
*An RCT to Evaluate Different Treatment Approaches to Mitigate the Progression of
Schistosomiasis Related Hepatic Fibrosis (SchiFT)*

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SIGNATURE PAGE

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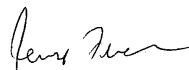
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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CMS	Clinical Material Services
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure

ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
MedDRA [®]	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to Participants)
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Participants Research
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PZQ	Praziquantel

QA	Quality Assurance
QC	Quality Control
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States
WHO	World Health Organization

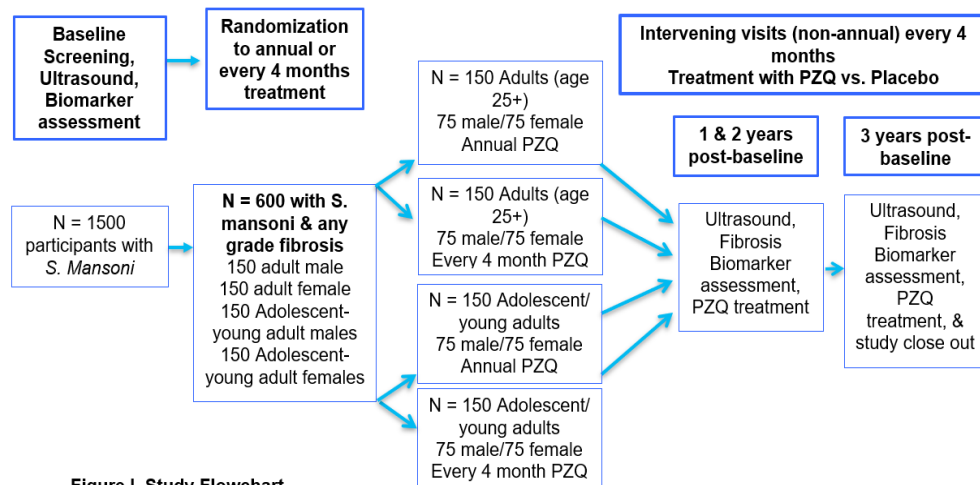
PROTOCOL SUMMARY

Title:	An RCT to Evaluate Different Treatment Approaches to Mitigate the Progression of Schistosomiasis Related Hepatic Fibrosis (SchiFT)
Design of the Study:	A randomized, double-blind placebo-controlled trial to evaluate different treatment frequencies (Praziquantel 40 mg/kg annually or every 4 months for 3 years) for Schistosomiasis associated hepatic fibrosis (mild, moderate or severe) as ascertained at baseline and annually by ultrasound among N=600 participants ages 15-40 from the Lake Albert region of Uganda. The primary outcome is reduction in the stage of hepatic fibrosis at three years.
Study Phase:	<i>III</i>
Study Population:	N=600 participants ages 15-40 from the Lake Albert region of Uganda with mild, moderate, or severe hepatic fibrosis due to <i>S. mansoni</i> as ascertained by ultrasound
Number of Sites:	1
Description of Study Product or Intervention:	• Praziquantel 40 mg/kg PO annually for three years • Praziquantel 40 mg/kg PO every 4 months for three years
Study Objectives:	Primary: • To evaluate the impact of two different treatment frequencies with Praziquantel (40 mg/kg) annually or every 4 months on improvement in grade of fibrosis from baseline to three years with improvement defined as any decline in grade of fibrosis e.g. moderate to mild, moderate to none.

	Secondary: 1. To evaluate the impact of two different treatment frequencies with Praziquantel (40 mg/kg) annually or every 4 months on improvement in grade portal hypertension baseline to three years 2. To evaluate the impact of two different treatment frequencies with Praziquantel (40 mg/kg) annually or every 4 months on improvement in stage of fibrosis after one and two years of treatment 3. To evaluate the impact of two different treatment frequencies with Praziquantel (40 mg/kg) annually or every 4 months on improvement in stage of portal hypertension after one and two years of treatment 4. To evaluate the impact of two different treatment frequencies with Praziquantel (40 mg/kg) annually or every 4 months on key biomarkers of fibrosis
Duration of Individual Participation:	37 months
Estimated duration of the study	46 months

Table 1: Treatment Arms

Trial Cohort (N=600 participants with <i>S. mansoni</i> and any grade of hepatic fibrosis)	ARM 1	N= 300 Stratified by age (15-24/25-40 years) and sex	Praziquantel annually at 40 mg/kg
	ARM 2	300 Stratified by age (15-24/25-40 years) and sex	Praziquantel every 4 months at 40 mg/kg

Figure 1: Schematic of Study Design

1 KEY ROLES

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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Global morbidity due to schistosomiasis and current approaches to treatment

Close to 190 million individuals globally are infected with one of three predominant species of schistosomes with an estimated 250,000 deaths annually.^{1,2} Both *S. japonicum*, endemic in Asia, and *S. mansoni*, endemic in multiple regions globally, cause intestinal schistosomiasis. Severe morbidity due to intestinal schistosomiasis is related to the location of adult worms in the mesenteric plexus. A significant number of eggs are swept into the liver, rather than migrating to the gut lumen for release into the environment.^{3,4} The human host creates granulomas around eggs, which evolve with fibroblasts to encircle the egg with collagenous material. This process, with often thousands of eggs deposited throughout the liver, culminates in hepatic fibrosis. When these occur around the portal vessels, blood flow through the liver is restricted culminating in portal hypertension, collateral vessels, esophageal varices with consequent hematemesis, which is the primary cause of death due to intestinal schistosomiasis. Of great relevance to this proposal, once established at higher grades, hepatic fibrosis is not reversible with a high case fatality rate.^{5,6}

Currently, preventative chemotherapy strategies with Praziquantel (PZQ) are the mainstay of treatment, with “mass drug administration” (MDA) delivered annually or bi-annually. With this approach, individuals are treated without formal diagnosis of schistosomiasis. Endemic nations globally do not currently have screening tools to identify individuals with hepatic fibrosis who may be at increased risk for progression to irreversible disease stages, and who are likely to benefit from more frequent treatment with PZQ.⁷⁻¹⁰ Also of concern, many MDA strategies globally focus on school-based treatment, leaving high risk adolescents and adults untreated, and delivery of MDA broadly is inconsistent due to drug shortages and lack of staff and transport to deliver MDA.¹¹

Burden of schistosomiasis-related liver fibrosis

In many regions of the world, hepatic fibrosis with periportal fibrosis is evident among children and adolescents.^{12,13} Our recently published study conducted in pre-school aged children in the proposed study area (Lake Albert) found evidence of incipient fibrosis with 17% having evidence of periportal thickening.¹⁴ There is also high occurrence of increased portal vein diameter, an early marker for portal hypertension has also been demonstrated in children.¹⁵ Studies from Tanzania found that 22.4-42.2% of adolescents and adults with schistosomiasis had periportal fibrosis as determined by ultrasound staging with the Niamey protocol,^{16,17} prompting recent calls to action to ensure the inclusion of adolescent and adult community members in MDA campaigns.^{5,18} Importantly, periportal fibrosis is a significant risk factor for portal hypertension, which in turn places individuals at significant risk for the development of varices and hematemesis, the ultimate cause of death due to schistosomiasis-related hepatic fibrosis.

A recent review across many regions noted that varices occur in up to 82% (11.1-100%) of individuals with periportal fibrosis.⁵ Of great concern, there is an estimated mortality rate of up to 29% among individuals with late stage varices.⁵ With the World Health Organization (WHO) 2020 NTD roadmap's focus on indicators of host morbidity and decreasing morbidity due to schistosomiasis,¹⁹ biomarkers to identify both individuals at high risk of progression of hepatic fibrosis as well as best treatment approaches before this is irreversible are urgently needed.³ Biomarkers could ultimately be developed into Point of Care Tests (POCTs) to identify individuals at risk of fibrosis progression and also support prioritization of community MDA, rather than isolated school-based MDA.

The risk for schistosomiasis-related hepatic fibrosis is multi-factorial with many studies demonstrating that fibrosis occurs at low/moderate intensities of infection.^{13,20,21} In fact, intensity is just one factor in a complex, multi-factorial disease phenotype^{21,22} with strong evidence to suggest that longevity of infection,^{20,21} host and parasite genetic factors,^{21,22} sex, and Th1/Th2 balance²³⁻²⁶ in response to infection, all impact the risk of severe morbidity and mortality. Distinct

strategies to identify and more aggressively treat individuals at high risk of progression of schistosomiasis-related hepatic fibrosis are urgently needed.

Screening for early identification to reverse progression of hepatic fibrosis

WHO's 2020 NTD Roadmap explicitly highlights the need for defined indicators of *host morbidity*.¹⁹ However, there are guidelines for the ongoing management of individuals with early and moderate stages of fibrosis, before the onset of overt symptoms related to periportal hypertension such as esophageal varices. Treatment with a single dose of PZQ with an option to re-treat six weeks later is recommended without further follow up.

Once these individuals progress to severe hepatic fibrosis and portal hypertension, these processes are largely irreversible with standard treatment with PZQ.⁷ This is supported by one study in which treatment up to every six months for *S. mansoni* led to reversal, as evaluated by the WHO "Niamey" ultrasound protocol, among individuals with mild or moderate fibrosis (69.7% & 40.6%, respectively), but did not occur among individuals with severe fibrosis (0%).⁷

For individuals with severe fibrosis and portal hypertension, referral to gastroenterologists is recommended. Importantly, this level of care is not available in most endemic areas globally and, even when available, interventions are risky and often unsuccessful.⁶ No studies have addressed whether treatment provided more frequently over longer periods of time in these early stages can mitigate this risk.

