in three cohorts in each study site: Cohort A) 10 µg rSm-p80 + 5 µg GLA-SE, Cohort B) 30 µg rSm-p80 + 5 µg GLA-SE and Cohort C) 100 µg rSm-p80 + 5 µg GLA-SE (Table 1). Each study cohort will include 20 participants randomized to receive either rSm-p80 product or placebo in a 3:1 ratio. All participants will receive three intramuscular injections of 0.5 mL of the designated study product / placebo, on Days 0, 28, and 56 (28 days apart).

Cohort A will receive the low-dose antigen formulation or placebo, Cohort B will receive the medium-dose antigen formulation or placebo, and Cohort C will receive the high-dose antigen formulation or placebo. The safety profile of any one formulation does not necessarily predict that of the others, therefore participants will be enrolled in a sequential dose-escalation manner, with safety review between each cohort's enrollment. After enrollment of participants in Cohort A, the SMC and site PIs will review blinded safety data of Cohort A collected through seven days after the second dose of study product injection and assess this data according to pre-specified criteria for halting dose escalation (Section 10.4.3). If halting criteria are not met, Cohort B participants will be enrolled. Dose escalation to Cohort C will follow the same procedures requiring SMC and Site PIs review. These safety data reviews and decision to progress to the next dose level will occur for each site separately. If dose escalation halting criteria *are* met at any point, the matter will be escalated to the DSMB, which will review the summarized safety data in an unblinded manner. The DSMB must provide their recommendation to the Sponsor for dose escalation before study product injection of the next cohort is initiated (Figure 1). The DSMB review is not required for dose escalation into the next cohort unless the halting criteria are met or the SMC requests for DSMB review.

8.2 IDENTITY OF CONTROL PRODUCT: PLACEBO

8.2.1 Composition

Sterile 0.9% sodium chloride will be used as placebo.

8.2.2 Preparation and Administration

[Refer to the MOP/Pharmacy Manual]

8.2.3 Dose Selection and Timing

Not applicable

8.3 PRODUCT LOGISTICS

8.3.1 Labeling and Packaging

The vaccine will be supplied in multi-dose vials and the placebo will be supplied in single dose vials and packaging. Labels on study products [rSm-p80 formulation or placebo] will have the following product information:

- Study code
- Name of product
- Route of injection
- Investigational use only statement ("For Clinical Trial Use Only").
- Storage conditions
- Batch #
- Name of Sponsor
- Expiry date, if applicable

8.3.2 Product Shipment, Storage, stability and Accountability

The logistics designee will contact the site PI or a designee to determine the dates and times of delivery of products.

8.3.2.1 Product Shipment

Vaccines must be kept in temperature-controlled environments at all times throughout the shipment process including in transit storage points/warehousing. Every vaccine shipment will include a temperature-monitoring device to verify maintenance of the cold chain during transit. On delivery of the product to the site, the delegated personnel in charge of product receipt must follow the instructions given in the

MOP/Pharmacy Manual, including checking that the cold chain was maintained during shipment (i.e., verification of the temperature recorders). If there is an indication that the cold chain was broken, the site designee personnel should immediately quarantine the product, alert the IVI, and request authorization from IVI to before using the product.

8.3.2.2 Product Storage & Stability

Study product will be shipped to the study pharmacy at the recommended temperature range using appropriate shipping configurations. At the site, products must be kept in a secure place with restricted access.

rSm-p80 for Injection

rSm-p80 for Injection, supplied as a lyophilized cake in glass vials with a protein content of 125 µg per vial must be stored between 2°C to 8°C; excursions up to 25°C are permitted for 4 hours.

Water for Injection

The sterile water for injection (WFI) vials are stored as per manufacturer's instructions provided in the package insert.

GLA-SE Adjuvant

GLA-SE adjuvant is provided as a stable oil-in-water emulsion (SE) and must be stored between 2°C to 8°C; excursions up to 25°C are permitted for 24 hours, but the product should never be frozen.

Placebo

Sterile 0.9% sodium chloride is supplied in glass vials and must be stored as per manufacturer's instructions provided in the package insert. Vaccines will be stored in a refrigerator at a temperature ranging from +2°C to +8°C. The vaccines must not be frozen.

The temperature must be monitored and documented for the entire time that the vaccine is at the trial site. In case of accidental freezing or disruption of the cold chain, vaccines must not be administered and must be quarantined, and the Investigator or authorized designee should contact sponsor for further instructions.

If deviations in storage temperature occur from the normal allowance for the pharmacy refrigerator/freezer, the site pharmacist or designee must quarantine affected product(s) and report the storage temperature excursion within 48 hours to the site PI and the IVI. The excursion must be evaluated and investigated, and action must be taken to restore and maintain the desired temperature limits. Pending the outcome of the investigation, the IND Sponsor will notify the site pharmacist or designee if clinical use of the affected product is acceptable, and the affected product may be removed from quarantine.

8.3.2.3 Product Accountability

Documentation

Each study site will be responsible for maintaining an accurate record of the treatment codes, inventory and an accountability record of the investigational study product supplies for this study.

Disposition

Empty vials and the unused portion of a vial must be verified by the monitor before being discarded in a biohazard container that will be incinerated or autoclaved in accordance with site policy. Any unopened vials that remain at the end of the study will be discarded at the discretion of the IND Sponsor in accordance with policies or guidance from the manufacturer that apply to investigational products (see details in pharmacy manual).

8.3.3 Product Preparation

The preparation and administration of the vaccines to participants enrolled into the study will only be done by the unblinded study personnel according to the procedures stipulated in this study protocol. The study personnel responsible for vaccine administration is qualified to perform this task and same will be documented by site investigator.

rSm-p80 will be appropriately formulated and mixed with diluted GLA-SE adjuvant. Once diluted, the rSm-p80 must be used within 24 hours. The rSm-p80 plus GLA-SE adjuvant admixture must be used within four hours of preparation. Both the diluted vaccine and vaccine plus adjuvant admixture will be held at 2ºC to 25ºC. The study product will be administered within 30 minutes after dispensing from temperature-controlled storage. For further details please refer to the MOP/Pharmacy manual.

8.3.4 Replacement Doses

In case a replacement dose is required (e.g., because the vial broke or particulate matter was observed in the vial), the site personnel must follow the instructions given in the MOP/Pharmacy Manual and contact the sponsor to get to obtain a new assigned product.

8.3.5 Disposal of Unused Products

Unused or wasted products will be either disposed of on the site in accordance with national regulations or returned to the Sponsor in accordance with the instructions in the MOP/Pharmacy Manual.

8.3.6 Recall of Products

In case the Sponsor makes a decision to launch a retrieval procedure, the Investigators will be informed of what needs to be done.

8.4 RANDOMIZATION AND ALLOCATION PROCEDURES

The randomization list will be generated by an independent statistician who is not directly involved in the study conduct. Eligible participants will be assigned to receive 1st dose of the study product. The randomization list will contain sequential numbers unique to each participant and the block randomization process will be employed to ensure an effective balance between the interventions. Only the independent statistician will have a complete set of randomization lists. The individual site lists will be kept under lock and key by the pharmacy staff at all clinical sites. At the end of the study after unblinding of the participants, the lists will be returned to the statistician.

Two types of randomization list, one with randomization number only, and the second with randomization number and vaccine allocation will be prepared. Participants in the study will be randomized. Randomization list “without the vaccine allocation” with numbers will only be shared with the blinded trial staff, for enrolling the trial participants and assigning them the randomization number. The randomization list “with the vaccine allocation” will be shared with the unblinded vaccine administrator (study nurse/).

Upon enrollment, in order to receive the study vaccine, participants will be sent to the vaccine administrator with their randomization number. The unblinded study nurse/pharmacist located in a different room will administer vaccine(s) to the participant according to the randomization list. The randomization number of the participant receiving the study vaccine will be written on the empty vaccine vial and on the vaccine accountability log for record and reconciliation.

Trial staff other than the unblinded study staff will remain blinded to vaccine administration. The unblinded study nurse/pharmacist staff will not be involved in the evaluation of vaccine safety and will not discuss with the investigator and clinical staff about vaccines administered.

