Clinicaltrials.Gov. NCT03893097

A proof-of-concept trial to evaluate artesunate-mefloquine as a novel alternative treatment for schistosomiasis in African children (SchistoSAM)

Revised Protocol
Version 1.5, 19-February-2020

Sponsor:	Institute of Tropical Medicine Nationalestraat 155 2000 Antwerpen Belgium
International Coordinating Investigator:	Dr. Emmanuel Bottieau
National Coordinating Investigator:	Pr. Souleymane Mboup
Principal Investigator:	Dr. Moustapha Mbow

ClinicalTrials.Gov.:	Registration will be done after study approval
Study Title:	A proof-of-concept trial to evaluate artesunate-mefloquine as a novel alternative treatment for schistosomiasis in African children
Study Acronym	SchistoSAM
Version:	Version 1.5
Previous version:	1.4, dd. 04/Jun/2019
Date:	19 February 2020
Study drugs:	Artesunate-mefloquine Praziquantel
Funder:	SOFI
Sponsor:	Institute of Tropical Medicine, Antwerp, Belgium

Sponsor study team

Institute of Tropical Medicine (ITM)

Name	Function	Institution	Contact details
Emmanuel Bottieau	Coordinating Investigator	ITM	ebottieau@itg.be
Katja Polman	Co-coordinating Investigator	ITM	kpolman@itg.be
Anna Rosanas	Head of Malaria Unit	ITM	arosanas@itg.be
Isabel Brosius	Clinician	ITM	ibrosius@itg.be
Clementine Roucher	Biomedical Research Assistant	ITM	croucher@itg.be
Yven van Herrewege	Head of the Clinical Trial Unit	ITM	yvanherrewege@itg.be
Annelies De Hondt	Clinical Trials Scientist	ITM	adehondt@itg.be
Christophe Burm	Data manager	ITM	cburm@itg.be
Diana Arango	Data reviewer	ITM	darango@itg.be
Achilleas Tsoumanis	Statistician	ITM	atsoumanis@itg.be

Site study team in Senegal

Institut de Recherche en Santé, de Surveillance Épidémiologique et de Formation (IRESSEF)

Name	Function	Institution	Contact details
Pr. Souleymane Mboup	National Coordinating Investigator	IRESSEF	Souleymane.mbow@iressef.org
Dr Moustapha Mbow	Country Principal Investigator	UCAD/IRESSEF	Moustapha2.mbow@ucad.edu.sn moustapha.mbow@iressef.org
Dr Badara Cissé	Epidemiologist	IRESSEF	badara.cisse@lshtm.ac.uk
Mr Babacar T. Faye	Data Manager	UCAD	babacarfaye626@gmail.com

STATEMENT OF COMPLIANCE

By signing this protocol, the Investigator(s) acknowledge(s) and agree(s):

This protocol contains the necessary information for conducting this clinical study. The Principal Investigator will conduct this study as detailed herein and will make every reasonable effort to complete the study within the time designated. The Principal Investigator commits to carry out the study in compliance with the protocol, amendments, applicable procedures and other study-related documents provided by the Sponsor, and in compliance with the Declaration of Helsinki, Good Clinical [Laboratory] Practice (GC[L]P), the EU General Data Protection Regulation (GDPR), the data protection regulation of the Comité National d'Ethique de Recherche en Santé of Senegal, the ESF/ALLEA Code of Conduct for Research Integrity, and applicable regulatory requirements.

The protocol and all relevant study information, which is provided by the Sponsor, will be made available to the physicians, nurses and other personnel who participate in conducting this study. The Investigator will use this material for their training so that they are fully informed regarding the drugs and the conduct of the study.

The Sponsor of this study – the Institute of Tropical Medicine in Antwerp, Belgium (ITM) – can at any time have access to the source documents from which Case Report Form information may have been generated and will be permitted to perform trial-related monitoring and audits. All study material will be maintained according to regulatory requirements and until the Sponsor advises that retention is no longer necessary.

DIRECTOR OF THE INSTITUTE OF TROPICAL MEDICINE:

Title, Name: Dr. Marc-Alain WIDDOWSON Date: _____

Signed:

COORDINATING INVESTIGATOR:

Title, Name: Dr. Emmanuel BOTTIEAU Date: _____

Signed:

CO-COORDINATING INVESTIGATOR:

Title, Name: Dr. Katja POLMAN Date: _____

Signed:

NATIONAL COORDINATING INVESTIGATOR:

Title, Name: Pr. Souleymane MBOUP Date: _____

Signed:

COUNTRY PRINCIPAL INVESTIGATOR:

Title, Name: Dr. Moustapha MBOW Date: _____

Signed:

Signing this document, I commit to carry out the trial in accordance with the protocol, Good Clinical Practice and applicable ethical and regulatory requirements. I also acknowledge the paragraph relevant to study confidentiality and authorize the Institute of Tropical Medicine, Antwerp, Belgium and the Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation in Senegal to record my data on a computerized system containing all the data pertinent to the study.

Table of Contents

LIST OF ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AM	Artesunate-Mefloquine
CAA	Circulating Anodic Antigen
CCA	Circulating Cathodic Antigen
CI	Confidence Interval
CR	Cure Rate
CRS	Clinical Research Scientist
C_t	Cycle threshold
CTU	Clinical Trial Unit
DBS	Dried Blood Spot
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DNDi	Drugs for Neglected Diseases initiative
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Form
ERR	Egg Reduction Rate
FDC	Fixed Drug Combination
FOB	Fecal Occult Blood
GCLP	Good Clinical Laboratory Practice
GCP	Good Clinical Practice
ICF	Informed Consent Form
IF	Investigator File
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IRESSEF	Institut de Recherche en Santé, de Surveillance Épidémiologique et de Formation
ITM	Institute of Tropical Medicine
ITS2	Internal Transcriber-Spacer-2
ITT	Intent To Treat
LAMP	Loop-mediated Isothermal Amplifications
MDA	Mass Drug Administration
(q)PCR	(quantitative) Polymerase chain reaction
PhHV-1	Phocin Herpes Virus 1
PI	Principal Investigator
POC	Point-Of-Care
PP	Per Protocol
PZQ	Praziquantel
QA	Quality Assurance
(S)AE	(Serious) Adverse Event
SAP	Statistical Analysis Plan
SDV	Source Data Verification
Sh	Schistosoma haematobium
Sm	Schistosoma mansoni
SNP	Single nucleotid polymorphism
SOP	Standard Operating Procedure
TB	Tuberculosis
TCA	Trichloro-Acetic Acid
UCP-LF	Up-Converting Phosphor Lateral Flow
WHO	World health organization
YLDs	Years Living with Disability

SYNOPSIS

HYPOTHESIS	1. The efficacy of one and of repeated courses of artesunate-mefloquine (AM) is respectively similar to or higher than that of a standard praziquantel (PZQ) treatment. 2. Novel DNA- and antigen-based diagnostics are more accurate than microscopy in assessing antischistosomal treatment response.
DESIGN	The **SchistoSAM** study is an open label, two-arm, individually-randomized controlled trial with a non-inferiority design.
STUDY SITE & POPULATION	The trial will be conducted in the northern region of Senegal, in the region of Saint Louis, in the commune of Richard Toll. A total of 726 children aged 6 to 14 years old and infected with schistosomiasis will be recruited from selected schools and randomized in two treatment arms (AM vs PZQ). Sample size was calculated to obtain 80% power on the parasitological cure rate endpoint after the first course of AM, taking into account a possible 20% loss to follow up. The sample size was calculated on the parasitological cure rate endpoint after the first course of AM, to test the hypothesis that AM is non-inferior to PZQ.
DURATION	Total duration: 4 years For each individual study participant, the study follow-up period will last 48 weeks. Considering a time frame for inclusion of 2 to 3 months, the trial will take up to 1 year and 2 months. Approximately, an additional 3 years will be necessary for additional testing of preserved samples, statistical analysis and reporting.
OBJECTIVES AND ENDPOINTS	***Primary*** To evaluate the efficacy and safety of a single course of AM compared to the standard PZQ regimen. *Endpoint*: cure rate (CR) and frequency and pattern of drug-related adverse events (AEs) and serious AEs (SAEs) ***Secondary*** To evaluate the safety and efficacy of two additional courses of AM compared to a single course of AM and to the standard PZQ regimen. *Endpoint*: CR, AEs and SAEs To determine the egg reduction rate (ERR) obtained after single and repeated courses of AM compared to the standard PZQ regimen. *Endpoint*: ERR To determine the parasitological efficacy of single and repeated courses of AM by *Schistosoma* species and by infection intensity. Endpoint: CR and ERR To assess the impact of repeated AM courses on schistosomiasis-related morbidity. *Endpoint*: prevalence and severity of morbidity To evaluate the diagnostic accuracy of novel schistosomiasis antigen- and DNA-based diagnostic assays compared to microscopy and composite reference standards. *Endpoint*: diagnostic accuracy