Compelling biomarker candidates with state-of-the art ultrasound protocol as gold standard

Fibrosis with dysregulated collagen metabolism is a hallmark of schistosome infection. However the dynamics of dysregulated collagen metabolism and their longitudinal relationship to cycles of infection, treatment and reinfection have not been examined. A recent review of the diagnosis and management of intestinal schistosomiasis noted that there are currently no available diagnostic

tools for the early diagnosis of patients with periportal fibrosis and early varices and emphasized the need for these to allow screening and aggressive provision of PZQ.⁵

Studies in other diseases have identified biomarkers that can non-invasively grade liver fibrosis (biopsy as gold standard). However, biomarkers performed differently in children and adults in Hepatitis C infection, stressing the need to examine both adolescents and adults.²⁷

We will address this gap by evaluating biomarkers involved in collagen deposition, remodeling, and degradation many of which have been implicated in schistosomiasis-related hepatic fibrosis,²⁸⁻³⁴ including studies in *S. japonicum*.³⁵⁻³⁷

Pathologic fibrosis represents a complex process in which fibrosis can result from increased production of analytes that are potent drivers of deposition (e.g. TGF- β 1,³⁸⁻⁴⁰ IL-13,^{35,40-42} MCP-1,⁴³⁻⁴⁵ MIP-1 α ^{40,43,46}), decreased production of analytes that inhibit collagen deposition (e.g. matrix metalloproteases (MMPs³¹)) and overproduction of analytes that inhibit degradation (e.g. Tissue Inhibitor of Metalloproteases (TIMPs^{31,37,40})). Importantly, in published³⁷ work from our group from a longitudinal cohort in The Philippines, TIMP-1 produced by PBMCs in response to SEA was a significant predictor of hepatic fibrosis one year after treatment with PZQ.³⁷ Because TIMP-1 inhibits most matrix metalloproteases (MMPs) which are responsible for collagen degradation, these data suggest that schistosome-associated hepatic fibrosis results in part from **excessive inhibition** of collagen remodeling. Cytokines also promote fibrosis in the context of human schistosomiasis,²⁸⁻³⁰ with some effects likely occurring through stimulation of Transforming Growth Factor-beta (TGF- β).⁴⁷ We will also evaluate other novel biomarkers such as microRNAs, Procollagen III amino terminal peptide, and hyaluronic acid.^{34,48-50}

An inhouse multiplexed “FibroPlex” assay that captures over 40 key analytes involved in fibroproliferation or degradation developed at Rhode Island Hospital^{35,37,51,52} will be set up at MRC/UVRI & LSHTM Uganda Research Unit where analysis will take place. These biomarkers will be assessed against ultrasound evaluation.

Effects of Praziquantel on hepatic fibrosis

Evidence from a meta-analysis of 33 studies reported association of periportal fibrosis with current infection status, highlighting the overall importance of treatment for schistosomiasis in mitigating fibrosis.⁵³ Animal models^{54,55} and human studies^{4,5,7-10} indicate that treatment with PZQ leads to slowing and even reversal of hepatic fibrosis, particularly when diagnosed in early stages. Given schistosome eggs are the primary driver of hepatic granuloma formation and fibrosis, mouse models demonstrate potent effects of PZQ with respect to egg viability.⁵⁶

In animal models, a single dose of PZQ dramatically reduced the viability of eggs in liver tissue (13.0%) when compared with untreated controls (85.3%), and no eggs in the liver were viable after a second dose nine days later.⁵⁶ This supports the *scientific premise* that PZQ has a multi-faceted effect on liver fibrosis through both direct killing of eggs as well as mature worms. This further supports the need to study more frequent treatment of individuals with fibrosis to ensure killing of eggs and mature worms due to repeated re-infections in endemic areas.

Studies in humans have demonstrated that participants treated at least annually for *S. mansoni*-associated hepatic fibrosis experience significant decreases in (33%), or resolution of (25%), hepatic fibrosis by ultrasound.⁹

In the SCORE studies, it was noted that even annual community-based treatment with PZQ resulted in only a slight decrease in pattern B fibrosis from 16.6% at baseline to 11.4% over five years among school aged children. Further, these studies did not have a group with more frequent than annual treatment and the “control” group, receiving school-based treatment every two years, experienced a slight increase in the prevalence of pattern B fibrosis. A prevalence of fibrosis of 11.4 % among children ages 11-12, despite annual treatment for five years, is unacceptably high and calls for studies as the one proposed to evaluate more frequent treatment strategies.

Support for the importance of more frequent treatment as proposed for this trial is also provided by studies that demonstrate that the likelihood of resolution of fibrosis is significantly related to a lower frequency of post-treatment recurrence, which can be disrupted by more frequent treatment

as proposed.⁷ Furthermore, a meta-analysis that included 71 studies addressing the impact of single treatment with PZQ on a range of morbidities, supports the scientific premise that PZQ treatment culminates in significant reversal of hepatic fibrosis, including ultrasound findings of periportal fibrosis.⁴

Overall, no studies have looked at treatment frequency more than annually as we propose and annual treatment leaves a significant proportion of individuals with no change in severity of fibrosis. It should also be noted that one study in *S. japonicum* actually noted that participants treated annually vs. once every two years had higher rate of grades I-III fibrosis progression (20.5 vs. 30.6%, significance not reported, respectively).⁵⁷ This study stands out as the only in the extant literature suggesting that more frequent treatment (annual vs every two years) resulted in greater rate of progression (significance testing not provided).

Finally, there is clinical equipoise as a) it is not clear that more frequent treatment will improve morbidity as this has never been studied with a greater frequency than annual treatment and b) one study demonstrated a higher rate of fibrosis progression in *S. japonicum* comparing annual to every two years treatment⁵⁷ as above.

This study will therefore address the following gaps:

- a) Lack of biomarkers to identify individuals with current fibrosis due to schistosomiasis and whether these individuals can be distinguished from individuals with fibrosis due to other causes (viral hepatitis, alcoholic cirrhosis)
- b) Lack of clarity with respect to optimal frequency of treatment among both children and adults, to stabilize or reverse fibrosis over longer period of follow up
- c) Lack of longitudinal data examining the impact of specific biomarkers and relationship to progression which will enhance understanding of pathobiological drivers of schistosomiasis-related hepatic fibrosis

- d) Lack of easy to deploy ways to identify individuals at risk for early stages of fibrosis other than ultrasound making serum biomarkers and the prospect for point of care tests attractive.

2.2 Scientific Rationale

We have chosen to use a standard dose of Praziquantel based on its established safety and efficacy and vary the treatment frequency as individuals who are treated annually are rapidly re-infected before their next annual treatment resulting in more egg deposition which drives hepatic fibrosis. The choice of every 4 months reflects a balance between a) what might ultimately be feasible in a region with extremely limited access to health care and the difficulties inherent in drug delivery to rural communities with b) a sufficient frequency to treat re-infections that occur almost continuously in this setting. Further, the primary driver of morbidity is egg deposition, and it takes about 2-3 months from the time of new infection to adult worm maturation and egg laying. These factors drive the frequency of treatment decision.

We stratify randomization by sex given many studies demonstrate a higher prevalence of fibrosis among men, even after adjusting for age, water contact exposure, and intensity of infection. Furthermore, we stratify by age groups (15-24 and 25-40 years) to ensure adequate power to understand efficacy of increased treatment among adolescents. As above, this is driven by the evidence that in many regions of the world, hepatic fibrosis with periportal fibrosis is evident even before adolescence.^{12,13} Studies from our group in Lake Albert¹⁴ and the Schistosomiasis Consortium for Operational Research and Evaluation¹⁵ demonstrated that even very young children (Ages 1-7) have evidence of increased portal vein diameter, an early marker for portal hypertension.

Specifically, treatment may have less significant impact on hepatic fibrosis among men or women which should be identified. With respect to age, we will limit the age range to 15 to 40. This is because later stages of fibrosis, as well as portal hypertension are rare among individual before the

end of puberty. We believe young children who have this phenotype may have unique genetic or other risk factors that will add to much heterogeneity to the study sample. Studies have suggested that biologic factors such as pubertal hormones such as dehydroepiandrosterone sulfate (DHEA-S)⁶⁰ may modify morbidity risk and this will add too much heterogeneity to the sample. Similarly, we have limited the upper age range as older adults may have other risk factors and co-morbidities that may modify both response to treatment and fibrosis biomarker profiles. Further, in many studies, duration of infection,^{21,22} rather than age per se, is found to be a key driver of fibrosis risk, particularly later stage fibrosis. As one of the impetuses for this study is to identify individuals with fibrosis while it is still reversible, we would like to focus on a younger age range, rather than older adults who are more likely to have irreversible disease based on duration of exposure.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

The overarching goals of this proposal are to conduct a **double blind, randomized controlled trial (RCT) (Phase III) among N=600 participants ages 15-40 in Lake Albert, Uganda**, that will examine **varying frequencies of treatment with Praziquantel (standard 40 mg/kg PO every 4 months or annually for three years)** to identify approaches that best reverse or halt fibrosis as captured by ultrasound annually.

After excluding other causes of hepatic fibrosis, we will randomize/enroll N = 600 participants for the RCT (N = 150 individuals in each age and sex strata (4 sub-groups)) who have compensated schistosomal liver fibrosis based on the WHO “Niaamey” ultrasound protocol for *S. mansoni*.

Using stratified randomization by subgroup treatment arms are as follows:

1. **Annual treatment (standard) arm:** The annual treatment study arm will receive active study agent (PZQ at 40 mg/kg) at baseline and one, two, and three years post randomization and placebo at intervening 4 month visits (i.e. 4, 8, 16, 20, 28, 32 months post baseline).
2. **Every 4 months treatment (intervention) arm:** This arm will receive praziquantel at a dose of 40 mg/kg every 4 months for 3 years. (i.e. at 4, 8, 12, 16, 20, 24, 28, 32 and 36 months post baseline).