8.5 BLINDING

The PI, study staff, and participants will be blinded as to receipt of study vaccine or placebo. The unblinded pharmacy staff preparing the study product syringes and the unblinded study nurse who is administering the product will not be involved in the safety assessment of participants and will be instructed not to comment on the experimental agent to study staff. For all participants, the volume of injection will be consistent at 0.5 mL. The unblinded site pharmacy staff and the unblinded study nurse must sign a confidentiality agreement not to discuss randomization codes or participant assignments.

The enrollment/randomization number with its treatment assignment is generated in advance by the independent study statistician. The randomization information is only available to the study nurse/pharmacist staff and independent statistician. Enrollment/Randomization numbers will be assigned sequentially by the study nurse/pharmacist upon confirmation of eligibility and enrollment into the study by the Investigator. All eCRFs and source documents will be labeled with the randomization number and study visit number. Personal identifying information linking the study number to an individual volunteer will not be captured on eCRF as study data.

8.6 UNBLINDING OF PARTICIPANTS

A request for unblinding, with its rationale, must be forwarded through the PI. The PI will evaluate the request and will notify the Study Medical Monitor (SMM). The SMM will evaluate the request and will advise the Sponsor regarding a course of action. The Sponsor will decide whether to approve the request for unblinding. In the case of the former, the Sponsor will authorize the independent statistician to provide this information to the PI. It should be noted that there are very few circumstances in which unblinding will be essential to the medical management of a vaccine (or placebo) recipient. In case of vaccine-related death or life threatening, serious adverse events (SAEs), knowledge of whether a participant received vaccine or placebo can be critical for the interpretation of the significance of clinical findings and thus impact decisions regarding continuation of study participation. In such cases, the assignment of a participant may be unblinded.

Episodes of unblinding, whether accidental or intentional, will be reported by the site investigator (either by email or fax) with an explanation to the sponsor who will in its turn inform IVI IRB. Apart from this, the Site PI is also responsible for informing their own IRB/IEC. Other participating IRB/IECs will be informed through Sponsor. Follow-up of such participants will continue throughout the duration of the trial. Study participants will be unblinded after the completion of the final analysis. A interim analysis will be undertaken after 4 weeks of last study product administration where both safety and immunogenicity will be assessed. The database will be locked for the final analysis after safety and immunogenicity data is cleaned and locked.

8.7 TREATMENT COMPLIANCE

In order to ensure that the vaccine doses administered comply with those planned, and that any non-compliance is documented so that it can be accounted for in the data analyses, the following measures must be applied:

- All vaccinations will be administered by qualified trial personnel.
- The person in charge of product management at the site will maintain accountability records of the product delivery to the trial site, product inventory at the site, dose(s) given to each participant, and the disposal of unused or wasted doses.

8.8 CONCOMITANT MEDICATION

Medications to be reported in the eCRF are concomitant prescription medications, over-the-counter medications and supplements. Only routine medications should be entered in the database at the time of study randomization. All approved vaccinations for routine health care must be entered in the database throughout the study, if any. Product administrations must be scheduled such that:

- No inactivated vaccine is received within 4 weeks before or after each product administration.
- No live attenuated vaccine is received within 4 weeks before or after each product administration.

Concomitant medications will be updated in the study database if there is an occurrence of an AE that requires expedited reporting or development of a new chronic medical condition that requires ongoing medical management. Otherwise, concomitant medications taken during the study, including those for Schistosomiasis infection, must be recorded in the participant study file.

9 LABORATORY PROCEDURES/EVALUATIONS

9.1 CLINICAL LABORATORY EVALUATIONS

The following clinical laboratory evaluations will be performed at the site with Clinical /Medical Laboratory according to the schedule specified in the Schedule of Events.

- Hematology [Complete blood count (CBC) with automated differential]: White blood cell (WBC) count, Red Blood Cell count (RBC), hemoglobin, and platelet count will be measured at screening and at visits specified in the SOE.
- Serum chemistry: alanine aminotransferase (ALT)/ aspartate aminotransferase (AST), Total Bilirubin (TB), Creatinine (CREAT), and C-reactive protein (CRP) will be measured at Screening. and at visits specified in the Schedule of Events
- Urinalysis: Urine samples will be tested by dipstick for blood, glucose and protein at Screening and at visits specified in the SOE.
- Serology: Antibodies to Hepatitis B surface antigen (HBsAg), Hepatitis C antibody and HIV antibody (HIV-1/HIV-2).
- Special tests: blood smear at screening and rapid diagnostic test prior to each dosing will be done for Malaria diagnosis. Urine filtration for detection of S. *haematobium*, and Stool examination using the microscopy (Kato-Katz) and the POC-CCA for detection S. *mansoni* will be performed at Screening. Stool examination by microscopy for the diagnosis of other soil-transmitted helminths will be done at screening. RT-PCR for the confirmation of S. *mansoni* and soil-transmitted helminth detection, will be done at screening visit or retrospectively. Schistosoma CAA will be measured retrospectively before each dose and 4 weeks after the third dose.
- Pregnancy Testing: For women of childbearing potential (WOCBP), a serum pregnancy test will be obtained at screening and a urine pregnancy test will be performed prior to any dosing. A negative result for urine β-HCG must be available prior to each administration of IP. If the β-HCG test is positive, indicating that the participant is pregnant prior to completing the specified dose regimen, then no further IP will be administered. Every attempt should be made to follow pregnant participants for the remainder of the study and to determine the outcome of the pregnancy.
- SARS-CoV-2 rapid antigen using naso-pharyngeal swab will be done at screening, in case of suspected symptoms/signs of COVID-19 before each vaccination, and at each unscheduled if deemed necessary by the study physician.

9.2 SPECIMEN PROCESSING, HANDLING, AND STORAGE

Venous blood will be collected from participants for screening biological parameters and immunogenicity assessments. For immunogenicity assessment, whole blood will be processed, and sera will be aliquoted and stored according to the standard operating procedures until shipment to central laboratory (when applicable)/ performance of immunogenicity assessment onsite. Pre-print study labels provided in advance will be attached on each of the serum aliquots.

Participants will be asked to provide consent for the use of samples for future testing or assay development specific to Schistosoma. Aliquots of all collected samples from this study may be retained for additional testing with antigens specific to Schistosoma for a maximum of 15 years after the Clinical Study Report completion, unless local rules, regulations, or guidelines require different timeframes or different procedures, in accord with participant consent.

9.3 SPECIMEN SHIPMENT

Shipments to the laboratories will be made only after appropriate monitoring and following notification of the Logistics Coordinator. Sera will be shipped frozen, using dry ice to maintain them in a frozen state, in the packaging container provided by the carrier. Temperatures will be monitored. Shipments must be compliant with the IATA (International Air Transport Association) regulations. Based upon the study specific requirement, a depot can be used. See the MOP for further details.

9.4 ASSESSMENT OF IMMUNOGENICITY

ELISA (anti - Sm-p80 IgG)

Vaccine-specific IgG antibody responses will be assessed by (ELISA using serum collected from participants (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 through 3 months (Days 0, 7, 28, 35, 56, 63, 84 and 224). aliquots. The goal will be to compare the serum IgG titers at each time point across Study Groups. . Titers of total IgG and IgG subclasses (IgG1-4) will be measured against the Sm-p80 protein. Endpoint titer will be calculated for each curve and duplicates will be averaged if within established variance limits.

ELISA (anti-schistosome and anti-Sm-p80 IgE)

While participants positive for schistosomiasis or Sm-p80-IgE-at baseline will not be excluded from the trial, it is important to understand their pre-vaccination sero-status. Indeed, allergic reactions to helminth vaccines have been observed in individuals with pre-existing vaccine-reactive IgE from natural exposure. Therefore, schistosoma-specific IgE antibody will be measured by enzyme-linked immunosorbent assay (ELISA) using serum collected from participants (blood collected in a serum separator tube) at day 0, 28 and 56 to evaluate the existing antibody levels in participants prior to each dose of vaccine. Similarly, vaccine-specific anti-Sm-p80-IgE will be assessed at baseline (pre-vaccination) and after vaccination to describe the kinetics and longevity of vaccine-induced IgE responses (Days 0, 7, 28, 35, 56, 63, 84 and 224). Method will be similar to IgG detection but using an HRPO-conjugated anti-human IgE as second stage to detect IgE responses.