	To determine the effect of repeated AM courses on prevalence, incidence and morbidity of malaria. <u>*Endpoint*</u>: prevalence, incidence of malaria, frequency and severity of anemia To monitor the prevalence of Pf molecular markers associated with mefloquine resistance and the potential reduced artesunate susceptibility. <u>*Endpoint*</u>: prevalence of *Plasmodium falciparum* (Pf) molecular resistant markers
INCLUSION & EXCLUSION CRITERIA	***Inclusion criteria*** 1. Children aged 6 - 14 years 2. Enrolled in selected primary schools in the district of Richard Toll 3. Infected with schistosomiasis (i.e. *Schistosoma* spp. eggs in urine and/or stool) 4. Informed consent signed by parents/guardian ***Exclusion criteria*** 1. Planned travel of more than 1 months within the first 4 months after enrolment 2. History of, or ongoing, epilepsy or psychiatric illness (i.e. recent history of depression, generalized anxiety disorder; history of psychosis, schizophrenia or other major psychiatric disorders) or known hypersensitivity to one of the three study drugs 3. Chronic medication for any reason 4. Any severe underlying illness, including severe malnutrition or severe chronic schistosomiasis, based on clinical judgement 5. Any febrile illness or clinical malaria 6. Exposure to PZQ or ACT within the three previous months.
SCREENING, RECRUITMENT & RANDOMIZATION	Screening for *Schistosoma* infection on stool and urine samples and initial clinical assessment will be performed in the villages in June 2019. Eligible children will be randomly assigned to one of the two study arms, using randomization envelopes.
STUDY DRUG	***Study drug*** AM, available in fixed dose tablets of 25/50 mg and 100/200 mg will be administered once daily for three days in a dose of 4 mg/kg artesunate and 8 mg/kg mefloquine. This treatment course will be repeated 2 times at 6-week intervals. ***Comparator*** PZQ, available in tablets of 600 mg, will be administered as a single dose of 40 mg/kg.
FOLLOW-UP	Trial participants will be regularly followed-up: • Before and after each dose of artesunate-mefloquine • At week 4, 10 and 16 for parasitological assessment and follow-up of AE and SAE's • At week 6 and 12 for clinical assessment before drug administration • At week 24 and 48 for morbidity assessment
SAFETY	PZQ is used widely as antischistosomal treatment in mass drug administration programs without safety concerns. AM shows an

	excellent safety and tolerability profile when used as malaria treatment. Safety and tolerability of the treatments will be evaluated by recording Adverse Events (AE's) and grading laboratory and vital signs evaluations.
STATISTICAL METHODS	The primary hypothesis for non-inferior efficacy, the efficacy of the two treatments over time and the cure rate will be assessed by calculating the two-sided 95% confidence interval (CI) for the difference in proportions as well as using a mixed effects logistic regression model. The analyses for ERR will be done using a linear mixed effects model with a random intercept and a logit link function. The diagnostic accuracy of both novel and classic diagnostic tests will be calculated together with 95% confidence intervals. The prevalence and patterns of resistant mutations between the two treatment groups will be compared using Chi-square or Fisher's exact test. ***Safety analyses*** The number of subjects experiencing a SAE, up to 4 weeks after PZQ and AM administration will be compared between treatment groups using Fisher's exact test. Safety will be analyzed using the all-subjects-treated approach for each time point separately.

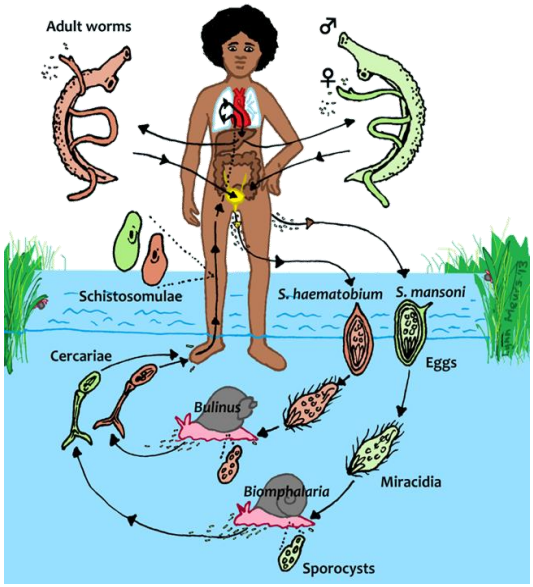
1. INTRODUCTION

1.1. Background & rationale

1.1.1. Treatment of schistosomiasis and the need for alternative drugs

Schistosomiasis is a chronic helminth infection that disproportionally affects poor and rural populations in sub-Saharan Africa, where it is almost exclusively caused by two species, *Schistosoma haematobium* (*Sh*) and/or *Schistosoma mansoni* (*Sm*). The transmission cycle requires contamination of surface water by excreta, specific freshwater snails as intermediate hosts, and human water contact. Infection occurs after *Schistosoma* larvae (cercariae) released by snails in surface water penetrate the host skin. These larvae then migrate in the tissues for about 5-7 weeks before developing in adult male and female worms in the veins, where they mate and start producing eggs.

Figure 1: Transmission cycle of schistosomiasis



Most morbidity and mortality associated with schistosomiasis results from organ lesions due to the host's immune response against schistosome eggs trapped in human tissues [1]. *Sh* causes primarily urogenital inflammation and scarring, leading to various complications, such as granulomatous cystitis, obstructive uropathy or infertility. In contrast, *Sm* affects mainly the intestines and liver, causing intestinal pseudopolyposis and periportal fibrosis with portal hypertension. Apart from "classic" pathologies, non-specific but disabling systemic morbidities, such as anemia, malnutrition and impaired physical and cognitive development, occur frequently [1–3].

According to the most recent estimates of the Global Burden of Disease Study in 2016, schistosomiasis affects about 200 million people worldwide [4]. In 2016, schistosomiasis accounted for approximately 10,000 deaths; 367,400 years of life lost; and 1,496,000 years lived with disability (YLDs) [4,5]. More than 90% of the global schistosomiasis burden is in sub-Saharan Africa, where the disease is among the top ten causes of YLDs [6].

In Senegal, both intestinal and urinary schistosomiasis are endemic, mainly in the north, the west and the south of the country. In the late 1980's, the northern region was confronted with a massive outbreak of *S. mansoni* infection, following the construction of the Diama dam on the Senegal River. Within a few years, the prevalence in Richard Toll, the epicenter of the epidemic went from 0% to almost 100%, with the highest intensities of infection ever described worldwide. Since then, the

infection rapidly spread throughout the northern region. A decade later, this severe *S. mansoni* epidemic was followed by a shift towards *S. haematobium* infection, which also reached record prevalences. Today, schistosomiasis is still highly endemic in Senegal, despite national control efforts. Both *S. mansoni* and *S. haematobium* are wide-spread, resulting in a large number of people with mixed infections in the communities [7].

Since the 1970s, treatment schistosomiasis has relied almost exclusively on praziquantel (PZQ). PZQ has demonstrated good efficacy [1] in a meta-analysis of 55 comparative and non-comparative trials involving > 19,000 participants, of whom 68% were school-age children [8]; results can be summarized as follows:

- For *Sh* infection, the WHO-recommended 40 mg/kg single dose of PZQ achieved a cure rate (CR) of 77.1% (95% confidence interval [CI] 68.4-85.1%; 2,645 participants/20 studies) and a mean egg reduction rate (ERR) of 94.1% (1,957 participants/19 studies).
- For *Sm* infection, the WHO-recommended 40 mg/kg single dose of PZQ achieved a CR of 76.7% (95% CI 71.9-81.2%; 6,377 participants/45 studies) and a mean ERR of 86.3% (5,546 participants/39 studies).
- For mixed *Sh/Sm* infection, CR was 63.5% (95% CI 48.2-77.0%; 379 participants/6 studies).

Another meta-analysis comparing school-age and preschool-age children in 47 comparative/non-comparative studies showed similar efficacy results in both age groups [9]. The WHO has set a tentative ERR threshold of 90% as an indicator of optimal efficacy [10]. In a systematic review of 10 studies, CR and ERR of *Sm* infection increased with repeated PZQ administrations, but such an effect was not observed for *Sh* [11].

Thanks to its safety, efficacy and low cost, PZQ has become the mainstay of schistosomiasis control. It is the drug of choice for mass drug administration (MDA) programs targeting high risk groups, including school-age children. PZQ is administered at regular intervals with the aim of reducing parasite load and preventing severe disease (the so-called 'preventive chemotherapy') [1,12]. Recommended time intervals between MDAs depends on the level of endemicity, but most of time PZQ is distributed annually to children 6 to 12 years of age, through school-based interventions. In 2015, PZQ has been distributed to 66.5 million of the estimated 218.8 million people requiring preventive chemotherapy worldwide, with a coverage of 42.2% in school-age children [13]. This is supposed to further increase in the coming years to reach the target 75% coverage in this age group and to extend treatment to pre-school children [9,14].