Randomization and blinding

The trial statistician will generate randomization codes by study number in blocks for each age/sex group and share with the pharmacist. Because the placebo and praziquantel tablets will be matched,

allocation to a given study arm will be concealed to participants and study staff, including ultrasonographers who are assessing study outcomes.

3.2 Study Objectives

The overall goals of this trial are to examine varying frequencies of treatment with Praziquantel (every 4 months or annually for three years) to identify approaches that best reverse or halt fibrosis as captured by ultrasound annually. We will also identify novel biomarkers using our multiplexed assay which captures over 40 analytes involved in fibro-degradation and fibro-proliferation, which best predict *progression* of fibrosis.

The **primary objective** for the trial will be to assess the impact of PZQ dosed annually vs every 4 months over three years on **grade of fibrosis** as captured by the Niamey protocol from baseline to three years with ultrasound performed annually. The primary endpoint is improvement in grade of fibrosis (any improvement) during this three-year period of follow up. Grades by image pattern are below and improvement, stability or worsening is based on changes in these categories from baseline.

3.2.1 Secondary

Secondary objectives are to:

- 1) assess the impact PZQ administered annually versus every 4 months over three years on presence and of severity portal hypertension status (absent, dilated or severely dilated present,) based on portal vein quotient
- 2) 2) assess the impact of PZQ administered annually versus every 4 months on grade of hepatic fibrosis after one and two years.
- 3) assess the impact of PZQ administered annually versus every 4 months presence and severity (absent, dilated, severely dilated) of portal hypertension after one and two years.

- 4) evaluate the impact of two different treatment frequencies with Praziquantel (40 mg/kg) annually or every 4 months on key biomarkers of fibrosis

3.3 Study Endpoints or Outcome Measures

The primary outcome will be any improvement in grade of fibrosis (mild, moderate, severe) from baseline to study close out at three years coded as Yes/No improvement for a rate of improvement across study arms.

Hepatic Fibrosis Grade: We will employ the “Niamey” protocol, which was co-authored by co-investigator, Dr. Joachim Richter, for the assessment of fibrosis in the context of *S. mansoni*.⁵⁸

Hepatosplenic schistosomiasis (HSS) encompasses a characteristic type of portal fibrosis, also called Symmer’s clay pipe fibrosis or periportal fibrosis, and its resulting complications such as portal hypertension.⁶ The Niamey protocol has a detailed scoring system for fibrosis that is based on both “image pattern” and degree of periportal fibrosis. For *S. mansoni*, fibrosis is characterized by grades (B- F) with grades C to F constituting mild to severe grades of fibrosis.

Since image pattern B is not specific to schistosomiasis, individuals with pattern B will not be included. Fibrosis will be graded with respect to location (periphery (C) & central (D, E, F)). These scores culminate in a **severity grading that maps to mild, moderate, and severe.**

Mild: Image Pattern C

Moderate: Image Pattern D

Severe: Image pattern E or F

Improvement is defined as change from baseline status as follows:

- E or F to D (severe to moderate)

Commented [AE1]: Confirm if he is co-investigator

-
- E or F to C (severe to mild)
 - E or F to A or B (severe to none/non specific)
 - D to C (moderate to mild)
 - D to A or B (moderate to none/non specific)
 - C to A or B (mild to none/non specific)

Stability is defined as no change from overall group (mild (C), moderate (D) or severe (E or F)) at defined follow up timepoints.

Progression is defined as change in baseline status as follows:

- C to D (mild to moderate)
- C to E or F (Mild to severe)
- D to E or F (Moderate to Severe)

Assessment of fibrosis is detailed in section 6.3.3.

3.3.1 Secondary

Clinical secondary outcomes include change in grade of portal hypertension from baseline.

Portal Hypertension: We will separately assess whether there is evidence of significant portal hypertension by ultrasound as per the Niamery protocol. The presence of portal hypertension will be defined by the portal vein quotient (adjusted for height). A quotient greater than 7.5 is indicative of portal hypertension.

Secondary outcomes include:

- a) resolution of portal hypertension after three years and
- b) improvement or stability in grade of fibrosis (as described above) after one and two years
- c) resolution of portal hypertension after one and two years

Assessment of portal hypertension is detailed in section 6.3.3.

3.3.2 Exploratory

We will also address the following objectives which do not represent clinical endpoints for the trial but rather identify key fibrosis biomarkers that predict the presence of hepatic fibrosis and its progression from baseline. These secondary/exploratory outcomes will use the aforementioned definition of change in fibrosis grade from baseline.

Biomarker identification: At baseline, we will cross-sectionally evaluate fibrosis biomarkers with our recently expanded FibroPlex (e.g microRNAs, hyaluronic acid)^{34,48-50} with over 40 analytes using Area Under the Curve Analyses to identify specific biomarkers and “cut offs” which accurately identify individuals with varying stages of liver fibrosis by ultrasound. We will then longitudinally evaluate fibrosis biomarkers that identify risk of progression from mild-moderate to higher grades of fibrosis as assessed by ultrasound annually stratified by treatment allocation among the N=600 RCT participants. This will allow identification of individuals at greatest risk of fibrosis progression before overt symptoms and while still reversible.

4 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

4.1 Study products

Two study products will be used as stated previously, namely:

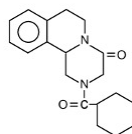
Praziquantel: The study agent is PZQ which comes in 600 mg oval tablets scored thrice to yield four pieces of 150mg each. This will be supplied by Macleods Pharmaceuticals, Marol Church Road, Andheri (East), Mumbai, India to ensure uniformity in composition and quality.

Placebo: A placebo matched by color, odor, taste and physical appearance will also be procured from Macleods Pharmaceuticals, Marol Church Road, Andheri (East), Mumbai, India.

4.2 Study Product Description

Praziquantel is a hygroscopic white to nearly white crystalline powder of bitter taste and is soluble in chloroform, dimethylsulfoxide and ethanol, and to a very low extent soluble in water. Inactive ingredients: corn starch, hypromellose, magnesium stearate, microcrystalline cellulose, polyethylene glycol, povidone, sodium lauryl sulfate, and titanium dioxide.

Praziquantel is a 2-(cyclohexylcarbonyl)-1,2,3,6,7, 11b-hexahydro-4H-pyrazino [2, 1-a] isoquinolin-4-one (C₁₉H₂₄N₂O₂) with anthelmintic properties.



4.2.1 Product Storage and Stability

Praziquantel should be stored below 86°F (30°C) because it is unstable above that temperature. It will be stored in a room with daily temperature monitoring. The placebo will be stored under similar conditions.

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

The investigational product will be administered at 40mg/kg. Study Pharmacist will be responsible for packing the product according to the randomization codes from the statistician and eventually dispensing it to the field team.

4.4 Pre-determined Modification of Study Intervention/Investigational Product for an Individual Participant

If women become pregnant during the study (tested before every 4 months at the time of interventional product administration), study activities will be temporarily held until after delivery.

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

From the Pharmacy, individual use doses/bottles needed for each study timepoint will be provided to the research team for specific trips to the field site in cooler boxes. The Pharmacist will be responsible for keeping records and documentation of the products, as well as supporting the field team in achieving this objective. The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records will be available for inspection by the assigned Study Monitor.

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

5 PARTICIPANT SELECTION, ENROLLMENT AND WITHDRAWAL

The study area is endemic with *S. mansoni*, with prevalence overall prevalence of over 50% accordingly to programmatic survey data.⁵⁹ Thus, we will need to screen approximately 3000 individuals to yield approximately 1500 who are infected. We shall use a conservative approximate of 40% of infected participants expected to have hepatic fibrosis that is due to *S. mansoni*, though the prevalence has been shown to be higher in some parts of the study area at 68%.⁶⁰ Thus, the 1500 infected will yield the 600 eligible for enrolment ($1500 * 0.40 = 600$).

Screening activities including history, physical exam, and liver ultrasound will have a separate screening informed consent process and individuals will be made aware that these are only screening activities and most will not be eligible for the longitudinal trial.

The *S. mansoni* infected individuals aged 15–40 years, they will be invited to the study field station for further screening activities including ultrasound for the presence of hepatic fibrosis. The ultimate target is enrolment of 600 individuals with *S. mansoni* infection and hepatic fibrosis. After allocation to the two main study groups (once versus three times annual treatment), they will be further categorized by age (15–24 and 25–40 years) and sex (male and female) to yield four sub-groups of 150 each. Recruiting in distinct age and sex sub-groups is important as many studies have demonstrated that men are at higher risk of hepatic fibrosis and it is possible that there will be effect modification by sex and age group. (See **Figure I, Study Flow Chart**).

Specifically, treatment may have less significant impact on hepatic fibrosis among men or women which should be identified. In addition, it is expected that there may be different genetic or other risk factors among individuals who experience hepatic fibrosis at younger ages. It is important to understand whether there is an interaction (effect modification) of treatment by age group as we expect that individuals who develop fibrosis at younger ages (adolescents) may have a genetic predisposition to fibrosis. Thus, in these younger individuals it is possible that increasing treatment

frequency may have a greater or lesser effect and different biomarkers may relate to this risk both at baseline, and its progression longitudinally.

Finally, there may be differences in response to treatment by age group based on genetic and other biologic factors such as levels of pubertal hormones such as dehydroepiandrosterone sulfate (DHEA-S).⁶¹ Identifying these key interactions will inform MDA strategies to include adolescents who could ultimately be identified as at risk with fibrosis POCTs and targeted for earlier and more frequent treatment.