Cytokine multiplex

Vaccine-specific cellular response may be assessed from PBMC's using a cytokine multiplex assay to assess T-helper type 1, 2, 17, Treg, and T-follicular helper representative cytokines (TNF, IFNγ, IL-2, IL-4, IL-5, IL-17A, IL-22, IL-21, and IL-10). Blood will be collected in a sodium heparin tube at specific time points selected to evaluate the kinetics of the response after each administration, peak response, and durability through 3 months (Days 0, 7, 28, 35, 56, and 63). This volume is sufficient to provide 1 specimen vial containing enough supernatant for analysis and repeat, if needed. The goal will be to characterize the cellular response elicited by vaccination and to compare the results from the different groups. To quantify antigen-specific cytokine secretion, PBMC's will be stimulated with Sm-p80, positive control (PHA), or left unstimulated. Supernatants will be harvested and frozen. Cytokine concentrations will be measured in the supernatant samples in batch at TTUHSC. Cytokine concentration from the unstimulated sample will be subtracted from the Sm-p80 samples to yield antigen-specific cytokine levels for each analyte.

T cell ELISpot

Vaccine-specific cellular responses may be evaluated using an IFN-γ ELISpot assay to enumerate antigen specific IFNγ-secreting cells. PBMCs may be collected from a subset of participants at baseline, prior to each vaccination on the day of vaccination, 7 days after each vaccination, 28 days and 168 days after the last vaccination (Days 0, 7, 28, 35, 56, 63, 84 and 224).

PBMC will be isolated and cryopreserved at specific time points selected to evaluate the kinetics of the response after each administration, peak response, and durability through 3 months. Approximately 17ml

(V2-V7) and 10ml (V8-V9) of blood is collected for the PBMC-based assays and will be sufficient for primary and backup samples for the analysis.

ASC B cell ELISpot

Vaccine-specific B cell responses may be assessed using ELISpot assays. PBMCs may be collected from a subset of participants at baseline, prior to each vaccination on the day of vaccination, 7 days after each vaccination, and 124 days after the last vaccination (Days 0, 7, 28, 35, 56, 63, 84 and 224). Enumeration of cytokine-secreting cells by IFNγ ELISpot (numbers of spot forming cells) will describe Sm-p80 specific cellular responses.

In addition, memory B cells will be expanded from PBMC and quantitated using this assay. The size of the memory B cell pool is quantified to indicate potential for durability using a memory B cell ELISpot assay. The ASC-response and memory B cell pools will be evaluated across Study Groups using PBMCs from specific time points selected to evaluate the kinetics of the response after each administration and peak response. 17ml blood are collected for all the PBMC-based assays and will be sufficient for ASC B cell ELISpot and memory B cell ELISpot.

RNA seq

Texas Tech University Health Sciences Center (TTUHSC) researchers in collaboration with Goeteborg Universitet (UofG) will use their acquired experience in systems biology approaches to explore schistosomiasis immune responses to explore molecules/signals that may play a role in the development of effective, adaptive schistosome immunity using an RNA Seq approach as a starting point for a broader systems biology approach. PBMCs transcriptional responses following vaccination will be studied. PBMC samples will be collected from a subset of participants after study product injections. Participant RNA will be subject to transcriptomics analysis. Differentially expressed genes compared to baseline will be identified by performing the moderate Student's t-test at each time point followed by further using the Benjamini-Hochberg method. Gene set enrichment analysis using the PBMC transcription modules and Ingenuity Pathway Analysis will be applied to the data to identify PBMC specific modules and functional pathways perturbed by vaccination with SchistoShield®. Subsequently, the correlation of enriched PBMC transcription modules in the transcriptome of participants following study product injection with the magnitude and quality of Sm-p80-specific antibody responses will be evaluated. This will provide information on early blood transcript signatures which may correlate with protective immunity to S. *mansoni* in humans.

***Schistosoma*-killing assay**

An in vitro worm killing assay will be performed using the sera collected from trial participants.[24,25] Antibody-Dependent Cell-Mediated killing of schistosomula in vitro will be tested using mechanically transformed schistosomula pre-treated with a volume of diluted antibody preparation and the resultant cultures incubated followed by enumeration of live and dead parasites. Efficiency of killing will be assessed by the difference between the percentage of dead schistosomula in the presence of active and inactivated complement.

10 ASSESSMENT OF SAFETY

10.1 SAFETY ASSESSMENT

The following procedures will be performed to monitor safety as listed in the SOE:

- **Demographic and medical history** (age, gender, baseline medical history of participants)
- **Physical examination** (height and weight, organ systems)
- **Vital signs** (body temperature, respiratory rate, pulse and blood pressure)
- **Diary card** will be used for participant for reporting outcomes.
- **Laboratory assessments** (hematology, biochemistry parameters and urine for routine urinalysis).

At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit.

10.1.1 Definition of Adverse Events (AE)

Adverse events (AE) are defined as any untoward medical occurrence which follows immunization, and which does not necessarily have a causal relationship with the administration of the vaccine. An AE may be any unfavorable or unintended sign, symptom, abnormal laboratory finding or disease.

Adverse Events include the following:

- Post-treatment complications that occur as a result of protocol mandated procedure after screening.
- Any pre-existing condition that increases in severity, or changes in nature during or as a consequence of the study product administration phase.
- Complications of pregnancy.

Adverse Events do not include the following:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion) performed; however, the condition that leads to the procedure is an AE.
- Pre-existing diseases or conditions or laboratory abnormalities present or detected before the Screening Visit that do not worsen.

- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions)
- Overdose without clinical sequelae
- Any medical condition or clinically significant laboratory abnormality with an onset date before the ICF is signed, is not an AE; it is considered to be pre-existing and will be documented on the medical history eCRF.
- Uncomplicated pregnancy
- An induced elective abortion to terminate a pregnancy without medical reason.

Solicited AEs are predetermined events, identified in the Investigator's Brochure, which may reflect safety concerns related to the investigational product. AEs that will be solicited by the participant and recorded in the diary card and reviewed by a blinded observer during the 7 days after each dose for this study include:

- Local reactions at the site of injection: Pain, tenderness, redness, swelling, pruritus and induration
- Systemic reactions: Fever, headache, nausea, vomiting, myalgia, arthralgia, malaise, dizziness, fatigue

Unsolicited AEs are all other adverse events (those that do not fall under the categories of solicited adverse reactions) that are identified by site staff, the PI and the Study Medical Monitor. These unsolicited AEs will be documented in the participant's clinic records and entered in the study eCRFs. They will be recorded during the 4 weeks after each dose.

Results will be expressed as frequency of the AEs and individual descriptions will be tabulated according to MedDRA organ class system and preferred term.

Adverse Event of Special Interest (AESI) are AEs that are considered by the sponsor to be relevant for the monitoring of the safety profile of the investigational vaccine.

AEs to be considered as AESI are listed in **Appendix B.**

10.1.2 Definition of Serious Adverse Events (SAE)

An AE or suspected adverse reaction is considered "serious" if at any dose (including overdose):

- Results in death,
- Is life-threatening[1] ,
- Requires inpatient hospitalization or prolongation of existing hospitalization[2]
- Results in persistent or significant disability/incapacity[3]
- Is a congenital anomaly/birth defect[4] ; or
- Is an important medical event that may jeopardize the participant or may require intervention to prevent one of the other outcomes listed above.

1. The term “life-threatening” refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
2. All medical events leading to hospitalizations will be recorded and reported as SAEs, with the exception of hospitalization planned before inclusion into the study or out-patient treatment with no hospitalization.
3. “Persistent or significant disability or incapacity” means that there is a substantial disruption of a person’s ability to carry out normal life functions.
4. Characteristic or abnormality existing at birth and found in the participant's offspring and not in the participant his/herself

Serious and severe are not synonymous. The term severe is often used to describe the intensity of a specific event as corresponding to Grade 3. This is not the same as serious which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient’s life or functioning. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations.

10.1.3 Definition of Unanticipated Problems (UP)

Non-serious UP: An UP that is not an Adverse Event (UP non-AE) is an unanticipated problem that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the participant, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, accidental destruction of study records or samples.