Despite the progress in schistosomiasis control attributed to preventive chemotherapy with PZQ, alternative therapeutics are urgently needed. The main reasons are [15,16] :

- PZQ is efficacious against all *Schistosoma* species, but only against the adult worms. Its activity against immature schistosome larvae is extremely weak, in the first few days up to 6-7 weeks after infection, before increasing progressively thereafter [17,18]. Treatment failures have usually been attributed to high reinfection rates by non-susceptible immature larvae, especially in high transmission areas, where large-scale and long-term use of PZQ monotherapy through repeated MDA in school-age children has not resulted in satisfactory community control so far [19].
- The extensive use of PZQ in monotherapy may result in the emergence of drug resistance [20]. PZQ resistance can be induced experimentally [21,22] and there have been sporadic reports on

[1] Efficacy is usually measured by the cure rate (CR: the proportion of egg-positive individuals, as assessed by urine and stool microscopy, who become egg-negative after treatment) and egg reduction rate (ERR: reduction of the post-treatment arithmetic and/or geometric mean egg count compared to pre-treatment mean count).

Schistosoma strains that have become tolerant or resistant, even though of little clinical relevance so far [23,24]. There is some recent evidence of a slow decrease of PZQ efficacy (below the ERR threshold of 90%) against *Sm* in Ugandan school-age children after increasing exposure to multiple MDA [25]. There is no guarantee that serious PZQ resistance will never appear [15].

- Despite much exploratory research, no new compounds are close to reaching Phase 1 clinical evaluation in the coming years [16,18]. Therefore, current clinical research tends to concentrate on new PZQ-based regimens such as higher dosages, prolonged administration or repeated courses [14], or on repurposing drugs used against other diseases [16]. So far however, most of the recently investigated drugs such as moxidectin, arachidonic acid or arterolane maleate/piperaquine have displayed unsatisfactory results, with ERRs far below the required 90% threshold [26].

There is thus a need to further investigate alternative antischistosomal therapies. Two drugs widely used as antimalarial therapy have demonstrated *in vitro* and *in vivo* activity against *Schistosoma sp.*: artemisinin derivatives and mefloquine [27,28].

- The antischistosomal effect of artemisinin-derivatives, such as artesunate and artemether has been studied since the 1980s in China. Adding artesunate/artemether to PZQ provides a superior efficacy against schistosomiasis, possibly due to the additional effect on immature larvae. In animal models, they showed excellent efficacy against the larval stages (> 90% reduction) and modest activity against adult worms (< 40% reduction) [29]. In human studies in China, the cure rates of artemisinin derivatives against *Schistosomiasis japonicum* was 73-96% [30], but such results could not be reproduced in Africa against *Sh* and *Sm* [31]. In three large clinical studies carried out in Africa on the effect of artesunate-based treatment on schistosomiasis, CR ranged from 14% to 59% and ERR from 35% to 93% [31]. Artemisinin derivatives had higher efficacy than placebo, but usually lower than PZQ, even when multiple courses were administered [27,31]. However, adding artesunate or artemether to PZQ consistently provided higher cure rates than PZQ alone for each *Schistosoma* species studied (*Sh*: 265 participants/2 studies; *Sm*: 75 participants/1 study and *S. japonicum*: 196 participants/2 studies), with an aggregated odd ratio of 2.07 (95% CI 1.27-3.36; p= 0.003) [32]. In addition, when artesunate and artemether were compared to placebo as chemoprophylactic agent against schistosomiasis, a significant protective effect was observed in all species, with an overall combined risk ratio of 0.11 (95% CI 0.06-0.22) and 0.25 (95% CI 0.16-0.40), respectively (P<0.001 for each). In a more recent study in Egypt, repeated administrations of artemether resulted in a substantial reduction of the incidence of new infections [33].
- Mefloquine on the other hand displays strong activity against both larval and adult stages of *Sm* in mice [34,35] and the combination of mefloquine and PZQ was superior to either drug used alone in a mice model [35]. Only two small underpowered trials have evaluated the activity of mefloquine monotherapy against human schistosomiasis. Mefloquine alone appears less active than PZQ but cure rates increase with repeated administration. In the first exploratory phase 2 study [36], a single course of mefloquine - 25 mg/kg administered to 19 Ivorian schoolchildren infected with *Sh* (including 8 with *Sm* coinfection) - had a lower efficacy than PZQ (ERR: 75% and CR: 21% versus 97% and 88% respectively). In the second preliminary trial [37], two courses of mefloquine - 15 mg/kg administered at one-month interval as intermittent preventive treatment against malaria to 30 Gabonese pregnant women with *Sh* infection - resulted in an ERR of 80% after one course and of 98% after the second course, with a "final" CR of 47% at 6-week post-second course.

The combination artesunate-mefloquine (AM) is one of the five artemisinin-based combination therapies (ACTs) currently recommended by the WHO for the treatment of uncomplicated *Plasmodium falciparum (Pf)* malaria. A three-day course of AM consistently reaches cure rate above 95%. AM is the first-line treatment against *Pf* malaria for more than 15 years in many Latin American and Asian countries. Recently, AM has been developed as fixed drug combination (FDC) by the Drugs for Neglected Diseases initiative (DNDi) and is now manufactured at low price by Cipla (India) under two different co-formulations (for children and adults) that were pre-qualified by WHO in 2012.

The use of AM as antischistosomal therapy has however hardly been evaluated so far and results are contradictory and inconclusive. In the exploratory trial previously mentioned [36], the efficacy of AM was similar to that of PZQ (ERR of 96% and CR of 61%). In another small (n=20/arm) exploratory trial in Ivorian schoolchildren with *Sh* infection [38], adding mefloquine or AM to PZQ increased the ERR from 90 to 96% but not the CR (surprisingly low at 30% in all three arms). However, the sample size was not adequate to properly assess possible superiority.

AM is usually not the first-line antimalarial treatment in African countries. While artemisinin derivates do not cause tolerance issues, there has been some concern about mefloquine-related adverse events (AEs), particularly the risk of serious neuropsychiatric side-effects. However, evidence from a systematic review [39], a pooled analysis of about 20,000 treated patients in Thailand [40] and a recent large multicenter RCT in Africa [41] consistently demonstrates that AEs are infrequent (1-2%) and serious neuropsychiatric events are very rare (< 1/1,000) in the pediatric population.

In the vast areas of sub-Saharan Africa where both schistosomiasis and malaria are co-endemic, there is no possibility to evaluate artesunate or mefloquine separately as antischistosomal therapy since the use of each drug in monotherapy could induce resistance of malaria parasites. So far, no molecular markers of resistance to artesunate and/or mefloquine have been detected in the most recent surveys in sub-Saharan Africa, in contrast to Southeast Asia where both drugs have long been used in monotherapies in the past. Finally, it is worth highlighting that the rates of schistosomiasis and malaria co-infection in school-age children range from 5% to 55% according to the areas in sub-Saharan Africa [42–44] and that evidence is mounting that morbidity of both conditions is cumulative, with higher frequencies of malaria attacks, anemia, hepatosplenic damage and urinary disorders [45–47]. However, in northern Senegal, where this study will be conducted, the incidence of malaria is less than 5 cases per 1000 inhabitants according to the report of the National Malaria Control Program (Annual Epidemiological Bulletin of Malaria in 2017). This, combined with the exclusion of febrile children, makes it highly unlikely that any selection pressure for AM will occur during this study.

Despite the *in vitro* and *in vivo* demonstration of activity of both drugs against larval and adult schistosomes, the benefit of the use of an AM combination in a clinical setting as an alternative antischistosomal therapy remains so far undetermined. It can be expected that in case of demonstrated dual activity of AM against malaria and schistosomiasis, clinical benefits would be even higher for patients with coinfection. Also, repeated administrations of AM, particularly during the rainy season when transmission of both schistosomiasis and malaria is highest, might have a cumulative effect not only on schistosomiasis and malaria morbidity but also on the rate of (asymptomatic) infections that is worth investigating. However, the risk of favoring resistance of malaria parasites has also to be scrutinized in co-endemic settings, even if this, as described above, seems extremely unlikely in the study area, the more when using a co-formulation at adequate antimalarial dosage.