5.1 Eligibility Criteria

5.1.1 Participant Inclusion Criteria

For enrolment (randomization) into the study, the participant must meet the following criteria:

1. *S. mansoni* infection as determined by urine Circulating Cathodic Antigen (CCA)
2. Otherwise healthy as determined by history and physical examination conducted by the study clinician
3. Not Pregnant
4. Age 15-40 years
5. Consent for individuals 18 years of age and older, and parental consent and adolescent assent for participants ages 15-17 years.
6. The presence of hepatic fibrosis due to *S. mansoni* (Niamey protocol grade C-F) and compensated (without late-stage portal hypertension) after ruling out other causes of hepatic fibrosis as below.

5.1.2 Participant Exclusion Criteria

All participants meeting any of the exclusion criteria at baseline will be excluded from study participation. Exclusion criteria for the baseline study is as follows:

1. History of upper gastrointestinal bleeding
2. Hepatic fibrosis that is not deemed to be due to schistosomiasis based on ultrasound and hepatitis serologies. For example, alcoholic cirrhosis or presence of chronic Hepatitis B or C by point of care test
3. Known neurocysticercosis or ocular cysticercosis

At baseline, participants will also be screened for HIV (based on self-reported status, unless a participant requests for an HIV test) and assessed for history/concomitant use of alcohol and medication with a focus on drugs that potentially affect plasma concentration of praziquantel by reducing (rifampicin, antiepileptics and dexamethasone) or increasing (cimetidine, ketoconazole, itraconazole and erythromycin). These variables will not form a basis for exclusion if they are NOT accompanied with liver fibrosis, but will inform the timing of administration of praziquantel or be adjusted for in the analysis

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

5.2.1 Withdrawal from the Study or Discontinuation of the Study Product

Participants may voluntarily withdraw their consent for study participation at any time without giving a reason or facing penalty and/or loss of access to standard services. Those who experience severe allergic reaction to any dose of the study agent given including but not limited to anaphylaxis will be withdrawn. Follow-up safety evaluations will be conducted, with consent of the participant. If a participant withdraws or is withdrawn prior to completion of the study, the reason for this decision will be recorded.

Participants will also be withdrawn from receiving the study product for any of the following:

- Meets individual halting criteria (reference to section 7.7)

-
- Medical condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the participant, interfere with the participant's successful completion of this study, or interfere with the evaluation
 - Lost to follow-up
 - Determined by a physician's discretion to require additional therapy not indicated in the protocol to ensure participant's health and well-being

If the participant consents, every attempt will be made to follow up safety events. The investigator will also seek and document permission on use of data and samples already collected.

5.2.2 Participant Replacement

Participants who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and administration of the study product will not be replaced. Women who become pregnant during the trial (after randomization) will not be withdrawn. Rather, study activities will be suspended until after delivery. The trial will offer appropriate support for access to antenatal, delivery and postnatal services.

6 STUDY PROCEDURES

6.1 Screening (up to 28 days prior to randomization)

Target communities in the districts of Kikuube, Hoima, Buliisa and Pakwach along the shores of Lake Albert will be approached for participation in the study. Entry meetings will be held with district leaders as well as key community gate keepers to inform them about the project and solicit their input to ensure successful and harmonious implementation. Periodic feedback sessions and dissemination of results will also be conducted in line with the study community engagement plan.

Study staff with training in Good clinical Practice and provision of informed consent will review the screening consent with potential participants as well as the screening assent with adolescents under the age of 18 and their parent/guardian for parental consent. Potential participants who have provided consent/assent will be screened for the presence of *S. mansoni*, as follows:

1. An informed consent will be administered for the screening procedures below.
2. Urine Circulating Cathodic Antigen (CCA) Test for the presence of *S. mansoni* infection will be administered in villages by study staff.
3. Individuals with *S. mansoni* infection will be invited to the field station with a private clinical intake area and laboratory for further eligibility screening including:
 - a. History and physical examination
 - b. Liver function tests and Complete blood count.
 - c. Point of care tests for Hepatitis B and C
 - d. Nutritional assessment (anthropometric measures)
4. Ultrasound of Liver and Spleen.
5. For women of child-bearing age: Urine sample for screening of pregnancy using rapid point-of-care test strips for Human Chorionic Gonadotropin (hCG).
6. A serum sample will be kept for confirmation of *S. mansoni* infection by Circulating Anodic Antigen (CAA) which is a quantitative antigen test.

Enrolment will be completed within 28 days of screening.

6.2 Enrollment

Participants who meet the eligibility criteria for enrolment (N=600) as detailed in section 5.1 will be consented based on an informed consent form that details procedures for enrolment and participation in the longitudinal part of the study. At baseline enrollment, this will include provision of the study agent which will be administered under direct observation, followed by monitoring for at least 2 hours post-dose to actively capture and treat any adverse events, and scheduling of the next visit in 4 months (window ± 2 weeks).

6.3 Planned Study Visits

- A. **Enrolment visit (Visit 1):** Participants who are deemed eligible for the trial (N=600) based on all the screening activities described above may be enrolled on the same day as screening to reduce inconvenience to study participants.
1. Randomization to the respective study groups
 2. Provision of the study agent under direct observation (all participants will receive PZQ at baseline), post-dose monitoring for at least 2 hours to actively capture and treat any adverse events.
 3. Blood sample collection and storage for analysis of biomarkers
 4. Scheduling of the next visit in 4 months (window ± 2 weeks).
- B. **Every 4 Month Study Visits (4 and 8 months from each annual visit = visits 2, 3, 5, 6, 8, 9)** Scheduled at 4, 8, 16, 20, 28, 32 months from baseline (window ± 2 weeks)
1. For women of child-bearing age: Urine sample for screening of pregnancy using rapid point-of-care test strips for Human Chorionic Gonadotropin (hCG).
 2. Provision of the study agent under direct observation (PZQ or placebo), post-dose monitoring for at least 2 hours to actively capture and treat any adverse events.
 3. Scheduling of the next visit in 4 months (window ± 2 weeks).

C. **Annual Visits** at 12, 24 months from baseline (window +/- 2 weeks) (see below for the 36-month visit = final study visit/close out). **Study visits 4 and 7**

1. Ultrasound of liver and spleen
2. History and physical examination
3. Liver function tests and Complete blood count
4. Blood sample collection and storage for analysis of biomarkers
5. Nutritional assessment (anthropometric measures)
6. Scheduling of the next visit in 4 months (window +/- 2 weeks)
7. Screening for Hepatitis B and C.
8. For women of child-bearing age: Urine sample for screening of pregnancy using rapid point-of-care test strips for Human Chorionic Gonadotropin (hCG).
9. Provision of the study agent under direct observation (all participants receive PZQ during annual visits), post-dose monitoring for at least for 2 hours to actively capture and treat any adverse events.

6.3.1 Follow-up

In between schedule study visits every 4 months, participants may report unsolicited adverse events including new onset hematemesis, other serious medical outcome that may warrant withdrawal, unexpected adverse events related to study drug administration occurring after the 2 hours observation period. Not scheduled.

6.3.2 Final Study Visit (Visit 10)

The final study visit will occur 3 years from the baseline/enrollment visit (study day 1) with a window of +/- 2 weeks.

1. Ultrasound of liver and spleen

-
2. History and physical examination
 3. Liver function tests and Complete blood count
 4. Blood sample collection and storage for analysis of biomarkers
 5. Nutritional assessment (anthropometric measures)
 6. For women of child-bearing age: Urine sample for screening of pregnancy using rapid point-of-care test strips for Human Chorionic Gonadotropin (hCG).
 7. Provision of the study agent under direct observation (all participants receive PZQ), post-dose monitoring for at least 2 hours to actively capture and treat any adverse events

6.3.3 Evaluation using ultrasound

Ultrasound scan will be performed at baseline to determine eligibility, and during annual follow up visits as detailed above. Sonographers with special training and experience in detecting schistosomiasis-related morbidity will carry out the procedure in the field. They will receive refresher training by Dr Joachim Richter, one of the authors of the Niamey protocol which is the WHO guiding document for detection and grading of schistosomiasis-related morbidity using ultrasound. Dr Richter will also be responsible for quality assurance by providing a second reading of the stored scans. The image pattern will be graded in line with **Figure 2** below.

The portal vein diameter will also be measured and used to derive the Portal Vein Quotient (PVQ) as follows:

$$PVQ = \frac{\text{Portal vein diameter}}{\text{Height}}$$

Where portal vein diameter is in millimeters (mm) and height in meters (m).

PVQ will be graded as follows, where dilatation implies presence of portal hypertension.

1. Normal: ≤ 7.5 mm/m

2. Dilated: 7.6 – 10 mm/m
3. Severely dilated: > 10 mm/m

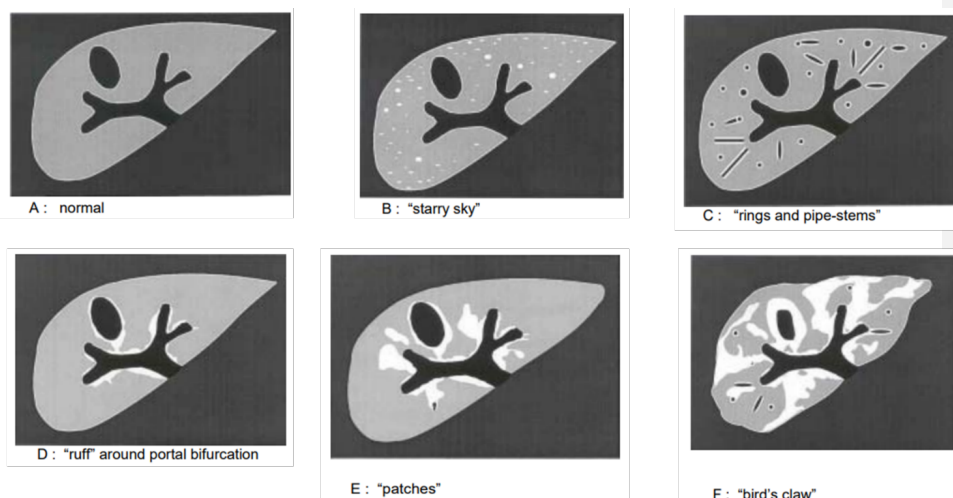


Figure 2: Grading of schistosomiasis-related liver fibrosis using ultrasound as per Niamery protocol: Grade B is considered non-specific to schistosomiasis and will be excluded from this study.