Serious Unanticipated problems include any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency of what is stated under adverse events in the protocol informed consent and Investigator's Brochure (IB).
- Related or possibly related to vaccination
- Suggests that participants or others will be at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Suspected unexpected serious adverse event (SUSAR) is defined as a serious adverse reaction whose nature or severity is not consistent with the applicable product information, Investigator's Brochure or the summary of product characteristics of an authorized product.

10.2 CLASSIFICATION OF AN ADVERSE EVENT

10.2.1 Severity of Event

All solicited AEs in the study will be graded for their severity and recorded in the eCRF as described in the tables below.

Table 5: Injection site reactogenicity grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade2)	Severe (Grade 3)
Pain — experienced without touching the injection site (spontaneous discomfort)	Subject is aware of pain b ut 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

Table 6: Injection site reactogenicity measurement

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 7: 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
Dizziness	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

Table 8: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever*	37.5 – 38.5°C	38.6 – 39.2°C	39.3 – 39.9°C

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

Signs and symptoms of unsolicited AEs will be recorded from the date of first vaccination until week 4 post vaccination for all participants.

All unsolicited adverse events observed by investigator and/or reported by participant after discussing with the investigator will be recorded in the eCRFs with their severity grading and relatedness to the study vaccine.

All SAEs irrespective of their causal association will also be graded for their severity.

10.2.2 Relationship to Investigational Product

For all collected AEs, the clinician who examines and evaluates the participant will determine the relationship of each AE with the investigational product based on plausible biologic mechanism, temporal relationship of occurrence after administration of the investigational product, identification of possible alternative etiologies including underlying disease, concurrent illness or concomitant medication, and his/her clinical judgment. The relationship of vaccination to AE will be determined based on the definitions below.

Not related: The AE is clearly or most probably caused by other etiologies such as participant's underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the AE is incompatible with a causal relationship; or the AE started before the first vaccination.

Related: There is a "reasonable possibility" that the AE was caused by the vaccine, meaning that there is evidence or arguments to suggest a causal relationship.

The same definitions are used for assessing the relationship between vaccination and SAEs.

Every site should follow its local regulatory authority guidance in case additional causality assessment categories are requested by the local regulatory authority.

10.2.3 Expectedness

The Study Medical Monitor in consultation with site principal investigator will be responsible for determining whether an AE is expected or unexpected. An Adverse Reaction will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

10.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

All participants will be observed for immediate local and systemic reactions for 60 minutes after each vaccination. For 7 consecutive days (Days 0-7) after each dose of study vaccine, the participant will be asked to record solicited local and systemic symptoms in the diary card. The study staff will remind participant of the importance of properly filling the diary card and to return the card at the next scheduled study visit. If they did not fill up or lost their card, participant will be interviewed for recall of symptoms with trained study staff during clinic visit on Day 8 after each vaccination.
For 28 consecutive days (Days 0-28) after each dose of study vaccine, unsolicited local and systemic symptoms will be recorded in the diary card.
The occurrence of an adverse event (AE) will come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (eCRF). Information to be collected includes event description, time of onset, symptoms and physical examination findings, clinician's assessment of severity, relationship to study product (assessed by the PI), medications given and time of resolution/stabilization of the event. All AEs occurring while on study will be documented appropriately regardless of relationship.

Any medical condition that is present at screening will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates or if frequency of intermittent condition increases at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The investigator will record all reportable events with start dates occurring any time after informed consent is obtained until the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

10.4 HALTING RULES

10.4.1 Study Halting Criteria

Further enrollment and study product administrations in study cohorts will be halted for DSMB review and discussion of all appropriate safety data if one or more participants in any treatment group experience any of the following adverse events 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 participant experiences ulceration, abscess, or necrosis at the injection site considered related to study product administration, 28 days post each vaccination.
- Any participant experiences laryngospasm, bronchospasm, or other anaphylaxis within 24 hours after administration of study product that is considered related to study product.
- Two or more participants 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 participants discontinue study product injections due to adverse events related to the study product.
- Three or more participants develop similar Grade 3 clinical or laboratory adverse event for longer than 24 hours related to the study product.
- Any participant develops a Potential Immune-Mediated Medical Conditions (PIMMC) considered related to study product.

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

- Two or more participants experience similar Grade 3 study product-related injection site reaction (excluding the size of redness and swelling/induration).
- Two or more of participants experience the same Grade 3 study product-related subjective systemic reaction, for which the severity (grade) is corroborated by study personnel.
- Two or more of participants experience the same Grade 3 study product-related quantitative systemic reaction.
- Two or more of participants 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 10.2.1 Severity of Event Grading scales for clinical safety laboratory AEs are included in Appendix A, Toxicity Tables. If any of the 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 DSMB to proceed. IVI, the study sponsor, retains the authority to suspend additional enrollment and administration of study product during the entire trial, as applicable.

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

10.4.2 Individual Halting Criteria

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

- If any participant meets any ineligibility criteria mentioned in section 7.2.7 study product injections will be discontinued.
- The participant has a systemic hypersensitivity reaction following a study product injection that is judged related to the study product.

- The participant has ulceration, abscess, or necrosis at the injection site related to study product administration.
- Grade 3 solicited or unsolicited AE, other than an injection site reaction, that occurs without alternative etiology following any study product injection.
- An unresolved or continuing Grade 3 injection site reaction, or an unresolved or continuing Grade 1 or Grade 2 adverse event, is permissible unless, in the opinion of the site principal investigator or appropriate sub-investigator or SMC, it would render study product injection unsafe or interfere with the evaluation of responses.
- Grade 3 clinical safety laboratory value (according to the Toxicity Tables Appendix A) 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 at the central (clinical) laboratory prior to the subsequent study product injection. If the clinical safety laboratory value decreases to Grade 2 or less, the participant 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.

10.4.3 Dose Escalation Halting Criteria

Participants are scheduled to receive three injections of the assigned study product, given 28 days apart. Dose escalation will proceed as described in Section 1.2.

If one or more dose escalation stopping rules are met, the DSMB will be convened to review the safety information and make recommendation whether dose escalation should proceed to enrollment of next cohort with higher dose.

- Any participant develops a Grade 3 solicited or unsolicited systemic AE or a Grade 3 clinical laboratory AE that is judged related to the study product, persisting at Grade 3 for more than 48 hours following each vaccine dose.
- Three or more participants assigned to same dose develop the same Grade 3 solicited injection site AE related to the study product, persisting at Grade 3 for more than 48 hours following each vaccine dose.

- Three or more participants assigned to same dose 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, persisting at Grade 2 for more than 48 hours following each vaccine dose.
- Any participant has an event meeting an individual halting criterion.
- There is no immunogenicity halting criteria for dose escalation.

10.5 REPORTING PROCEDURES

10.5.1 Adverse Event Recording and Reporting

Adverse events, solicited AEs, and SAEs will be assessed at all study visits, documented in the source record, and recorded in the eCRF using accepted medical terms and/or the diagnosis that accurately characterize the event. When the diagnosis is known the AE term recorded in the eCRF will be the diagnosis rather than constellation of symptoms. The PI will assess all AEs for seriousness, relationship to investigational product, severity, and other possible causes.

The timeframe for the collection of AEs and SAEs begins at the first administration of investigational product through to the end of the trial. The adverse events occurring in this study will be collected and recorded in the source document and eCRF according to the MOP.

When an AE has not resolved by the next visit it will be documented in the eCRF as ongoing. Documentation will include date of onset, detailed description of the event and relevant history and physical examination, severity, attribution of the AE, treatment given and date the AE improved or resolved. The medical monitor will review the AEs reported regularly and clarify with PI if there are queries. The data manager will review all AEs for consistency and provide summary of AEs to the study medical monitor periodically. Non-clinically significant AEs still ongoing as the end of the study will be listed as continuing. SAEs continuing at the end of the study will be followed to resolution or stabilization. Details of AE reporting are included in the MOP.

The PI, sub-investigators, and site staff will exercise due diligence in ascertaining, accurately recording and promptly entering data on the eCRF for all AEs of all study participants. As data becomes available from the participant, the clinic and laboratories, adverse events should be recorded and entered by the site staff

on regular basis. site investigators will review, in a timely manner, the AE source data and determine the severity of the event and relation to the study agent. Site investigators will contact the study medical monitor for consultation of AEs as required.