1.1.2. Diagnosis of schistosomiasis and the need for alternative tools

Historically, schistosomiasis diagnosis has relied on the microscopic detection of *Schistosoma* eggs in urine or stool. The **Kato-Katz technique** is the standard method for assessing the prevalence and intensity of infection with *S. mansoni* in endemic countries, and **urine filtration** for the detection of urogenital infections due to *S. haematobium*. Both methods are relatively simple and inexpensive, almost 100% specific, and sufficiently sensitive to detect moderate and heavy *Schistosoma* infections. However, egg output strongly fluctuates and repeated examinations are needed to obtain an accurate assessment of infection. Moreover, light infections are easily missed, resulting in an underestimation of the true prevalence, particularly in low-prevalence settings, or inaccurate assessments of treatment efficacy [48–51].

Treatment monitoring

With the current scaling up of MDA programs, the need for highly accurate diagnostic tools is evident. Highly sensitive diagnostic tools to detect light-intensity infections are pivotal for an accurate assessment of antischistosomal treatment response. Considerable progress has been made in recent years, and a number of promising novel tools have been developed, which are in different stages of innovation, standardization, validation or application. These new tools are either DNA- or antigen-based (see below):

- The past decade, the detection and quantification of *Schistosoma*-specific DNA in stool and urine samples using **real-time Polymerase Chain Reaction (PCR)** has proven to be a powerful tool for the accurate diagnosis of schistosomiasis [52]. Specificity is virtually 100%, and sensitivities range from equal to, up to or substantially higher than, traditional microscopy techniques, the latter particularly in low-transmission settings and light-intensity infections [51,53,54]. Considering the high diagnostic accuracy and stability of real-time PCR over multiple time periods, the assay could be a valuable alternative to the current microscopic 'gold standard' for diagnosis of schistosome infections. Moreover, it can be run in a high through-put set-up, providing a valuable post-hoc diagnostic tool for clinical and epidemiological studies [54,55]. So far, data on the performance of real-time PCR as a monitoring tool after chemotherapy are limited[55]. A major disadvantage of real-time PCR is that it requires expensive laboratory equipment and highly skilled personnel [56]. An interesting alternative could be the use of loop-mediated isothermal amplifications (LAMP) technology, which could be relatively easily adapted for field diagnosis in schistosomiasis-endemic areas [51,57–59].
- Since 2008, a lateral flow immunochromatographic point-of-care (POC) test detecting *Schistosoma* circulating cathodic antigen (CCA) in urine has been developed and is now commercially available. This so-called **POC-CCA assay** is a rapid, user-friendly test designed for semi-quantitative field diagnosis of intestinal schistosomiasis. Accumulated evidence suggests that a single POC-CCA test is considerably more sensitive than conventional stool microscopy based on Kato–Katz for *S. mansoni* diagnosis, especially when infection intensities are low and after antischistosomal treatment [51,60–62]. The test has already shown its value as a diagnostic alternative for *S. mansoni* prevalence mapping in national control programs [62,63]. However, its suitability for measuring antischistosomal drug efficacy still needs to be investigated in more detail.
- Moreover, an up-converting phosphor lateral flow assay has recently been developed, which detects circulating anodic antigen (**UCP-LF CAA**) of all *Schistosoma* species in serum and urine at very low parasite loads, indicative of single worm infections [64]. Previous studies have indicated that circulating antigen levels are a better indication and more stable measure of schistosome worm burdens than egg counts [65,66]. Using urine as a non-invasive sample methodology, prevalence based on the UCP-LF CAA assay has recently shown to be three-fold higher than the prevalence detected with a single urine filtration [67]. This ultrasensitive test is currently being developed in a high-throughput rapid test format, which is already in development. The UCP-LF CAA technology, both under its current format or in its new rapid test version in development, still needs more validation in endemic settings, particularly in low-intensity areas and after treatment.

There is thus a need to define the utility of new methods, more sensitive than microscopy, in assessing treatment response.

Morbidity monitoring

Assessing schistosomiasis morbidity is usually done by **ultrasonography**. It is a well-established, widely accepted and accurate technique and is currently the diagnostic tool of choice for detecting organ-specific lesions associated with chronic schistosomiasis (*S. haematobium*: urinary tract; *S. mansoni*: liver and spleen). [68,69]. Moreover, ultrasound examination (using portable machines) has proven to be feasible in operational research [70].

Field-applicable indirect tools are increasingly used to provide sensitive surrogate markers of *Sh* or *Sm* morbidity. Reagent strips for detecting **microhematuria** in urine are useful for the indirect diagnosis of *S. haematobium* infection and have since long been suggested as appropriate adjuncts for monitoring urogenital schistosomiasis morbidity [71,72]. More recently, two fecal point-of-care assays, namely **point-of-care fecal occult blood tests** and fecal calprotectin tests, have been proposed as simple, field-applicable instruments to demonstrate intestinal schistosomiasis [73].

Further research is needed to evaluate the usefulness of these tests as field-adapted morbidity monitoring tools after antischistosomal treatment.

1.2. Hypothesis

Our research hypothesis is that through its combined effect on larval and adult schistosomes, the efficacy of one course of AM is at least similar to, and in case of repeated AM courses, higher than that of a standard PZQ treatment.

Secondly, we hypothesize that novel DNA- and antigen-based diagnostics are more accurate than conventional microscopy in assessing the actual antischistosomal treatment response.

2. STUDY OBJECTIVES

2.1. Primary objective

The primary objective of the **SchistoSAM** (**Schisto**somiasis **S**enegal **A**rtesunate-**M**efloquine) study is to evaluate the efficacy and safety of a single course of artesunate-mefloquine (AM, intervention arm) for the treatment of schistosomiasis in Senegalese primary school-age children, compared to the standard PZQ regimen (control arm).

Endpoint measures:

- Efficacy: Parasitological cure rate (CR, as assessed by microscopy, according to WHO standard) after administration of PZQ and after one AM course (at week 4 – see study flowchart).
- Safety: Frequency and pattern of drug-related adverse events (AEs) and serious AEs (SAEs) up to 4 weeks after PZQ and AM administration.

2.2. Secondary objectives

1. To evaluate the safety and cumulative efficacy of two additional courses of AM (at 6-week intervals each) for the treatment of schistosomiasis, compared to a single course of AM, and compared to the standard PZQ regimen (control arm).

   Endpoint measures:

   - Efficacy: CR, as assessed by microscopy, after the second and after the third AM administration (at week 10 and 16, 24 and 48 – see study flowchart),
   - Safety: Frequency and pattern of AEs and SAEs up to 4 weeks after the second and third AM administration.

2. To determine the egg reduction rate (ERR) obtained after single and repeated courses of AM compared to the standard PZQ regimen.

   Endpoint measure: ERR after administration of PZQ and after each AM course (at week 4, 10, 16, 24 and 48 – see study flowchart).

3. To determine the parasitological efficacy of single and repeated courses of AM by *Schistosoma* species and by infection intensity.

   Endpoint measures: CR and ERR by initial *Schistosoma* infection intensity after PZQ administration and single and repeated courses of AM. (measured at week 4, 10 and 16 after first AM administration)

4. To assess the impact of repeated AM courses on schistosomiasis-related morbidity.

   Endpoint measures: Prevalence and severity of general and organ-specific schistosomiasis morbidity (as assessed by clinical evaluation, ultrasound, point-of-care morbidity markers and hemoglobin level) at week 24 and week 48 compared to baseline and compared to the control arm.

5. To determine the diagnostic performance of novel schistosomiasis antigen- and DNA-based diagnostic assays to monitor antischistosomal treatment response.

   Endpoint measures: Sensitivity and specificity of the different conventional and novel diagnostic tests at baseline and at defined time points after treatment compared to conventional microscopy, and to composite reference standards.

6. To determine the effect of repeated AM courses on prevalence of *P. falciparum* infection as well as on incidence and morbidity of clinical malaria in school-age children with schistosomiasis.

   Endpoint measures:

   - Prevalence of malaria infection, as assessed by molecular testing of dried blood spots at baseline and week 24 and 48.
   - Incidence of clinical malaria, as assessed by the number of incident malaria cases diagnosed through passive case detection during the study period (by standard malaria rapid tests and molecular diagnostics on dried blood spots).
   - Frequency and severity of anemia, as assessed by determination of hemoglobin levels at baseline and at week 24 and 48 after initial treatment.

7. To monitor the prevalence of Pf molecular markers associated with mefloquine resistance and the potential emergence of reduced artesunate susceptibility.

   Endpoint measures:

   - Prevalence and patterns of mutations in the K13 gene (for artemisinin susceptibility) and increased copy number of the Pfmdr1 gene or other relevant mutations (for mefloquine resistance) observed in the molecular surveys (baseline, week 24 and week 48).
   - Presence and patterns of mutations observed in incident malaria cases.