Severity of fibrosis is defined as:

Mild: Image Pattern C

Moderate: Image Pattern D

Severe: Image pattern E or F

6.3.4 Early Termination Visit

Early termination may result from a decision by the participant or the investigators as detailed in section 7.7. Under these circumstances, permission will be sought for use of data already collected and also safety follow up where required.

6.4 Unscheduled Study Visits

Unscheduled study visits may occur if participants report unsolicited adverse events including new onset hematemesis, other serious medical outcome that may warrant withdrawal, unexpected adverse events related to study drug administration occurring after the 2 hours observation period.

6.5 Protocol Deviations

A protocol deviation will be defined as any noncompliance with the clinical trial protocol, GCP, or protocol-specific Manual of Procedures (MOP) requirements. The noncompliance may be either on the participant, investigator, or study site staff. As a result of deviations, corrective actions will be developed and implemented promptly. It will be the responsibility of the site Principal Investigator and delegated study personnel to use continuous vigilance to identify and report protocol deviations. All individual protocol deviations will be addressed in participant study records. Protocol deviations will be sent to the local IRB/IEC and regulatory authorities as per existing guidelines. The site Principal Investigator and other study personnel will be responsible for knowing and adhering to IRB/IEC and regulatory requirements.

7 LABORATORY EVALUATIONS

7.1.1 Clinical Laboratory Evaluations

The following clinical laboratory evaluations will be conducted at our field laboratory in Lake Albert which has portable Piccolo Express Chemistry Analyzer (Abbot Laboratories) and Sysmex pocH-100i Automated Hematology Analyzer (McKesson).

Complete Blood Count: hemoglobin, hematocrit, white blood cells (WBC) with differential count, platelet count and red cell indices measured on Sysmex pocH-100i Automated Hematology Analyzer annually.

Liver function tests: total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT) measured using the Piccolo Express Hepatic function test cartridge annually.

Serology: Hepatitis B infection will be determined by presence of Hep B Surface Antigen using a POCT (SD Bioline WB™ HBsAg POCT strip (Korea) and Hepatitis C using the INSTI™ HCV antibody POCT (BioLytical, Ferndale, WA) on the day of the baseline visit for eligibility determination. These tests will be performed annually.

7.1.1.1 Laboratory Specimen Preparation, Handling, and Storage

During screening activities, 1ml of urine will be collected in a container pre-labeled with the participant's study ID. This will be used for screening for Circulating Cathodic Antigen (CCA) for diagnosis of *S. mansoni* infection.

At enrolment and annual follow-up visits, approximately 20ml of blood will be collected. This will be subdivided into purple top EDTA tube for plasma, yellow/gold top Serum Separator Tube (SST) for serum and green top Lithium Heparin vacutainers for PBMCs and plasma. Plasma, serum, and peripheral blood mononuclear cells (PBMCs) will be obtained and aliquots stored in liquid nitrogen in the field research site for transportation back to UVRI Uganda where they will be stored in a -80°C freezer awaiting analysis. Stored plasma, serum and PBMCs will be used in future

exploratory immunological assessments and metabolic profiling to understand immunopathology associated with fibrosis. Multiplexed fibrosis assays will be run approximately every six months until three months after study close out.

Hematology and liver function tests will also be done using part of the whole blood initially collected. Details of sample management will be outlined in the laboratory manual.

7.1.1.2 Laboratory sample Shipping

Samples will be transported from Lake Albert to UVRI by road in liquid nitrogen and stored at -80°C. We expect to set up the assays at the laboratory at MRC/UVRI & LSHTM Uganda Research Unit when appropriate are available. More complex immunological work and metabolic profiling will be conducted at our collaborator laboratory if necessary and if funds allow.

7.1.2 Laboratory Assays

We will assess biomarkers related to progression of hepatic fibrosis and differences by treatment group. Samples for the assays will be taken annually. To test for *S. mansoni* infection by CCA, we shall use the Point of Care Circulating Cathodic Antigen (Schisto POC-CCA®) test (Rapid Medical Diagnostics, Pretoria, South Africa), while serum CAA will be quantified using the up-converting phosphor-lateral flow circulating anodic antigen (UCP-LF CAA) assay (Leiden University Medical Centre, Netherlands)

Target markers of fibrosis for this clinical trial.

We will evaluate biomarkers that will identify individuals with *S. mansoni* associated hepatic fibrosis as diagnosed by ultrasound at baseline, as well as biomarkers that identify risk of progression from mild-moderate to higher grades as assessed by ultrasound annually for the three

years of follow up. We have developed a multiplexed assay (FibroPlex) that captures over 40 key analytes involved in fibro-proliferation, remodeling, and degradation that uniquely includes direct measures of collagen synthesis and degradation.⁶² We have also added novel biomarkers that have been more specifically related to hepatic fibrosis in the context of schistosomiasis including Procollagen III amino terminal peptide and hyaluronic acid that we will leverage.^{34,49}

Representative examples of each class are provided below:

- *Fibroproliferation:*

- **TGF- β 1:** potent profibrogenic cytokine. Promotes hepatic stellate cells activation, upregulates TIMPs, down-modulates MMP-s. Tissue fibrosis is primarily associated with TGF- β 1 isoform³⁸⁻⁴⁰.
- **IL-13:** Th2 cytokine. Promotes alternative activation of macrophages, induces TGF- β 1 production^{40,41,63} Increased levels in serum predict hepatic fibrosis in schistosome infected individuals.³⁵
- **Collagen type IV:** component of basement membrane (Disse space). Soluble Coll IV levels increased in schistosomiasis-induced hepatic fibrosis⁶⁴
- **Procollagen III:** has been associated with increased levels among patients with schistosomiasis, specifically increased in individuals with higher grades of fibrosis.³⁴
- **Hyaluronic acid:** is regulated by TGF- β and elevated in many fibrotic diseases including *S. mansoni*, including increased serum levels by ultrasound determined grade of fibrosis using the Niamey protocol⁴⁹

- *Remodeling/Effect Modifiers:*

- **BMP-7:** natural TGF- β antagonist⁶³. Adenovirus expression of BMP-7 suppresses the development of hepatic fibrosis in rats⁶⁵

-
- **CTGF**: enhancer of TGF- β 1, inhibits BMP-7.⁶⁶
 - **TIMP-1**: specific tissue inhibitor of MMPs with 4 isoforms, type 1 inhibits all MMPs except for MMP-2
 - *Fibro-degradation*
 - **MMP-1**: degradation and remodeling of ECM (4). MMP-1 degrades fibrillar collagens (like Coll I).⁶⁷ The imbalance between TIMPs and MMPs is an important determinant of ECM deposition and breakdown.

In addition to the aforementioned biomarkers which could ultimately be developed as lateral flow POCTs, we will also examine newer, innovative markers of fibrosis both at baseline and longitudinally to better understand the biology of fibrosis. MiRNAs in the context of schistosomiasis-related fibrosis have both pro- and anti fibrogenic roles.⁴⁸ We will evaluate MiRNAs associated with both pro-fibrogenic (MiR-21 and miR-96, miR-351) and anti-fibrogenic roles (miR-203-3p, Let-7b). MiRNAs could also serve as biomarkers for diagnosing current fibrosis before overt clinical symptoms as well as those who are greater risk of progression, for more aggressive treatment MiRNAs could also more sensitively capture response to therapy in the trial and ultimately may be developed as therapeutic targets.^{48,50} We will employ RT-PCR to capture relevant miRNAs.

ASSESSMENT OF SAFETY

7.2 Assessing and Recording Safety Parameters

Given PZQ is FDA approved at the dose used for the study (40 mg/kg), safety parameters are not primary endpoints. Safety will be assessed by the frequency and severity of actively monitored and passively captured adverse events continuously in the two hours after every investigational product administration throughout the duration of the trial.

7.2.1 Adverse Events (AEs)

We will use the ICH E6 definition of an AE as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it will be recorded as an AE.

7.2.2 Reactogenicity

Reactogenicity events are AEs that are common and known to occur following administration of praziquantel. They include low grade fever, vomiting, abdominal pain, nausea, malaise, myalgias, and fatigue as detailed above. They will be graded according to the severity scale above.

7.2.3 Serious Adverse Events (SAEs)

We will employ the following definition of Serious adverse event or serious suspected adverse reaction: An adverse event or suspected adverse reaction will be considered “serious” if, in the view of the investigator, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or

significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

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

- Death
- Life-threatening: An AE is considered “life-threatening” if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

AEs, SAEs, SAR and other events will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study clinician listed on the Form FDA 1572 or by the Institution as the site Principal Investigator or Sub-Investigator.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study physician (for IND studies, a physician listed on the Form FDA 1572 as the site Principal Investigator or Sub-Investigator).

- Reviewed and evaluated by DMID, the DSMB, IRB/IEC and National Drug Authority of Uganda.