10.5.2 Serious Adverse Event Reporting

The site PI will complete a SAE Form within the following time frame:
All SAE will be recorded on the SAE Form and submitted to the sponsor within 24 hours of initial receipt of the information (weekends and holidays are not included) and addressed to:

Birkneh Tadesse
Project Technical Lead
International Vaccine Institute
SNU Research Park, San 4-8, Nakseongdae-dong, Gwanak-gu, 08823 Seoul 151-919 Korea,
And
Dr. Mohamadou Siribie (For Madagascar)
Study Medical Monitor
International Vaccine Institute
SNU Research Park, San 4-8, Nakseongdae-dong, Gwanak-gu, 08823 Seoul 151-919 Korea,
And
Dr. Asma Binte Aziz (For Burkina Faso)
Study Medical Monitor
International Vaccine Institute
SNU Research Park, San 4-8, Nakseongdae-dong, Gwanak-gu, 08823 Seoul 151-919 Korea,

All serious adverse events (SAEs) should be reported immediately to the sponsor. The immediate reports should be followed promptly by detailed, written reports. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subjects rather than by the subjects' names, personal identification numbers, and/or addresses. The investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the national regulatory authority in Burkina Faso, national pharmacovigilance center in Madagascar and the local IECs in Burkina Faso and Madagascar.

All information (which may include special investigations and treatment received) will be recorded on the SAE form and submitted to NRA and site IRB/IEC. All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or to be stable. Other supporting documentation of the event may be requested by the sponsor and should be provided as soon as possible. SAE reporting to IVI IRB is not mandatory and will be reported with the annual renewal report.

- The sponsor will be responsible for notifying the IVI IRB of Suspected Unexpected Serious Adverse Reactions (SUSAR) within 24 hours of initial receipt of the information (weekends and holidays are not included).

The SUSAR report will include the following information:

- Protocol information: protocol number and date,
- A detailed description of the event, incident, experience, or outcome,
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an unexpected problem.

10.5.3 Safety Oversight

An internal **SMC** will be responsible to oversee the vaccine safety patterns during the course of the clinical trial. The SMC will be composed of individuals with appropriate expertise. The SMC will review blinded safety data on a regular basis according to the guidelines of the SMC charter. The SMC will send a summary of safety review findings to the site PIs and the Study Medical Monitor.

An independent **DSMB** will be constituted of experts from various fields of Medicine external to sponsor's organization. The DSMB will oversee the study in terms of safety data as per DSMB charter. DSMB will provide recommendation to sponsor regarding halting or termination of the study in case of any safety signals. DSMB chair will issue a recommendation letter after each meeting.

11 STUDY MONITORING AND AUDITING

Study monitoring and auditing will be performed in accordance with the sponsor's procedures, GCP guidelines and any other applicable regulatory requirements. Clinical Monitoring plan (CMP) and monitoring procedures developed by Sponsor or representative will be followed in order to comply with GCP guidelines and to ensure acceptability of stud data by regulatory authorities.

Upon successful ethical and regulatory approval of the protocol and establishment of the Regulatory File, the Investigators and the Sponsor's team or a representative will meet at the site-initiation visit to discuss the trial protocol and the detailed trial procedures. Emphasis will be placed on inclusion and exclusion criteria, visit timing, vaccination and follow up schedules, safety procedures, informed consent procedures, SAE reporting procedures, diary cards requirements, CRF and eCRF completion requirements, and the handling of blood samples and products. Investigators and/or their study staff will be trained on the study protocol and all applicable study procedures before the inclusion of the first participant in the center.

During the study execution, the IVI study team or representative (e.g., a contract research organization) as frequently as necessary will maintain regular correspondence with the site and will perform the monitoring activities to the study progress as Clinical monitoring plan (but not limited to);

- To ensure that the investigator and the study staff understand and accept their defined responsibilities,
- to ensure the rights and well-being of study participants are protected; to verify adequate, accurate and complete data collection; protocol compliance and to determine that the study is being conducted in conformance with applicable regulatory requirements,
- To verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records
- To verify the data entered into the CRFs with the hospital or clinic records (source documents). The nature and location of all source documents will be identified. Monitor must get the direct access to source documentation (medical records) for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. If electronic records are maintained at the investigational site, the method of verification must be discussed with the investigational staff.

- Investigator must ensure that all clinical and research records must be available for review by the sponsor's representative, local IRB/IEC representatives, and other regulatory agencies as part of their responsibilities for insuring the protection of research participants.

Findings from the review of CRFs and source documents will be discussed with the investigational staff and must documented in visit report and follow up letter. The IVI study team expects that, during monitoring visits, the relevant investigational staff will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor should meet with the investigator on a regular basis during the study to provide feedback on the study conduct.

At the end of the trial, a close-out visit will be performed to ensure that:

- The center has all the documents necessary for archiving.
- All samples have been shipped to the appropriate laboratories.
- All unused materials and products have been either destroyed or returned to the Sponsor.

11.1 QUALITY ASSURANCE AND QUALITY CONTROL

Quality Assurance (QA) oversight will be required at all stages of the trial process per ICH E6 (R2) and/or local government GCP requirements.

Quality Control (QC) procedures will be implemented beginning with the data entry system and data QC checks. Investigator and Sponsor or its designee (e.g., CRO) will ensure the periodic review activities (i.e., QC checks) to ensure that the protocol, Good Clinical Practices and local regulatory requirements are being followed. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

During study conduct, the Sponsor or its designee (e.g., CRO) will conduct periodic monitoring visits (i.e., QC checks) to ensure that the protocol, Good Clinical Practice, local regulatory requirements and sponsor's controlled documents (e.g., Standard Operating Procedure) are being followed. The monitors will review source documents to confirm that the data recorded on CRFs/eCRFs are accurate.

In addition to on-going QA oversight, selected investigator sites will be subjected to quality assurance audits performed by the sponsor or its designee, and/or by inspection by regulatory authorities and/or notified bodies/notified health authorities.

The investigational sites will provide direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor; inspection by local and regulatory authorities and/or notified bodies.

12 STATISTICAL CONSIDERATIONS

12.1 STUDY END POINTS

12.1.1 Primary Endpoints

- Proportion of participants with of any Serious Adverse Events (SAEs)/ adverse events of special interest (AESI) from the time of the first study vaccination through the final study visit.
- Proportion of participants with immediate adverse events (reactogenicity events) within 60 min from the time of each study vaccination
- Proportion of participants with solicited local and solicited systemic AEs as measured for 7 days (inclusive) following immunization with the three different dose formulations.
- Proportion of participants with unsolicited AEs from the time of vaccination until 28 days post immunization with the three different dose formulations.
- Proportion of participants with clinical safety laboratory adverse events measured at 7 days and 28 days after each study vaccination.

12.1.2 Secondary Endpoints

- For Sm-p80 IgG antibodies, seroconversion rate at approximately 4 weeks (28 days) after each dose of study vaccination as compared to baseline

  (Seroconversion defined as 4-fold rise in Sm-p80-specific total IgG antibodies after investigational product administration compared to baseline (D0))

- For Sm-p80 IgG antibodies, seroconversion rate at approximately 24 weeks after third dose of study vaccination as compared to baseline
- Geometric Mean Titers (GMTs) of serum Sm-p80 IgG antibodies at approximately 4 weeks after each dose of study vaccination.
- Geometric Mean Titers (GMTs) of serum Sm-p80 IgG antibodies at approximately 24 weeks after third dose of study vaccination.

12.2.3 Exploratory Endpoints

- For Sm-p80-specific cellular responses, enumeration of cytokine-secreting cells by IFNγ ELISpot (numbers of spot-forming cells) at specified time points
- For Sm-p80 IgE antibodies, seroconversion rate at 4 weeks (28 days) after each dose of study vaccination as compared to baseline
- Innate and adaptive immune signatures depicting the changes in a sub-set of immunized participants using PBMC samples collected at specified time points as measured by RNA seq and quantification of cytokines.
- T-cell cytokine and chemokine responses from PBMC samples collected at specified time points in a subset of participants.
- Enumeration of antibody-secreting and memory B cells from PBMC samples collected at specified time points in a subset of participants.
- Enumeration of the ability of Sm-p80-specific antibodies from human participants to kill schistosome larvae in vitro (schistosomule-killing assay)
- Identification of immune signature markers identified by RNA Seq that are related to protective efficacy in animal passive transfer studies to identify markers that can be used for future down-selection of other schistosome vaccine products
- Determination of the changes in the circulating anodic antigen (CAA) levels before vaccination as compared with the levels 4 weeks after the 3rd dose of vaccination.