3. STUDY DESIGN

The **SchistoSAM** study is an open label, two-arm, individually-randomized controlled trial among infected primary school-age children in northern Senegal, with a non-inferiority design.
The study will take place during the rainy season (July-October) to maximize the potential benefits of repeated AM administrations on both schistosomiasis and malaria.
At baseline, prior to the first drug administration, all children fulfilling the study criteria (chapter 4.2) will undergo a full parasitological and morbidity assessment of:

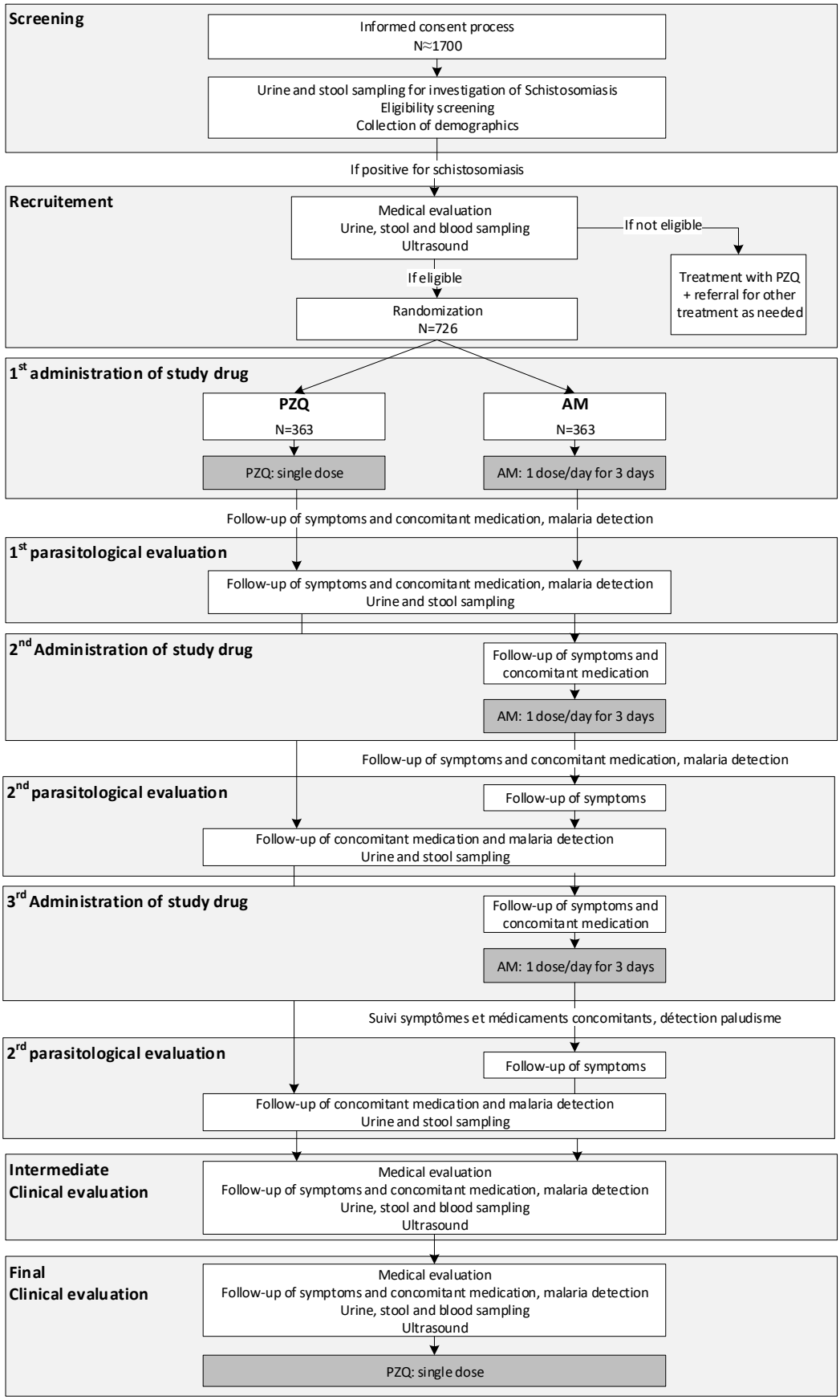
1. schistosomiasis infection: by microscopy (stool Kato-Katz and urine filtration) and point-of-care assays on site (POC-CCA) as well as additional diagnostics on preserved urine/stool samples (a UCP-LF CAA assay and a real time stool and urine PCR)
2. schistosomiasis systemic and organ-specific morbidity: by clinical evaluation, ultrasound, point-of-care morbidity markers and hemoglobin level
3. malaria infection: by determination of prevalence through molecular testing on preserved blood samples, and prevalence/pattern of molecular markers of resistance
4. malaria morbidity: by measurement of proportion and severity of anemia

Subsequently, children will be randomized and assigned to one of the two following study arms:

5. **AM arm**: Three-day course of AM (A: 4 mg/kg and M: 8 mg/kg per day, given once a day for three days) administered at baseline and repeated twice at 6-week intervals (intervention).
6. **PZQ arm**: Single dose of PZQ (40 mg/kg) administered at study inclusion (control arm/standard of care).

The 6-weeks interval between AM administrations corresponds to the time lapse for larval maturation (from infection to adult worms). In addition, this 6-week period should minimize/prevent the risk of cumulative mefloquine-associated toxicity.
Parasitological assessments of schistosomiasis (urine and stool microscopy and POC-CAA) will take place in both arms at week 4, 10 and 16 after first administration of AM or PZQ. Full parasitological assessment of schistosomiasis and malaria (urine, stool and blood analysis) will be repeated at week 24 and 48 post-inclusion.
Morbidity assessment will be repeated as described here above at week 24 and 48 after initial treatment. These assessments are described in more detail in chapter 5.
At week 48 post-inclusion, the children of both arms will receive a dose of PZQ. National control programs will be informed the study outcomes, including on the prevalence of schistosomiasis infection, to assist them in the organization of mass drug administration for non-study participants. This MDA will be coordinated by the national control program according to guidelines in force in Senegal. [74,75]
Also, children in the study testing positive for schistosomiasis by microscopy or POC-CCA at baseline, but who cannot participate to the study, will be treated with PZQ and, if needed, referred to health facilities for further care.

Figure 1: Study flowchart



There will be no blinding of the investigators nor participants because of the practical difficulty and costs to get a double dummy, since the intervention regimen is very different from the control arm. However, the results of the successive parasitological assessments will be masked to the clinical teams in the study sites, so that their evaluation of drug safety and tolerability will not be influenced if the efficacy data appear favorable. Similarly, the laboratory study team will be blinded for the treatment arm allocation.

Adverse events up to four weeks after the last dose of study medication (PZQ or AM) will be carefully monitored and SAEs will be immediately reported to the principal investigator, the project coordinator and the Data and Safety Monitoring Board (DSMB) (see chapter 7 for more details on safety reporting). Before each additional AM administration, the DSMB will receive by mail a list of the frequency, pattern and severity of AEs observed in both treatment arms, to be able to react accordingly.

4. PARTICIPANTS, POPULATION & SELECTION

4.1. Settings, selection & recruitment of the study population

The trial will be conducted in the northern region of Senegal, in the commune of Richard Toll located in the department of Dagana and the Saint-Louis region. Schistosomiasis in this region is highly endemic with mixed *Sm* and *Sh* infections and reported prevalence of >50% in the general population and up to 80% in school-age children [76]. Malaria transmission is seasonal and very low (annual incidence < 5/1,000 inhabitants) in this region, when assessed by light microscopy or rapid diagnostic test. Prevalence of Pf infection was rather low (1%) with more sensitive molecular assays in a neighboring northeastern area [76], although substantially higher (up to 15%) in neighboring southwestern regions [77]. The rainy season lasts usually from June to October in this region. This area with high prevalence and incidence of both *Schistosoma* species (and very low incidence of malaria) is then considered as particularly suited to safely test a "new" antimalarial drug with dual activity in a proof-of-concept trial. Such a setting provides a unique opportunity to obtain very robust data on efficacy against schistosomiasis while minimizing the risk of malaria resistance (the latter being very unlikely since AM is a potent ACT). However, scrutinizing the effect of AM on *Pf* infection (including asymptomatic malaria) and resistance remains key in the project.[76].

The trial will be conducted by the "Institut de Recherche en Santé de Surveillance Épidémiologique et de Formation" (IRESSEF) with the support of ITM. IRESSEF - accredited ISO 15189/15190 - is a 'not-for-profit' public institution hosting the African Network for HIV Research (RARS) which includes 22 West and Central African countries. IRESSEF staff has long and strong experience in research on HIV, Tuberculosis, Malaria and Neglected Diseases. IRESSEF possesses resources and infrastructures to conduct technically demanding studies including units of malaria, clinical trial, epidemiology, data management, and IT. It has scientific and technical partnerships with prestigious universities such as Harvard, Oxford, Imperial College, as well as national institutions and universities including among other the Ministry of Health (with whom it has signed a partnership agreement) and the Cheikh Anta Diop University of Dakar. Since 2007 under LBV-UCAD, scientists from IRESSEF have collaborated with the ITM in various projects in the field of schistosomiasis in Northern Senegal (DGD/EU/SchistoINIR). IRESSEF is presently coordinating the second phase of the West African Network for TB, AIDS and Malaria (WANETAM2) - funded by EDCTP.