7.2.3.1 Adverse Events Grading and relationship to study product

Safety events will be graded as detailed in the table below:

Table 2 – Severity grading for AE/SAE/SARs and other safety events

Grade	Severity	Definition
Grade 1	Mild	asymptomatic or mild symptoms; no or minimal interference with usual social & functional activities, intervention not indicated
Grade 2	Moderate	moderate symptoms causing greater than minimal interference with usual social & functional activities, intervention indicated
Grade 3	Severe	severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated
Grade 4	Potentially Life Threatening	symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability or death
Grade 5	Death	Death

The relationship to the study product will be assessed and classified as follows:

- Not related:** an event not related to the use of study vaccine
- Doubtfully (unlikely):** An event for which an alternative explanation is more likely e.g. con meds/disease or the relationship in time suggests that a causal relationship is unlikely

-
- iii) **Possibly:** An event that might be due to the use of study vaccine. An alternative explanation e.g. con meds/disease is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
 - iv) **Probably:** An event that might be due to the use of study vaccine. The relationship in time is suggestive (e.g. confirmed by de-challenge). An alternative explanation e.g. con meds/disease is less likely.
 - v) **Definitely:** An event that is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation e.g. concomitant medication/disease. The relationship in time is very suggestive (e.g. it is confirmed by de-challenge and re-challenge)

7.3 Specification of Safety Parameters

Given PZQ is FDA approved at the dose used for the study (40 mg/kg), safety parameters are not primary endpoints. Safety will be assessed by the frequency and severity of actively monitored and passively captured adverse events continuously in the 2 hours after every investigational product administration throughout the duration of the trial.

Safety will be assessed by the frequency and severity of adverse events up to two hours after study product administration.

7.3.1 Solicited Events

Solicited events are AEs that are common and known to occur following administration of praziquantel. Safety will be assessed by the frequency and severity of actively monitored (solicited) adverse events continuously in the 2 hours after every investigational product administration. We will specifically capture known reactogenicity to PZQ including but not limited

to abdominal pain, vomiting, diarrhea, headache, low grade fever, malaise, and myalgia. We will provide acetaminophen which mitigates headache, low grade fever, and myalgia.

7.3.2 Unsolicited Events

All participants will be asked to speak to study staff about any adverse events for two hours after administration of the study product.

7.3.3 New-Onset Chronic Medical Conditions (NOCMCs)

Participants in this study are at risk for developing uncompensated hepatic fibrosis with esophageal varices and bleeding since all will have some degree of hepatic fibrosis (and many portal hypertension) at baseline. If participants develop this, they will need to be referred to care for medical (e.g. propranolol) or surgical interventions (esophageal “banding” as available). These participants would be withdrawn if this is developed.

7.4 Reporting Procedures

7.4.1 Reporting Serious Adverse Events

SAEs will be reported to the sponsor within 24-hours of site awareness and to the ethics and regulatory authorities in Uganda within seven days. These include Uganda Virus Research Institute REC, UNCST and National Drug Authority, in line with UNCST guidelines on research involving humans (2025).

All SAEs will be reported throughout participation of the individual in the study, whether solicited or unsolicited. SAE followed until resolution. If a participant withdraws or is withdrawn from the trial when an SAE is still ongoing, permission will be sought for further safety follow up where necessary. Linkage to appropriate care will also be done. Resolution of an SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic. This study will not be under IND as it uses an FDA approved dose of PZQ of 40 mg/kg.

7.5 Type and Duration of Follow-up of participants after Adverse Events

AEs will be assessed and followed from initial recognition of the AE through end of the protocol defined follow-up period, however, based on the half-life of PZQ (less than 4 hours) we only expect these to last through the day of dosing for AE that are reactogenic to the dose. SAEs will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined the follow-up period. Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

7.6 Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

1. **Anemia** Individuals found to be anemic will be treated as per country clinical guidelines.
2. **Uncompensated hepatic fibrosis.** Participants with esophageal varices and bleeding on ultrasound at baseline or any annual visit will need to be referred to care for medical (e.g. propranolol) or surgical interventions (esophageal “banding” as available).
3. **Hepatitis B and C**-Though no treatment is amiable for these viral infections, it is crucial that women of reproductive age be notified so that their newborns may receive timely Hepatis B vaccination and repeat doses in infancy. In addition, both are sexually transmitted viruses and individuals should be aware and advised to use condoms, in addition to partner testing.

7.7 Halting Rules

Given that PZQ is approved by FDA and WHO, and licensed in Uganda at the applied dose for treatment of schistosomiasis, we do not expect significant rates of SAEs that would necessitate halting the study due to immediate safety concerns. Therefore, halting rules are based on difference

in the longitudinal impact on fibrosis with a focus on worsening of grade of fibrosis **in either treatment arm** from baseline.

With respect to **futility**, it is possible that the impact of more frequent treatment increases after one and two years of the RCT and it is important to capture the true longitudinal impact of this to inform changes in public health approaches, particularly an assessment of the true benefit of more frequent treatment over time to inform MDA approaches. It is possible that participants continue to experience incremental improvement in grade of fibrosis even up to three years. Findings may also support more community, rather than school-based treatment to target older children and adults, even if we see improvements in fibrosis with annual treatment for three years. Given this, we do not propose halting for futility. Please see below for more details.

7.7.1 Study Halting Criteria

We will employ halting rules to address the possibility, though unlikely, that more frequent treatment will culminate in increased rates of fibrosis progression from baseline. After 100 participants have had one year follow up visits that include ultrasound, we will assess the rate of **progression** or worsening of fibrosis from baseline to one year after follow up and again after two years of follow up as a safety risk.

While WHO recommends annual treatment for individuals living in endemic areas with prevalence greater than 10%, there is a concern that the increased frequency in the three treatments per year arm could pose a safety risk of increased fibrosis. To mitigate this concern, we will perform an interim analysis after 100 participants have completed one year of treatment. Specifically, we will examine the change in fibrosis severity between the two dosing arms, under the concern that participants who received three annual treatments will show worse fibrosis after one year. Repeated measures regression analysis will be used, modeling average fibrosis severity at baseline and one year follow-up. Evidence of a safety concern will be demonstrated by an

interaction term which is a product of time and treatment condition. This coefficient represents the difference in differences, suggesting that participants who received three PZQ treatments annually showed a greater increase in fibrosis severity from baseline to follow up than those who only received a single treatment annually.

Aware that repeated hypothesis testing can increase the likelihood of a type one error, an alpha spending approach will be used. The goal is to ensure only a 5% chance of a false positive significance test, however performing multiple tests may result in significance simply due to performing multiple tests. Alpha spending addresses this by reducing the critical value used to determine significance. Instead of deeming a test significant if $p < 0.05$, a smaller p value will be required. This reduces the likelihood that any one significance test will be significant such that the overall probability of significance across all tests performed retains 5% type one error rate. This procedure is referred to as spending because the critical p values used for the interim analysis and the final analysis are designed to add together to be equal to 0.05. In effect, each interim analysis spends type one error rate, directly reducing the p value that needs to be observed in the final analysis to be considered significant.

In this case, we used recommendations from O'Brien and Fleming for determining what critical values to use. Performing the interim analysis at 100 participants enrolled represents one-sixth of the 600 participants for total enrollment. As such, the interim analysis will be considered significant if the two tailed probability is less than $0.05 / 6 = p < 0.008$. In this event, the trial will be stopped out of concern for participant safety. Having spent 0.008 on the interim analysis, the final analysis will only be determined significant if the two tailed probability is less than $0.05 - 0.008 = p < 0.042$. This will ensure participants are safe during study participation, while mitigating the possibility that results are significant due to repeated testing.

In this case, we used recommendations from O'Brien and Fleming for determining what critical values to use. Performing the interim analysis at 200 participants enrolled represents one third of the 600 participants for total enrollment. As such, the interim analysis will be considered

significant if the two tailed probability is less than $0.05 / 3 = p < 0.017$. In this event, the trial will be stopped out of concern for participant safety. Having spent 0.017 on the interim analysis, the final analysis will only be determined significant if the two tailed probability is less than $0.05 - 0.017 = p < 0.033$. This will ensure participants are safe during study participation, while mitigating the possibility that results are significant due to repeated testing.

Overall, the study may be halted if new evidence emerges that renders continuation of the study futile or unethical. This may arise from discovery of alternate treatment for schistosomiasis-related fibrosis which is considered overwhelmingly more efficacious.

7.7.2 Individual Halting Criteria

A participant will be discontinued from the study if he/she experiences a severe allergic/anaphylactic reaction to any dose of the study product. Participants with uncompensated hepatic fibrosis with esophageal varices and bleeding need to be referred to care for medical (e.g. propranolol) or surgical interventions (esophageal “banding” as available). These participants would be withdrawn and referred for care if this is developed during the study. If women become pregnant, study procedures which include blood draws, ultrasound, and treatment will be deferred until after delivery when full study participation will resume. Pregnant women will be referred and appropriately supported to receive antenatal, delivery and post-natal care services. Furthermore, a participant may be withdrawn if the investigators deem that they have not complied with study procedures under reasonable circumstances, for example, deliberate use of praziquantel outside the study. A participant may also decide to withdraw participation at any point without giving reason to the study team. Irrespective of the reason for withdrawal, permission will be sought and appropriately documented, for further use of data already collected and further safety follow up where necessary.

7.8 Safety Oversight

7.8.1 Independent Safety Monitor (ISM)

For this clinical trial an ISM is not required.

7.8.2 Data and Safety Monitoring Board (DSMB)

Safety oversight will be conducted by a DSMB that will be an independent group with expertise to interpret data from this study and will monitor study progress as well as participant safety. The DSMB members will be separate and independent of study personnel and will not have scientific, financial or other conflict of interest related to this study. The DSMB will consist of at least three voting members, including a biostatistician experienced in statistical methods for clinical trials and a clinician with relevant expertise. A charter will be developed, detailing the operational details of the DSMB.