12.2 SAMPLE SIZE

A total of 120 participants will be enrolled in the study. The sample sizes in each study site are distributed by study cohorts and treatment arms. In each cohort, randomization will be performed in 3:1 allocation with 15 participants in the rSm-p80 + GLA-SE arm and 5 participants in the placebo arm.

Cohort A with 20 participants (10 µg Ag/5 µg Adjuvant, 15 in rSm-p80 + GLA-SE group and 5 in placebo)
Cohort B with 20 participants (30 µg Ag/5 µg Adjuvant, 15 in rSm-p80 + GLA-SE group and 5 in placebo)
Cohort C with 20 participants (100 µg Ag/5 µg Adjuvant, 15 in rSm-p80 + GLA-SE group and 5 in placebo)

No formal power analysis is applicable to this study, as descriptive statistics will be used to summarize the data. A sample size of 30 participants who received a low, medium, or high antigen-dose formulation of the study product will provide 95% confidence that the true incidence of SAEs is <12%, if no SAE are observed in those dose cohorts. A sample size of 90 participants who received the candidate vaccine, irrespective of antigen-dose formulation, among all cohorts will provide 95% confidence that the true incidence of SAEs is <5%, if no SAEs are observed.

12.3 STATISTICAL ANALYSIS PLAN

This is a Phase 1b, multicenter, randomized, placebo-controlled, observer-blinded, dose escalation study to evaluate the safety, tolerability, and immunogenicity of the rSm-p80 + GLA-SE (SchistoShield®) candidate vaccine in healthy adults in Burkina Faso and Madagascar.

In order to obtain a preliminary assessment of the safety and immunogenicity of the IP without waiting until the end of the trial, interim analysis will be conducted independently at each site after the last participant completes Visit 8 (i.e., 4-week follow-up visit post last study IP administration). The study personnel will remain blinded until the database is locked, except for those who will perform the interim analysis. These individuals will not be involved in the communication and the decision-making process after unblinding. In addition, an independent statistician will support the interim analysis process. Additional details such as the designated personnel and level of unblinding will be described in the data integrity document supporting interim analysis.

The database will be locked for final analysis when all participants have completed approximately 32 weeks of enrollment, and after safety and immunogenicity data are cleaned, prior to database lock.

Immunogenicity and safety data up to week 32 will be included in final analysis. Additional statistical analysis details will be described in the statistical analysis plan (SAP) and any deviation(s) from the original SAP will be described and justified in the final study report.

12.4 STATISTICAL HYPOTHESES

No formal statistical hypothesis is not tested in this study.

12.5 ANALYSIS DATASETS

The safety analysis set (SAF) includes all participants who receive at least one dose of the SmP80+GLA-SE formulation or Placebo. Participants for the safety analysis will be grouped in accordance with the dose of rSm-p80 + GLA-SE that they received. All safety analyses are based on the SAF.

The Full Analysis Set (FAS) is a modified intention to treat (mITT) analysis set includes all participants who receive at least one dose of rSm-p80 + GLA-SE or Placebo and have at least one post baseline immunogenicity data available. The FAS will be used for the secondary and exploratory immunogenicity endpoints.

The per-protocol analysis set (PPS) is comprised of subset of the FAS who receive all their planned administrations and who have no SMM-assessed important protocol deviations. Analyses on the PPS will be considered supportive of the corresponding immunogenicity analyses. Participants excluded from the PPS will be identified and documented prior to locking the study database. A sensitivity analysis using the PPS will be conducted for the primary and exploratory immunogenicity endpoints.

12.6 DESCRIPTION OF STATISTICAL METHODS

12.6.1 General Approach

The primary objective of the study is to evaluate tolerability and safety of rSm-p80+GLA-SE administered intramuscularly in healthy adults.

Unless otherwise specified, standard descriptive statistics will be computed for all endpoints and other observed values. The standard descriptive statistics for continuous variables include number of observations analyzed, mean, standard deviation, median, minimum, and maximum. The standard descriptive statistics for categorical variables include frequency distribution with the number and percentage of participants included in each category.

The binary outcome measures of safety and immunogenicity will be summarized with frequency, proportion and associated 95% confidence interval. The continuous outcome measures of immunogenicity will be summarized with geometric mean and associated 95% confidence interval. Missing immunogenicity data will not be imputed for the analysis. Missing data will not be imputed or replaced, and calculations will be based reported values.

12.6.2 Baseline Descriptive Statistics

Demographic characteristics and other baseline data of participants enrolled and randomized will be tabulated by study cohort and study arm for the participants in the PPS and FAS. Continuous variables (i.e., age, height and weight) and categorical variables (i.e., sex) will be summarized by standard descriptive statistics.

12.6.3 Primary Endpoint Analysis

The primary endpoint analyses are safety analyses of treatment emergent adverse events (TEAEs). TEAEs are defined for this study as any following AEs/AESIs/SAEs that occur from the time of the first study vaccination of rSm-p80+GLA-SE or placebo :

- SAEs and AESIs from the time of the first study vaccination through the final study visit
- Immediate AEs (Reactogenicity Events) within 60 minutes from the time of each study vaccination
- Solicited AEs (injection site and systemic reactogenicity events) from the time of each study vaccination through 7 days (inclusive) after each study vaccination
- Unsolicited AEs from the time of each study vaccination through 4 weeks (28 days) after each study vaccination
- Clinical safety laboratory AEs (significant laboratory abnormality) at 1 week (7 days) and 4 weeks (28 days) after each study vaccination.

All TEAEs will be summarized by frequency, percentage and associated 95% confidence interval by study cohort and study arm. The frequencies will also be presented separately by dose regimen and will be depicted by system organ class and preferred term. Additional frequencies will be presented with respect to maximum severity and relationship to study product.

All of these primary endpoint analyses will be conducted on the participants in the Safety Analysis Set (SAS).

12.6.4 Secondary Endpoints Analysis

The secondary endpoint analyses are immunogenicity analyses of anti-Sm-p80 IgG antibody levels measured at 4 weeks (28 days) after each dose of study vaccination and at 24 weeks (168 days) after the third dose of study vaccination. These analyses will be summarized descriptively by study cohort and study arm using seroconversion rates and associated 95% confidence interval. The GMT and 95% confidence interval of Sm-p80 IgG response at 4 weeks (28 days) after each dose of study vaccination and at 24 weeks (168 days) after the third dose of study vaccination will be summarized descriptively by study cohort and study arm.

12.6.5 Adherence and Retention Analyses

Summaries of Participants Disposition will be based on all participants who provide informed consent in the study. A flow diagram of participant disposition (CONSORT flow diagram) will illustrate the progress of participants through the study duration from initial screening for eligibility to the completion of the final primary outcome assessment. Number and percentage by vaccine group will be given for participants in the SAS, FAS and PPS, and reasons for study discontinuation.

12.6.6 Planned Interim Analysis (if applicable)

An interim analysis consisting of both group information supporting safety endpoints and in support of immunogenicity endpoints, will be conducted after the completion of the visit 8 (D84 ±7) inclusive of all final participant (i.e., four weeks post-third vaccination dose) for each site where both safety and immunogenicity data are made available.

The final analysis will be performed once all participants have completed the last study visit, (i.e., visit 9 (D224 ±14)) post database is locked.

12.6.7 Additional Sub-Group Analysis

The subgroup analysis will be carried out to assess the difference/consistency of the immunogenicity and safety across subgroups. The potential difference in safety and immunogenicity by age groups (20 to <45 years old, ≥45 years old), and gender (female, male) will be assessed.

12.6.8 Multiple Comparison/Multiplicity

No adjustment for multiplicity will be made for this study as it is not applicable.