The commune of Richard Toll compasses about 37 primary schools in total, with a number of pupils ranging from 50 to more than 200 per school. Because schistosomiasis transmission is typically focal and evolving, the prevalence of schistosomiasis will be however re-assessed according to the WHO methodology. Schools will be selected according to their size, proximity to Richard Toll and prevalence of schistosomiasis. A list of priority schools will then be established, where selection and recruitment of children for the clinical trial will take place.
School directors and teaching staff will be informed during a preparatory visit, in close collaboration with the study teams. Specific information on the targeted disease (schistosomiasis and malaria) and the risk/benefits of the trial will then be shared in a culturally-sensitive way with local communities. They will have the possibility to ask questions. The members of the study team from IRESSEF in Senegal who have already established contact with local communities will facilitate this sharing of information.
The parents/guardians of the school children will be invited for a general information session and an individual informed consent process (detailed informed consent procedure in chapter 5.2). Those that consent will give a stool and urine sample for identification of schistosomiasis infection.
Eligible children positive for schistosomiasis will then be invited to come for medical and initial parasitological and morbidity assessment, and enrolment in one of the two randomly assigned study arms. Children that tested positive for *Schistosomiasis* but are not eligible will be offered standard

treatment with PZQ. We will keep screening until we achieve the necessary 726 enrollments (363 in each arm; details of sample size in chapter 4.3).

4.2. Inclusion and exclusion criteria

Inclusion criteria

1. Children ≥6 and ≤14 years of age
2. Enrolled in one of the selected primary schools in the district of Richard Toll
3. Infected with schistosomiasis (i.e. presence of *Schistosoma* spp. eggs in urine and/or stool)
4. Informed consent from parents/guardians signed

Exclusion criteria

1. Planned travel of more than 1 months within the first 4 months after enrolment
2. History of, or ongoing, epilepsy or psychiatric illness (i.e. recent history of depression, generalized anxiety disorder; history of psychosis, schizophrenia or other major psychiatric disorders) or known hypersensitivity to one of the three study drugs
3. Chronic medication for any reason
4. Any severe underlying illness, including severe malnutrition or severe chronic schistosomiasis, based on clinical judgement
5. Any febrile illness or clinical malaria
6. Exposure to PZQ or ACT within the three previous months.

As mentioned above, children with schistosomiasis but excluded from the study will be treated with PZQ according to standard of care in Senegal. Children found with other illnesses or severe schistosomiasis will be referred to adequate health facilities for further care.

4.3. Sample size

The sample size was calculated on the parasitological cure rate endpoint after the first course of AM, to test the hypothesis that AM is non-inferior to PZQ: at least a 65% cure rate with AM as compared to a 75% cure rate with PZQ according to the following rationale. We consider that a cure rate (CR) of 65% is clinically acceptable as threshold for non-inferiority for the AM combination, for the following reasons: a cure rate above 65% would be very satisfactory compared to all alternative drugs evaluated so far against schistosomiasis, and an efficacy slightly lower than that of PZQ would be largely compensated by the positive clinical effect due to concomitant malaria suppression. Note that this is likely to be marginal in North Senegal where malaria transmission is low, but this “dual” effect could be a major added-value in many other co-endemic regions with higher malaria transmission

Sample size was calculated by simulation to obtain 80% power. The required sample size is 300 schoolchildren per arm. To account for a 20% loss to follow up, the total number of children to be recruited will be 726 (363 per arm). With the same sample size, superiority of repeated courses of AM to PZQ (cure rate of at least 85% after 3 courses) can also be demonstrated with a similar power (i.e. secondary endpoint).

4.4. Randomization

A block randomization schedule - to ensure balance of the two arms among the study sample, stratified for the two species of Schistosomiasis, will be prepared by the sponsor biostatistician. The individual randomization number and treatment arm of each subject will be stored in sealed envelopes.

Randomization will be done at the time of enrollment using randomization envelopes, specifying the treatment arm. This is an open-label study, so both the study team members as well as the participant will immediately be informed about the assignment to one of the two study arms. The randomization list will be prepared using SAS 9.4 (SAS Institute, Cary NC).

4.5. Withdrawal and termination of the study

Reasons for withdrawal
Participants may be withdrawn from the study if:

- The parents/guardians of the participant withdraw the consent
- The participant develops epilepsy, psychiatric illness or severe gastric intolerance, or any other illness that could have a negative effect on the participant's health before the last dose of study medication
- The participant has vomited the initial and repeated dose of study medication
- The participant needs to get started on chronic medication for any reason before the last dose of study medication

Participants will only be considered lost-to-follow up after the week 48 follow-up visit window has passed. Participants who miss one or more study visits will remain in the study. The missed visits will be skipped, and the participant will continue with the original visit schedule (see chapter 5). Participants will be actively followed-up to attend all study visits.

Handling of Withdrawals
Participants withdrawing for medical reasons will be referred to adequate health facilities for further care if necessary.

Unless consent is withdrawn, efforts will be made to schedule, for these participants, at least the week 24 and week 48 follow-up visits. Collection and follow-up of adverse events and serious adverse events is further described in chapter 7.

Participants who have been withdrawn from the study will be offered a standard dose of PZQ.

Data collected up to the time of withdrawal will remain in the study data base and be used for analysis whether or not the participant continues with the follow-up visits.

Termination of study
In case the members of the DSMB (see chapter 7.2), based on preliminary data presented to them, show concern about the continuation of the trial (for safety or any other reason) they may collectively advice the Sponsor to prematurely terminate the study. Following ethical shortcomings in the conduct of the project, the ethics committees in Senegal or Belgium could also decide to stop the study.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The ECs will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

5. STUDY PROCEDURES

5.1. Study/visit schedule

Screening

- Informed consent procedure and signing the ICF by parents/guardians
- First urine and stool sampling for investigation of schistosomiasis
- Eligibility screening (inclusion/exclusion criteria):
    - Collection of demographics and medical history data

Recruitment

- Medical evaluation, with special emphasis on exclusion criteria
- Ultrasound examination (by a radiologist experienced in *Schistosomiasis* assessment)
- Blood (finger prick)*, urine and stool sampling**
- Hemoglobin measurement
- Parasitological assessment by microscopy and POC-CCA
- Urine reagent strip testing
- Point-of-care fecal occult blood
- Randomization in the AM or PZQ arm

First administration of IMP (Day 0)

- Systematic questionnaire on concomitant medication and symptoms
- Administration of study medication, under directly observed therapy:
    - AM arm: first dose of A at 4 mg/kg and M at 8 mg/kg
    - PZQ arm: single dose of PZQ (40 mg/kg)

First administration of the IMP (Day 1 & 2) (AM arm only)

- Administration of study medication in AM arm: second and third doses of A at 4 mg/kg and M at 8 mg/kg
- Follow-up for AE and SAEs***

Follow-up for AE and SAE (Day 7)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis

Evaluation of parasitological efficacy after initial treatment (week 4)

- Urine and stool sampling**
- Parasitological assessment by microscopy and POC-CCA
- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis
- Follow-up for AEs and SAEs*** (retrospective assessment)

Second administration of the IMP, day 0 (AM arm only) (week 6)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis
- Administration of study medication in AM arm: first dose of A at 4 mg/kg and M at 8 mg/kg

Second administration of the IMP, day 1 & 2 (AM arm only) (week 6)

- Systematic questionnaire on concomitant medication and symptoms
- Administration of study medication in AM arm: second and third doses of A at 4 mg/kg and M at 8 mg/kg
- Follow-up for AE and SAEs***

Follow-up for AE and SAE (Day 7)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis

Evaluation of parasitological efficacy after second round of AM (week 10)

- Urine and stool sampling**
- Parasitological assessment by microscopy and POC-CCA
- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis
- Follow-up for AEs and SAEs***

Third administration of the IMP, day 0 (AM arm only) (week 12)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis

- Administration of study medication in AM arm: first dose of A at 4 mg/kg and M at 8 mg/kg

Third administration of the IMP, day 1 & 2 (AM arm only) (week 12)

- Systematic questionnaire on concomitant medication and symptoms
- Administration of study medication in AM arm: second and third dose of A at 4 mg/kg and M at 8 mg/kg
- Follow-up for AE and SAEs***

Follow-up for AE and SAE (Day 7)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis

Evaluation of parasitological efficacy after third round of AM (week 16)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis
- Urine and stool sampling**
- Parasitological assessment by microscopy and POC-CCA
- Follow-up for AEs and SAEs***

Intermediate evaluation of clinical efficacy (week 24)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis
- Medical evaluation
- Ultrasound examination
- Blood (finger prick)*, urine and stool sampling**
- Hemoglobin measurement
- Parasitological assessment by microscopy and POC-CCA
- Urine reagent strip testing
- Point-of-care fecal occult blood

Final evaluation of clinical efficacy (week 48)

- Systematic questionnaire on concomitant medication, symptoms and malaria diagnosis
- Medical evaluation
- Ultrasound examination
- Blood (finger prick)*, urine and stool sampling**
- Hemoglobin measurement
- Parasitological assessment by microscopy and POC-CCA
- Urine reagent strip testing
- Point-of-care fecal occult blood
- PZQ for all the children enrolled in the study and MDA to the rest of the community under the coordination of the national control program, according to WHO guidelines[74,75]

A schematic overview of the visit schedule and related procedures can be found in the 'Table 1: Schedule of assessments' below.