ETHICS AND HUMAN SUBJECTS PROTECTION

7.9 Institutional Review Board/Independent Ethics Committee

Approval for this protocol will be obtained from ethics and regulatory authorities in Uganda and from Rhode Island Hospital IRB and supporting documentation sent to NIH before initiating recruitment procedures. In Uganda, approval will be sought from the following entities: a) Uganda Virus Research Institute Research Ethics Committee (UVRI-REC), b) Uganda National Council for Science and Technology (UNCST) and c) National Drug Authority (NDA).

The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations.

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

The IRB/IEC will determine that adequate provisions are made for soliciting the permission of each child's parent(s) or legal guardian.

7.10 Informed Consent Process

Written informed consent will be obtained from each participant aged 18-years or older. For children aged 15-17 years, assent will be sought from the child in addition to consent from a parent or authorized caretaker. Consent for study procedures and activities will involve the following:

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- Consent or assent (in addition to parental consent) for screening for diagnosis of schistosomiasis and liver fibrosis.
 - Consent or assent (in addition to parental consent) for enrolment (randomization), administration of study drug, sample collection and other follow up procedures.
 - Upon enrolment, each participant will also consent or assent (in addition to parental consent) for storage of samples for future use.

Informed consent forms will be IRB-approved, with evidence of a stamp indicating expiry of approval. Participants will be required to sign the informed consent form prior to starting any study-specific procedures.

Step-by-step procedures for obtaining informed consent will be detailed in the relevant SOP.

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

We will seek consent of the parent/guardian assent for all adolescent participants below the age of 18 years. This will be followed by obtaining assent from the participant. It is only after both have given written permission as confirmed by parental consent and assent forms that any study-related activities and procedures will be conducted.

7.10.2 Other Informed Consent Procedures

Illiterate Participants

We expect many participants in the proposed study will be illiterate. Participants who are not literate will request for an impartial witness to read and relay information from the consent form and information sheet, after which the participant will be asked to consent based on his/her understanding of the procedures as witnessed by a third party according to Office of Human

Research Protections guidelines (Title 45, Part 46). They will indicate their agreement to participate by thumb print on the consent document.

7.11 Consent for Future Use of Stored Specimens and Data

Aliquots of residual serum samples will be stored for future use specimens from residual samples (after the protocol assays are completed). These will be stored de-identified with links to other clinical and laboratory meta-data. A separate consent or assent (in addition to parental consent) form will be signed for permission to store samples for future use.

7.12 Confidentiality

Confidential data includes documentation, investigation data, participant's clinical information, and all other information generated during participation in the study. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the participant. Participant confidentiality will be maintained when study results are published or discussed in conferences. The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

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

7.13 Costs, Participant Compensation, and Research Related Injuries

Participants will incur no costs for study-related tests, procedures, and study product while taking part in this trial. Participants will either be transported using the study vehicle or receive transport reimbursement based on distance. Additionally, each participant will receive Uganda Shillings 20,000 as compensation for time for each scheduled visit. The same rates for transport and compensation for time will apply to unscheduled visits initiated by the study staff. Parents/guardians who accompany participants under 18 years of age for such procedures as consent will be transported accordingly and also receive Uganda shillings 20,000 as compensation for time separate from the primary participant. For unscheduled visits such as sick visits that are initiated by the participant, transport reimbursement will be provided and the cost of treatment or referral incurred by the study.

If it is determined by the site principal investigator that an injury occurred to a participant as a direct result of the tests or treatment or other aspects of participation in the trial, then referrals to appropriate health care facilities will be provided at no cost to the participant. In line with the laws of Uganda, the study shall procure clinical trial insurance that will be responsible for compensation of research-related injuries as may be required. No financial compensation will be directly provided by the NIAID, NIH.

8 STATISTICAL CONSIDERATIONS

8.1 Study Hypotheses

Primary Endpoint

The study hypothesizes (primary endpoint) that participants treated every 4 months with PZQ vs annually for three years will experience a higher rate of improvement in grade of fibrosis (mild, moderate, severe) as assessed by liver ultrasound.

Secondary Endpoints

The study hypothesizes (secondary endpoint) that participants treated every 4 months with PZQ vs annually for three years will experience a higher rate of resolution of portal hypertension as assessed by liver ultrasound.

The study hypothesizes (primary endpoint) that participants treated every 4 months with PZQ vs annually for three years will experience a higher rate of improvement in grade of fibrosis (mild, moderate, severe) as assessed by liver ultrasound after one and two years. Superiority.

The study hypothesizes (secondary endpoint) that participants treated every 4 months with PZQ vs annually for three years will experience a higher rate of resolution of portal hypertension as assessed by liver ultrasound after one and two years.

8.2 Sample Size Considerations

Our proposed sample size for the trial ($N = 600$) is based on the primary outcome of interest which is improvement in grade of fibrosis from baseline to three years comparing annual vs. every 4 months treatment. Improvement will be defined as any decline in grade of fibrosis eg moderate to mild, moderate to none. We will compare the overall percentage of individuals in each group who experienced improvement. We will recruit $N=600$ ($150 * 4 = 600$) to allow an estimated lost to

follow up of 20% based on our previous shorter studies (18 months) with approximately 12% loss to follow up. Recruiting $N = 600$ will yield approximately 480 participants or 120 in each age/sex sub-group in the analytic data set ($600 - (600 \times 0.20) = 480$). As above, when we reach approximately $N=140$ in each age/sex stratum, we will end screening in the relevant stratum and continue to screen and recruit into the trial for strata still requiring participants. Databases will be programmed to alert when $N=140$ is reached for each stratum so screening can be modified in real time.

Using studies that have demonstrated improvement among 58% of participants with single, annual treatment for *S. mansoni*,⁹ we will have 91% power to detect a null difference of proportions of 15% between groups (58 vs. 43%), with $\alpha = 0.05$, even with an expected loss to follow up of 20%. Importantly, we would like to examine treatment effects by both age group (15-24 and 25-40 years) and biologic sex. In these two sets of sub-groups, we will have $480/2 = 240$ participants in each sub-group. For these sub-group analyses, we will have 88% power to detect an absolute treatment effect of 20% within each sub-group with $\alpha=0.05$. We will have 80% power to detect effect modification, i.e. a difference in treatment effect, of approximately 25% between sub-groups.

We will not stratify analyses based on baseline severity of fibrosis as all participants will have some grade of fibrosis and the outcome of interest is any improvement in grade.

Key secondary outcomes include:

- a) percent of participants experiencing decrease in stage of portal hypertension at one and two year timepoints,
- b) percent of participants experiencing improvement in grade of fibrosis and portal hypertension after one and two years of treatment and
- c) changes in key biomarkers of fibrosis

We expect similar sample size or power calculations for the secondary endpoint (a) above as for the primary endpoint of change in grade of fibrosis over three years. We expect lower power to

detect differences for the secondary endpoints (b) captured after only one or two years since the effect size (percent of individuals experiencing a change after only one year) will be lower. However, we have increased the overall sample size for the study to include analyses by two main sub-groups (age groups and sex) as above. We expect that we will have the power to detect differences at one and two years for secondary endpoints, though perhaps not by age and sex.

With respect to sample size for the evaluation of biomarkers that best identify individuals at risk of fibrosis progression, we will limit these analyses to the group randomized to once annual treatment as this is the cohort for whom we would ultimately like to identify those who will need more frequent treatment. Specifically, we are interested in predicting which individuals will experience progression of their schistosomiasis-related fibrosis vs those who do not progress or experience improvement. To identify a cut-off to discriminate individuals with progression of hepatic fibrosis from individuals without this diagnosis for a given biomarker, we first estimate an allocation ratio (the ratio of the number of cases (fibrosis progression) to number of controls (no fibrosis progression)). Based on our previous longitudinal study of fibrosis, we found that approximately 15% experienced progression one year after a single treatment, giving an allocation ratio of 5.66.³⁷

We use this allocation ratio, a pre-specified $\alpha = 0.05$ and power = 0.90, and target AUC of 0.7. With these parameters we would need a sample size of 133 to determine that the optimal “cut off” to identify individuals at risk of fibrosis progression. We will have N=300 randomized to the annual treatment arm, providing sufficient power to examine biomarkers by age and sex with an estimated 20% loss to follow up.

8.3 Treatment Assignment Procedures

8.3.1 Randomization Procedures

The trial biostatistician will generate and provide randomization codes by study number in blocks stratified by age (15-24 and 25-40 years)/sex sub-categories to the pharmacist. The pharmacist will use the randomization list to package the study agent product (praziquantel or placebo) in containers pre-labelled with each participant's study ID according to the visit time point. Neither the investigators nor the participant will be able to tell the randomization group because the randomization will be concealed and the placebo matched to the praziquantel.

8.4 Planned Interim Analyses

8.4.1 Interim Safety Review

Praziquantel is approved by FDA and is safe for community-wide distribution during MDA programs at a dose of 40 mg/kg. Despite the increased frequency (every 4 months) in comparison to routine practice, the half-life of praziquantel is only 0.8 - 1.5 hours, implying there is no concern for elevated drug levels four months after treatment.

We will conduct interim safety reviews to address the possibility, though unlikely, that more frequent treatment will culminate in increased rates of fibrosis progression from baseline. After 100 participants have had one year follow up visits that include ultrasound, we will assess the rate of **progression** or worsening of fibrosis from baseline to one year after follow up and again after two years of follow up as a safety risk. While WHO recommends annual treatment for individuals living in endemic areas with prevalence greater than 10%, there is a concern that the increased frequency in the three treatments per year arm could pose a safety risk of increased fibrosis. To mitigate this concern, we will perform an interim analysis after 100 participants have completed one year of treatment. Specifically, we will examine the change in fibrosis severity between the

two dosing arms, under the concern that participants who received three treatments will show worse fibrosis after one year.