12.6.9 Exploratory Endpoints Analyses

Antigen specific cellular immune responses by IFNγ ELISpot, innate and adaptive immune signatures, and T and B cell response will be analyzed descriptively by study cohort and study arm using the geometric mean and associated 95% confidence intervals.
Exploratory endpoint analysis will be conducted on sub-group.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DOCUMENTS

Data recorded on the eCRF will be verified by checking the eCRF entries against source documents (i.e., all original records, laboratory reports, medical records, participants diaries) in order to ensure data completeness and accuracy as required by study protocol. Source documents will be stored at the clinical site in a secured place under lock and key. The investigator and/or site staff must make eCRFs and source documents of participants enrolled in this study available for inspection by IVI representative at the time of each monitoring visit.

At a minimum, source documentation must be available to substantiate participant identification, eligibility and participation, proper informed consent procedures, dates of visits, adherence to protocol procedures, adequate reporting and follow-up of adverse events, administration of concomitant medication, study vaccine receipt/administration/return records, study vaccine administration information, and date of completion and reason. Specific items required as source documents will be reviewed with the investigator before the study.

The source documents must also be available for inspection, verification and copying, as required by regulations, by officials of the regulatory health authorities (e.g., NRA, others) and/or IRBs/IECs and for

audit by IVI Quality Management and funding agency (e.g., European Commission H2020). The investigator and study site staff must comply with applicable privacy, data protection and medical confidentiality laws for use and disclosure of information related to the study and enrolled participants.

The participant must also allow access to his/her medical records. Each participant should be informed of this prior to the start of the study by administration of the informed consent process per ICH E6 (R2).

Each participant will have a complete source documentation of records including study logbooks, ICF, lab reports and test results for the entire study period. Appropriate source documents will be prepared by study staff. These records must be available to the IVI and regulatory authorities upon request for review.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

An Electronic Data Capture (EDC) system will be used for recording eCRF data for each participant enrolled in the study. The Principal Investigator is responsible to ensure the accuracy, completeness, legibility and timeliness of the eCRF data captured in EDC. The captured eCRF data derived from source documents will be consistent with the source documents. In case of discrepancies, data will be clarified and corrected. The IVI will provide guidance to investigator on making corrections to the eCRF.

Study staff will extract all data collected in source documents and workbooks for computerization into the EDC. Data will be entered into the EDC system specially created for the study where an internet connection is stable. The EDC system must be compliant with industry regulations such as 21 CFR Part 11, EMA Annex 11, GAMP, etc. The entire data collection and handling will be monitored through the implementation of individual credentials to maintain appropriate database access and ensure database integrity. Edit checks will be programmed in the EDC system to identified data entry errors during transcription, including range and consistency checks wherever applicable.

All sequential changes made will be captured in audit trail in the EDC system which will also provide error reports and summary reports for each activity. The data will be automatically stored onto IVI server. Data security for this EDC system will be managed and ensured by IVI. Data entry and cleaning will be conducted at the sites. Final data cleaning, data freezing/database lock and data analysis will be performed at the IVI. Unblinding of study vaccines will be carried out after the database lock for the final analysis.

14.2 STUDY RECORDS RETENTION

The site PI will retain all study records that support eCRFs for this study (ie., ICFs, source documents, IP dispensing records) required by a sponsor and by the applicable regulations in a secure and safe facility. The PI will consult IVI representative before a disposal of any study records and will notify the sponsor of any change in the location, disposition, or custody of the study files. These documents should be retained for equal or more than 3 years after the approval of a marketing application or at least 2 years has been

elapsed since the formal discontinuation of clinical development of the investigational product. (ICH E6 (R2)). IVI will inform the site PI when these documents no longer need to be retained (ICH E6 (R2)).

If a site PI retires, relocates or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a qualified person who will accept this responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. The PI will be responsible for retaining sufficient information about each participant, i.e., name, address, telephone number, and participant identifier in the study, so that the sponsor and/or other regulatory authorities may have access to this information should the need arise.

If site express its inability to retain those study documents beyond certain time period due to space constraints or due to an unanticipated event, then IVI will take the help of external archiving vendor.

14.3 PUBLICATION AND DATA SHARING POLICY

IVI assures that the key design elements of this protocol will be posted in a publicly accessible database such as Clinicaltrials.gov. All data collected during this study will be used to support this vaccine development plan until licensure and WHO prequalification. All individual data will stay strictly confidential. Analyzed data may be presented in scientific conferences and published in peer-reviewed scientific journals with a priority given to open access publications to ensure maximum accessibility to the scientific and public health community. Anyone wishing to publish or present data obtained during and/or after completion of the study will conform to study site policies and then forward the publication for review and approval to IVI and manufacturer.

15 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

15.1 REGULATORY AND ETHICAL COMPLIANCE

The investigators will ensure that this study is conducted in full conformity with the ICH E6 GCP Guidelines, Council for International Organizations of Medical Science (CIOMS), local country's ethical policy statement or the Declaration of Helsinki, whichever provides the most protection to human participants. Sponsor will ensure the availability of Clinical Trial Insurance Policy to cover the risks to participants and/or any other eventualities pertaining to study.

This study will be undertaken only after IRB/IEC and the regulatory authority have given full approval of the final protocol, amendments (if any), the informed consent form, subject diary card, applicable recruiting materials, and subject compensation programs, and the sponsor has received a copy of this approval.

In Burkina Faso, the protocol will be submitted to the following ethics committees and regulatory authority:

Comité d'éthique pour la recherche en santé au Burkina Faso (CERS)
Under the authority of the Ministry of Health and Public Hygiene and the Ministry of Higher Education, Research and Innovation in Burkina Faso.
01 P.O. BOX 7009, Ouagadougou, Burkina Faso

Agence Nationale de la Regulation Pharmaceutique (ANRP) the National Regulatory Authority
under the authority of the Ministry of Health and Public Hygiene in Burkina Faso.
01 P.O. BOX 7009, Ouagadougou, Burkina Faso
Web site: *www.anrp.bf*

In Madagascar, the study protocol will be submitted to:

Comité d'éthique pour la Recherche Biomédicale à Madagascar (CERBM)
Under the authorithy of the Ministry of Public Health of Madagascar,
Address: Agence du Médicament de Madagascar, Ministère de la Santé Publique, 8 Rue Karija TSARALALANA ANTANANARIVO 101

15.2 PARTICIPANT AND DATA CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB or pharmaceutical company supplying study product 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 participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical site and by IVI Data Management will be secured and password protected.

No personal identifier will be used in any publication or communication used to support this research study. The participant's identification number will be used in the event it becomes necessary to identify data specific to a single participant.

15.3. RESEARCH USE OF STORED HUMAN SAMPLES OR SPECIMENS

Intended Use: Samples and data collected under this protocol may be used to study immune responses to the vaccines administered and for safety purpose if deemed necessary per medical judgement of the PI or special request from the sponsor or IRB/IECs. No genetic testing will be performed.

Storage: Samples and data will be stored at Site and VASA consortium partner labs using codes assigned by the investigators. Data will be kept in password-protected computers. Investigators will have access to the stored samples only when proper records are made to keep sample chain of custody intact.

Disposition at the completion of the study: All stored samples will be kept at Site laboratory for long term storage. Study participants who request destruction of samples will be notified of compliance with such request and all supporting details will be maintained for tracking.

15.4 FUTURE USE OF STORED SPECIMENS

With the participant's approval and as approved by Site and IVI IRB, the identified biological samples will be stored in VASA consortium partner labs for future use. The lab will be attributed a code that will allow linking the biological specimens with the phenotypic and clinical data from each participant, maintaining the masking of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to bio-sample storage will not be possible after the study is completed.

The stored samples may be used for additional assessment of immunogenicity, study of possible immune correlates of protection, validation of assays, testing of new assays, and for safety purpose, depending on the consent status of the samples for future research.