** Dried blood spot (DBS) will also be collected on filter papers and will be shipped to ITM for further molecular analysis (malaria diagnosis and presence of mefloquine resistance markers / markers of susceptibility to artemisinin)*

*** Urine and stool samples will be aliquoted, preserved and shipped to ITM for further schistosomiasis diagnostic assays (eg. urine CAA lateral-flow assay – UCP-LF CAA – and a real-time stool and urine PCR)*

**** Throughout the trial, (S)AEs up until 4 weeks after the last administration of study medication (PZQ and AM arm) will be taken into account. In the AM arm, on the second and third administration day, this will be actively monitored. Plus, for both arms, efforts will be made to proactively monitor the adverse events within one week after PZQ and first AM administration, by an additional visit at day 7. If no contact is possible, (S)AEs will be gathered at the next scheduled study visit. More details on safety collection and reporting are described in chapter 7. A study specific SOP will be created to elaborate on the detection, the handling, and the reporting of AEs and SAEs.*

Table 1: Schedule of assessments

		Screening	Recruitment	1st admin IMP (Day 0)	1st admin IMP (Day 1 & 2[3])	Follow-up AE & SAE Day 7	Parasitological evaluation 1	2nd admin IMP, day 0[3]	2nd admin IMP day 1 & 2[3]	Follow-up AE & SAE Day 7	Parasitological evaluation 2	3rd admin IMP, day 0[3]	3rd admin IMP day 1 & 2[3]	Follow-up AE & SAE Day 7	Parasitological evaluation 3	Intermediar efficacy evaluation	Final efficacy evaluation
Informed consent		X															
Eligibility		X	X														
Demographics		X															
Medical history			X														
Medical evaluation			X													X	X
Hemoglobin measurement			X													X	X
Microscopy (urine and stool)		X	X				X				X				X	X	X
POC-CCA			X				X				X				X	X	X
Urine reagent strip test			X													X	X
Fecal occult blood			X													X	X
Ultrasound			X													X	X
Randomization			X														
Concomitant medication check				X	X	X	X	X	X	X	X	X	X	X	X	X	X
Symptom check				X	X	X	X	X	X	X	X	X	X	X	X	X	X
Malaria diagnosis check						X	X	X		X	X	X		X	X	X	X
Study drug administration	**AM arm**			AM	AM			AM	AM			AM	AM				PZQ
	PZQ arm			PZQ													PZQ
(S)AE follow-up					X[1]	X	X		X[1]	X	X		X[1]	X	X		

[1] (S)AE follow-up done before second and third study drug administration.

[2] (S)AE follow-up done up to 4 weeks after the last study drug administration. However, efforts will be made to proactively monitor the adverse events within a week after PZQ and each AM administration, by an additional visit at day 7. If participants could not be reached, (S)AEs will be collected during the study visit.
[3] Only applicable for AM arm

5.2. Obtaining free informed consent

Parents/guardians of the children will be invited for a general information session about the study. Subsequently – using a comprehensive information sheet – the parents/guardians and the children themselves will individually be verbally informed by a trained and delegated member of the study team, in understandable language, about the study objectives, how the project will be conducted, and the implications of the research. Only after the parents/guardian are confident that they want their child to be enrolled in the study after all their questions have been answered satisfactory, written consent will be obtained. Because all participants will be between 6 and 14 years old, the informed consent procedure will take place with the parents or guardian of the participant. The verbal consent of the child (assent) will be requested and documented by the study team member. Only after written informed consent from the parent/guardian and the verbal consent (assent) from the child, the latter may be included in the trial and study specific procedures may be performed. It should be noted that at the time of the medical evaluation at the initial visit preceding the administration of the medicinal products, the investigating doctor will again ask again whether the study has been properly understood and whether clarifications are necessary. If participants maintain their agreement, the physician will countersign the informed consent document and proceed with randomization. If participants refuse at this time, they will not be enrolled

Although the consent of both parents is advisable, the written consent of one of the parents will be acceptable.
Because the concept of legal guardianship is usually not formally established in rural settings in Senegal, for the trial, a person is considered as guardian of the child if he/she takes responsibility for the child, contributes to the child's maintenance and is accepted by the community as the child's care giver. If the parent/guardian is illiterate, a member of the team will explain to him/her in the language he/she understands the information related to the study and the terms of the consent form. An independent literate witness will attend the consent interview, and will sign the informed consent form (in addition to a thumbprint of the parent/guardian) confirming that the information that was provided is correct.

The content of the ICF will be compliant with the most recent ICH GCP Guidelines and Declaration of Helsinki, as well as any ethical/regulatory requirements in Senegal, and will include information on the purpose of the study, the procedures to be followed, the risks and benefits of participation, the voluntary nature of participation, the storage and shipment of the samples, the confidentiality and data protection, etc. A separate section of the ICF is dedicated to the long-term storage and secondary use of the collected samples, to which parents/guardians may or may not consent.

A separate section of the informed consent form is the assent form, in which the study is explained in a way that can be understood by the target population of the study.

A study specific SOP will be developed with detailed guidance for site staff on how to perform the informed consent procedure.

5.3. Specific procedures and activities

Data collection and sampling will be done in collaboration with the medical staff from the health centers and the community health workers. Registration, collection of demographics and medical history data as well as blood sampling, clinical examination, drugs administration and ultrasound will be performed on site in a designated private room (probably in a health post) by an experienced health staff.

Standardized interview
All parents/guardians will be asked to provide demographic information of their child (age, gender, residence, etc.) and information on his/her medical history (past or ongoing treatment, disease, history of epilepsy etc). All information will be collected in a standardized way.

Sampling strategy
Blood sampling:
A finger prick of blood (about 200 µl) will be taken from each child to assess hemoglobin concentration/anemia using a portable hemoglobinometer (HemoCue) and categorized according to WHO criteria [78]. Four separate drops of blood will also be put on Whatman 3MM® filter paper and stored at room temperature until shipment and molecular analysis at ITM.
Stool and urine sampling:
Two fresh stool and urine samples will be collected on consecutive days. The samples will be adequately stored until use by the laboratory.

Clinical examination
Clinical examination will include measurement of weight, height and temperature, observation of signs of anemia and abdominal examination to evaluate size and consistency of liver and spleen, as well as the presence of stigmata of portal hypertension such as ascites and collateral blood vessels. Hepatomegaly is defined as liver palpability (or detectability by percussion) of more than 2cm below the costal margin. For enlargement of the spleen, Hackett's classification can be used.

Organ specific pathology (ultrasound examination)
All children will receive an ultrasound examination in order to detect organ-specific lesions related to *Sm* and *Sh*, respectively

- hepatomegaly, periportal fibrosis, portal hypertension (dilatation of the portal and splenic veins and porto-systemic collaterals), portal vein thrombosis, reactive splenic hyperplasia or intestinal wall thickening for *Sm,* and
- urinary tract lesions such as formation of intravesical masses, calcification of the bladder wall, dilatation of the ureter and hydronephrosis for *Sh.*

A suitable place will be prepared on site for ultrasound examinations and they will be all performed by a qualified person, following the scores from the WHO guidelines [79].

Study specific SOPs will be developed with detailed guidance for site staff on how to perform the above-mentioned study procedures.

5.4. Laboratory procedures

5.4.1. Field tests

Microscopy
Parasitological examination of urine and stool by microscopy will be applied for the detection of *Sh* and *Sm* eggs to assess the primary endpoint (treatment efficacy), since it remains the WHO-established standard for diagnosis of schistosomiasis and assessment of treatment response. For each participant, two urine and two stool samples will be collected at two different days. Per feces sample, two Kato-Katz slides of 25 mg fecal material will be prepared and microscopically examined for *S. mansoni* eggs*;* each urine sample will be analyzed by the standard filtration technique for the detection of *S. haematobium* eggs. *Sm* and *Sh* infection intensity (average number of eggs per 25 mg gram of stool and per 10 ml of urine respectively) will be determined to determine the intensity of the infection according to WHO criteria [74]. Microscopy will be performed in the laboratory of the Public Health

facility in Richard Toll by experienced lab technicians, after specific training from an ITM expert. Other intestinal parasites detected by the two methods will also be recorded. For quality control, a random representative number of the slides read per day will be re-read by a second microscopist and the results will be compared for quality control. In case of discordant results, a third observation will be required to validate the result.