The Data and Safety Monitoring Board (DSM) will also review adverse event rates, and SAEs to ensure safety of participants. They will also review other elements that could affect the integrity of the trial, including participant attrition and protocol adherence.

8.4.2 Interim Efficacy Review

Interim analyses for efficacy are planned 100 participants have completed their one year follow up visit with ultrasound. Suggested meeting schedule for the DSMB would be approximately 3 months after the baseline enrollment. At that meeting, we suggest a review of recruitment, enrollment, and reactogenicity at the time of the first dosing. We then suggest meeting about one year later, after 100 participants have completed their one-year post baseline ultrasounds have been completed to review safety data and participant progress/attrition, and yearly for two more years to review halting rules, safety and participant progress/attrition. Suggested schedule as follows:

1. Meeting one: Charter review including agreement on remit and schedule of meetings. Before recruitment and enrollment.
2. Meeting two: (three months after initial participants enrolled into trial). Three months after baseline visit to recruitment, enrollment, and reactogenicity at the time of the first dosing.
3. Meeting three: (about 15 months after initial participants enrolled into trial) Approximately one year after meeting two when about N=100 participants have completed their one year post baseline treatment ultrasounds have been completed to review halting rules, safety data, protocol fidelity, and participant progress/attrition after one year of follow up for about 100 participants.
4. Meeting four: (27 months after initial participants enrolled into trial). Approximately one year after meeting two to review safety data, protocol fidelity and participant progress/attrition after two years of follow up.

8.5 Final Analysis Plan

We will first conduct descriptive analyses to evaluate the distribution of potential confounders (including baseline age, sex, nutritional status, grade of fibrosis) by treatment allocation.

For the primary outcome, we will compare the percent of individuals who experience any reduction in grade of fibrosis across the two treatment arms, adjusting for age and sex as the randomization is stratified by these factors. Though we expect randomization will culminate in balancing of other potential confounders, we will additionally adjust for these in binomial regression models if there are significant differences ($P < 0.01$ at baseline).

Binomial regression is used as percentages are “bounded” such that it is preferable to use binary (0/1) for each participant who does or does not experience improvement in fibrosis at three years. We will employ the same approach for the secondary outcome, portal hypertension at three years as well as secondary outcomes of progression of fibrosis at one and two years.

We will also evaluate the role of effect modification by age group (15-24 and 25-40 years) and sex. Interactive terms will be fit on a multiplicative scale. We do not have specific hypotheses to evaluate interaction terms on an additive scale ie we do not hypothesize that the combined effect of sex and age group is larger than the *sum* of the individual effects of the two exposures. Unless there are large differences (effect size) in different sub-groups it is unlikely that policies can be derived that differ by subgroups due to the complexity this adds to implementation. Thus, it is only likely to be relevant if the effect size differences are large, which we are powered to detect. For the secondary outcomes of progression of fibrosis at one and two years, we will employ similar binomial regression approaches, though we may not have sufficient power to conduct sub-group analyses as it is likely that effect sizes for these outcomes will be lower given the shorter timeframe for these outcomes.

If interactions are significant, we will stratify analyses to present the effect size for key subgroups (age and sex strata) such that clear approaches can be recommended for distinct groups as needed. Intention to treat analyses will be applied. More details will be provided in a Statistical Analysis Plan developed by study Biostatistician.

9 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

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

10 QUALITY CONTROL AND QUALITY ASSURANCE

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

A designated monitor will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to the sponsor, PIs and DSMB, as requested.

Much of the data collected will be entered into tablets directly. The database will be programmed with automatic checks that will ensure that data input is logical, complete and within acceptable limits. These are programmed to alert in “real time” during data entry.

For ultrasound assessment, in the first year of the trial Dr. Joachim Richter, one of the authors of the Niamey protocol, will complete inter-rater reliability assessments to ensure all ultrasonographers are reliably capturing the correct grade of fibrosis and portal hypertension.

Standard Operating Procedures (SOPs) for standardization of study procedures and activities.

Commented [AE2]: Quality control procedures for ultrasound need to be further defined.

11 DATA MANAGEMENT

The Principal Investigators will be responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Data collection will be the responsibility of the study personnel at the participating clinical study site under the supervision of the site principal investigator.

The data management center at MRC/UVRI & LSHTM Uganda Research Unit will be responsible for data management, quality review, analysis, and reporting of the study data with oversight from the site PI, Data Manager and co-investigators. Weekly checks on data in REDCap will be done with queries assigned to the study implementation team who will be able to correct any errors within the time stipulated in the data management SOP.

12 CLINICAL MONITORING

Site monitoring will be conducted to ensure that the human participants' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. The study sponsor will appoint an External Monitor for the purpose.

13 PUBLICATION AND DISSEMINATION POLICY

Prior to the collection of any data, the MPIs and co-investigators will meet and draft a MOP outlining the planned primary manuscripts including the primary outcome manuscript. A draft title is generated, and a list of likely authors is provided specifying first, final, and corresponding authors for each. Care will be taken to include co-investigators from MRC Uganda on all manuscripts including a prominent role (e.g. first author) on key manuscripts.

The Trial will be registered on <https://ClinicalTrials.gov> no later than 21 days after the enrollment of the first participant and results will be submitted no later than 12 months following the primary completion date. Following completion of the study, the lead Principal Investigator will publish the results of this research in a scientific journal. The publication will be submitted to the National Library of Medicine's PubMed Central (<http://www.ncbi.nlm.nih.gov/pmc/>) an electronic version of the final, peer-reviewed manuscripts upon acceptance for publication as per NIH requirement.

For this trial the responsible party is Jennifer Friedman who will register the trial and post results.

Research findings will also be disseminated at Uganda Virus Research Institute, Ministry of Health Uganda, participating districts and communities. This will be done with involvement of the Uganda National Council for Science and Technology (UNCST), while maintaining confidentiality of the study participants. In the participating communities, effort will be made to translate the findings into the appropriate local language and convert the information into pictorial format for ease of comprehension.

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15 APPENDICES

Schedule of Events

Appendix A. Schedule of Events

Evaluation	Final Screening/ Enrollment Visit 1 Day 1	Interval Visits Every 4 months (Visit # 2,3,5,6,8,9)	Annual Visits at 12, 24 months (Visit # 4, 7, 10)	Study close out Visit 10
Signed consent form	X			
Confirmation of eligibility criteria	X			
Medical history by study Physician	X		X	X
Physical exam by study Physician	X		X	X
Concomitant medications	X	X	X	X
Liver Ultrasound	X		X	X
Complete Blood Count	X		X	X
Liver function tests	X		X	X
Serology for Hepatitis B and C	X			
Aliquot for fibrosis biomarkers collected	X		X	X
Treatment for Soil Transmitted Helminths as needed	X		X	X
Treatment for Iron Deficiency as needed	X		X	X
Study intervention	X	X	X	
Observation for 2 hours to actively solicit AE post dose	X	X	X	X
All participants with <i>S. mansoni</i> treated with PZQ (close out)				X
Schedule next visit in four months	X	X	X	

List of Tests to be Done

Complete Blood Count: hemoglobin, hematocrit, white blood cells (WBC) with differential count, platelet count and red cell indices measured on Sysmex pocH-100i Automated Hematology Analyzer

Liver function tests: total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT) measured using the Piccolo Express Hepatic function test cartridge.

Serology: Hepatitis B infection will be determined by presence of Hep B Surface Antigen using a POCT (SD Bioline WBTM HBsAg POCT strip (Korea)) and Hepatitis C using the INSTITM HCV antibody POCT (BioLytical, Ferndale, WA). Both on the day of the baseline visit for eligibility determination.

Circulating Cathodic Antigen- this POCT will be done as an initial field based urine screening to determine if individuals are infected with *S. mansoni*.

Fibrosis Biomarkers – Will be assessed at baseline and annually including at study close out

Study Intervention – Administered at baseline and every 4 months (PZQ or Placebo) and annually at 12 and 24 months post baseline (visit 1).

Appendix B. TOXICITY TABLE

Laboratory outcome	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life threatening
Liver function tests; ALT, AST increase by factor of	1.1 -2.5 X Upper limit of normal (38.5-90.9 U/l)	2.6 -5.0 X upper limit of normal (91.0-178.4 U/l)	5.1 -10 x upper limit of normal (178.5-350 U/l)	X10 X upper limit of normal < 350 U/l
Bilirubin when liver function tests are normal; increase by factor of	1.1-1.5 X Upper limit of normal (1.1–1.5 mg/dl)	1.6-2.0 X Upper limit of normal (1.6-1.9 mg/dl)	2.0-3.0 X Upper limit of normal (2.0-3.0 mg/dl)	> 3.0 X Upper limit of normal > 3.0 mg/dl
Bilirubin when accompanied by any increase in liver function test; increase by factor of	1.1-1.25 X Upper limit of normal (1.1-1.25 mg/dl)	1.26-1.5 X Upper limit of normal (1.26-1.5 mg/dl)	1.51-1.75 X Upper limit of normal (1.51-1.75 mg/dl)	> 1.75 X Upper limit of normal >1.75 mg/dl
White blood cell count high (cell/mm ³)	10,800 - 15,000	15,001 - 20,000	20,001 - 25, 000	> 25,000
White blood cell count low (cell/mm ³)	2,500 - 3,500	1,500 - 2,499	1000 – 1,499	< 1,000
Platelet count low (cell/mm ³)	125,000 - 140,000	100,000 - 124,000	25,000 - 99,000	< 25,000