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17 AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date Issued	Authors of Changes	Summary of Changes Made
Initial release	1.0	07/NOV/2022	NA	NA
1	2.0	16/JAN/2023	FM, BT, MS, TB, AA, TG, HS, ELP, SBS & RR	• Correction of Typo errors • Updated IVI, GRAS and UofA personnel list • Updated participant age group, Updated Enrollment/ Vaccination Rule, exclusion criteria, schedule of event table, Immunogenicity blood volume & IP storage conditions • Updated section 3.3, 3.4, 5.0, 7.1, 7.2, 8.2, 8.3, 9.1, 10.5, 11.1, 12.6, 13 & 15.1.
2	3.0	26JUN2023	FM, BT, MS, TB, AA, TG. HS, ELP, RR, SBS	• Correction of Typo errors. • Update of the SoE with change in the blood volume for immunogenicity assessment. • Change of the adjuvant manufacturer from IDRI to AAHI. • Update of the exclusion criteria (6, 8 and 13). • Update of the section 3.3.2, 5, 7.1.3.2, 7.1.3.3, 7.2.3, 7.2.6, 8.3.1, 8.3.2.3, 8.8, 9.1, 9.4, 10.1.1. • Update in GRAS Study Coordinator • Update Sm-p80 as rSm-p80

3	4.0	06NOV2024	FM, BT, MS, TY, ELP, JP, DRK, DK, AR	• Update of IVI team member list • Update of the trial period • Clarification on the safety data reviews and decision to progress to the next dose level independently of each site. • Clarification of the screening visit in relation to the first dose visit. • Update of the clinical data • Update of the statistical analysis plan and the planned interim analysis sections. • Inclusion of the interim analysis of safety and immunogenicity data. • Change of BDM (Biostatistics and Data management to DSI (Data Science and Innovation)

18 APPENDICES

APPENDIX A:

Schedule of Study Procedures / Schedule of Events (SOE)

Visit Number	V0[&]	V1[π]	V2	V3	V4	V5	V6	V7	V8	V9	UV[a]
Visit Description	Pre-screening	Screening	Vac 1	Vac 1+7 days	Vac 2	Vac 2 +7 days	Vac 3	Vac 3+7	One month follow-up visit, post V6	Six months follow-up visit, post visit V6	Unscheduled Visit
Visit Day (D) ± Window	[v]	D -28 to -1	D0	D7+3	D28+3	D35+3	D56+3	D63+3	D84±7	D224±14	-
Visit Week	[§]	-4 ~ -1	0	1	4	5	8	9	12	32	-
Pre-screening informed consent	X										
Deworming with Praziquantel & Albendazole	X										
Informed Consent		X									
Inclusion & Exclusion Criteria		X									
Temporary Exclusion Criteria Review			X		X		X				
Medical History		X	X								
Full Physical Examination		X	X	X	X	X	X	X	X	X	X
Demographic, Height, Weight		X									
Vital Signs		X	X	X	X	X	X	X	X	X	X
Serology (HCV, HBsAg, HIV)		X									
Urinalysis [b]		X		X	X	X	X	X	X		
Pregnancy test (PT)[%]		X	X		X		X				
Malaria diagnosis [β]		X	X		X		X				
Blood sample for CAA[c]			X		X		X		X		
Stool sample for Schistosoma eggs and STH detection [d]		X									
Urine sample for POC-CCA and Schistosoma eggs detection.		X									
SARS-CoV-2 Rapid Antigen test		X	X [¶]		X [¶]		X [¶]				X [¶]
Use of effective contraceptive		X	X	X	X	X	X	X	X		
Enrollment & Randomization			X								

Visit Number	V0[&]	V1[π]	V2	V3	V4	V5	V6	V7	V8	V9	UV[a]
Visit Description	**Pre-screening**	**Screening**	**Vac 1**	**Vac 1+7 days**	**Vac 2**	**Vac 2 +7 days**	**Vac 3**	**Vac 3+7**	**One month follow-up visit, post V6**	**Six months follow-up visit, post visit V6**	**Unscheduled Visit**
Visit Day (D) ± Window	[¥]	**D -28 to -1**	**D0**	**D7+3**	**D28+3**	**D35+3**	**D56+3**	**D63+3**	**D84±7**	**D224±14**	-
Visit Week	[§]	**-4 ~ -1**	**0**	**1**	**4**	**5**	**8**	**9**	**12**	**32**	-
Safety Blood Samples (SBS) for Hematology [e] and Serum Chemistry [f]		**SBS1**		**SBS2**	**SBS3**	**SBS4**	**SBS5**	**SBS6**	**SBS7**		
Immunogenicity Blood Samples (IBS)			**IBS1**	**IBS2**	**IBS3**	**IBS4**	**IBS5**	**IBS6**	**IBS7**	**IBS8**	
Study Product Administration			**X**		**X**		**X**				
Post Vaccination 60 min observation			**X**		**X**		**X**				
Solicited adverse events			**X**	**X**	**X**	**X**	**X**	**X**			
Unsolicited adverse events within 28 days			**X**	**X**	**X**	**X**	**X**	**X**	**X**		**X**
Serious Adverse Event (SAE)			To be monitored throughout study duration following first dose.								
Adverse event of special interest (AESI)			To be monitored throughout study duration following first dose.								
Participant Diary Card (DC) distribution			**DC1**		**DC2**		**DC3**				
Participant Diary Card (DC) collection and verification				**DC1**		**DC2**		**DC3**			
Review of concomitant medications		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Safety Blood Volume at screening											
EDTA Hematology		3 mL									
Serum Serum chemistry, Serology, Serum pregnancy test		3 mL									
Safety Blood Volume after enrollment											
EDTA Hematology				3 mL	3 mL	3 mL	3 mL	3 mL	3 mL		
Serum Serum chemistry				3 mL	3 mL	3 mL	3 mL	3 mL	3 mL		
Immunogenicity Blood Volume											
Serum Antibody assays			9 mL	9 mL	9 mL	9 mL	9 mL	9 mL	9 mL	5 mL	
NaHep [#] PBMC isolation for T and B cell assays, RNAseq and cytokine multiplex Assay			17 mL	17 mL	17 mL	17 mL	17 mL	17 mL	10 mL	10 mL	
Total volume per visit (Excluding PBMC subset)		6 mL	9 mL	15 mL	15 mL	15 mL	15 mL	15 mL	15 mL	5 mL	

Visit Number	V0[&]	V1[π]	V2	V3	V4	V5	V6	V7	V8	V9	UV[a]
Visit Description	Pre-screening	Screening	Vac 1	Vac 1+7 days	Vac 2	Vac 2 +7 days	Vac 3	Vac 3+7	One month follow-up visit, post V6	Six months follow-up visit, post visit V6	Unscheduled Visit
Visit Day (D) ± Window	[¥]	D -28 to -1	D0	D7+3	D28+3	D35+3	D56+3	D63+3	D84±7	D224±14	-
Visit Week	[§]	-4 ~ -1	0	1	4	5	8	9	12	32	-
	[&, ¥, §] **Visit 0:** Pre-screening activities (which should be conducted -56 to -42 days or -8 to -6 weeks before the screening visit) will follow local standard of care to de-worm potential study participants prior to formal study screening; further details in section 7.1.3.2.. [π] Visit 1 (screening visit) should happen within 28 days prior to Visit 2 (first vaccination visit). In other words, the first dose should be given within 28 days from the day of screening. [as] During unscheduled visit, blood sample and naso-pharyngeal swab will be collected for further investigation when deemed necessary by the study physician. [b] Urinalysis: blood, glucose and protein by dipstick [%] Pregnancy test will be performed on all female participants of childbearing age at screening and before each study vaccination. Serum pregnancy test will be carried out at screening and urine pregnancy test at vaccination visits. [β] Malaria diagnosis will be done using blood smear at the screening visit and rapid diagnostic test before each vaccination. [¶] SARS-CoV-2 rapid antigen test will be done in case of suspected symptoms/signs of COVID-19 before each vaccination, and at unscheduled visit if deemed necessary by the study physician. [c] Circulating Anodic Antigen (CAA) assay will be done retrospectively using the serum. [d] Detection of Schistosoma mansoni infection will be done by Kato-Katz and POC-CCA confirmed using the RT-PCR. Soiled transmitted helminths infections will be detected by Kato-Katz and confirmed by the RT-PCR. [e] Hematology: hemoglobin, complete blood count with differential, and platelets [f] Serum chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), creatinine (CREAT) and C-reactive protein (CRP). [#] Blood collection for PBMC isolation, RNAseq and cytokine multiplex Assay will only be conducted in a subset of participants. Phone calls will be arranged after vaccine administration to ensure completion of the Participant's DC and to check their health status. No deviation will be considered if the phone call could not be completed on the exact dates following vaccination visit.									

APPENDIX B

Adverse Events of Special Interest (AESIs).

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)
- 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
- 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., noninfectious
- 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
- Acute febrile neutrophilic dermatosis (Sweet's syndrome)
- Vitiligo

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