POC-CCA

A lateral flow immunochromatographic point-of-care assay (POC-CCA) will be used for the detection of *Schistosoma* circulating cathodic antigens in urine. The commercially available rapid immunochromatographic dipstick will be used (Rapid Medical Diagnostics, Pretoria, South Africa) and tests will be performed according to the manufacturer instructions. Briefly, 2 drops of urine will be transferred into the cassette and the results will be read after 20 minutes. The results will be categorized as negative or positive (trace CCA results will be considered positive) and for the positive, based on the intensity of the band, a concentration of the sample will be estimated in ng/ml (data unpublished), as a semi-quantitative measure of intensity of infection.

Urine reagent strip test

A urine heme reagent dipstick test (medi-test Combi - 9® Macherey-Nagel) will be used as an indirect diagnosis of *Sh* infection morbidity by detecting micro-hematuria. The dipstick will be read against the key on the dipstick jar few seconds after being in the urine and the results on microhematuria will be registered as negative, 1+, 2+ or 3+. The macroscopic aspect (color, turbidity, hematuria) of the urine will be observed and registered; the presence of glucose, leucocytes, nitrite, blood and proteins will also be registered as given by the dipstick.

Fecal occult blood

Fecal occult blood (FOB) chromatographic detection tests will be used as indirect surrogate markers of *Sm* intestinal morbidity as an indicator of bowel morbidity. A commercially chromatographic test will be employed for FOB detection (Mission test®, Acon Laboratories, San Diego, CA). Two small drops of feces suspension will be applied to the test after feces homogenization in a liquid buffer and results will be visually read after five minutes and categorized as negative (-), trace (+/-) and positive (+).

5.4.2. Testing at ITM

An aliquot of stool (3g) and two aliquots of urine (1 ml of urine sediments for the PCR and 1 ml of filtrated urine for the UCP-LF CAA) will be transferred in cryotubes labelled with the individual code given to each participant and stored at -20°C. They will be shipped to on dry ice to ITM, where additional diagnostic assays will be performed.

UCP-LF CAA

An up-converting phosphor lateral flow assay detecting all *Schistosoma* species circulating anodic antigen (UCP-LF CAA) (UCAA500) will be performed on urine as an ultrasensitive quantitative test. It will be used in a user-friendly lateral flow assay format. Briefly, 500 µl of urine will be mixed with 100 µl of 12% (*w/v*) trichloro-acetic acid (TCA) in order to remove interfering proteins and to dissociate potential immune complexes. After centrifugation, 500 µl supernatant will be transferred into an Amicon Ultra-0.5 Centrifugal Filter Device (Merck Millipore) and concentrated to approximately 20 µl. The concentrated samples will be examined by the dry-format assay using dried reagents, freshly reconstituted with water. Urine samples concentrates will then be mixed with CAA-specific UCP reporter conjugate solution and incubated for 1h on a microtiter plate thermo-shaker after which a LF strip will be placed in each well. Then the strips will be scanned for UCP reporter signals with a dedicated fluorescent strip

reader. Results will be expressed as a ratio value between the T line and the Fc line. A TCA-soluble fraction of *S. mansoni* and *S. haematobium* adult worm antigen with known CAA concentration will be used as a reference standard for the quantification of the antigen [64,80–82].

PCR for Schistosoma detection

A multiplex real-time PCR on stool and urine samples will be realized for the detection and quantification of *Schistosoma*-specific DNA and as a post-hoc reference standard for microscopy. This PCR will target the *Schistosoma*-specific internal transcriber-spacer-2 (ITS2) sequence of *S. mansoni*, *S. haematobium* and *S. intercalatum*. This ITS2-based PCR has been validated[83] is virtually 100% specific and will be performed as described previously[84]. First, DNA will be extracted using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany). The phocin herpes virus 1 (PhHV-1) will be used as an internal control and will be added to the lysis buffer as well as primers specific for this virus and a corresponding PhHV-1-specific, Cy5 double-labelled detection probe will be included in each reaction mixture. Then, amplification, detection and data analysis will be performed with the CFX96 Real-Time System version 1.1 (Bio-Rad, Hercules, CA). Negative and positive control samples will be always included in each PCR run. The PCR output will be the cycle-threshold (C_t) value, representing the amplification cycle in which the level of fluorescence signal exceeds the background fluorescence. Hence, low C_t value will correspond to high parasite-specific DNA loads in the sample tested, and vice versa.

PCR for malaria detection and Pf genetic analysis

A *Plasmodium falciparum* qPCR will be performed after DNA extraction from dried blood spot (DBS) samples to measure the prevalence of malaria during each full parasitological assessment (baseline, week 24 and week 48) as well as to assess the incidence of clinical malaria during the study.

DNA extraction from the DBS will be performed by QiaAMP DNA mini kit (Qiagen, Germany) as per the manufacturer's protocols for DBS.

Pf infection will be detected and quantified through an ultra-sensitive real-time quantitative PCR targeting a conserved acidic terminal sequence of the var genes (varATS, 59 copies/genome). Briefly 5µl of PCR water, 10 µl of 2 x Taqman Universal PCR mastermix (Applied Biosystems, New Jersey, USA), 1.6 µl of 10 µM forward and reverse primers, 0.8 µl of 10 µM probe and 5 µl of parasite DNA will be vortexed and run on CFX 96 Touch TM Real-Time System (Bio-Rad Laboratories, CA, USA)[85,86].

In the positive malaria cases detected by qPCR, pfmdr1 gene amplification, a marker of mefloquine/piperaquine resistance, will be determined by qPCR based on methods described previously[87]. In addition, single nucleotide polymorphisms (SNPs) in pfmdr1 will be detected through Taqman real-time PCR. Each set of reactions will include a 3D7 *P. falciparum* strain used as a calibrator and a positive DNA extract with an already estimated pfmdr1 copy number.

In the same way, artesunate decreasing susceptibility will be monitored by detecting mutations in the kelch propeller domain of the K13 gene of *Plasmodium falciparum*. Thus the amplification the K13-propeller domain will be used to detect k13 mutations by sequencing using the Sanger sequencing approach[88]. Electrophoregrams will be analysed on both strands with CEQ 2000 genetic analysis system software (Beckman Coulter, Villepinte, France), using PF3D7-1343700 as reference sequence. Isolates with mixed allele will be considered as mutants[89].

For each test, diagnostic accuracy will be assessed, as well as treatment response (cure rate, egg reduction rate), in each treatment arm and at each time point. Results of all tests will be compared to each other, to conventional microscopy, and to composite reference standards.

All analysis of clinical trial samples will be carried out in compliance with Good Clinical Laboratory Practice (GCLP). A Lab Analytical Plan will be available, in line with WHO-GCLP. Study specific SOPs will be developed with detailed guidance for site staff on how to perform the above-mentioned laboratory procedures.

6. STUDY INVESTIGATIONAL PRODUCT

6.1. Purchasing and administration

The investigational fixed drug combination artesunate-mefloquine (AM) as well as praziquantel (PZQ), manufactured by the Indian company Cipla, will be used for the study. Both drugs received WHO prequalification. The drugs will be donated by the company. In any way, the company will not intervene in the design of the study, nor in the analysis and dissemination of the results).

The Investigational Medicinal Products (IMP) will be shipped directly from the manufacturer to the study site, following the good distribution practices.

Praziquantel is available in a formulation of 600 mg per tablet and will be administered as a single dose, adjusted according to the weight of the participant with a best approximation to the target dose of 40 mg/kg.

Participant's weight	Number of Praziquantel tablets
≥12 - <19kg	1
≥19 - <27kg	1 ½
≥27 - <34kg	2
≥34 - <42kg	2 ½
≥42kg	3

The investigational AM combination is available in two fixed dose formulations, e.g. artesunate-mefloquine 25/50 mg and artesunate-mefloquine 100/200 mg.
The recommended target dose for each drug is 4 mg/kg for artesunate and 8 mg/kg for mefloquine per day given once day for three days, corresponding to total doses of 12 mg/kg and 24 mg/kg, respectively. The dose should be adjusted according to the weight of the participant.

Participant's weight	Number of artesunate-mefloquine tablets
≥15 – <22kg	**3** 25/50mg tablets/day
≥22 – <28kg	**1** 100/200mg tablet/day
≥28 – <34kg	**1** 100/200mg tablet + **1** 25/50mg tablet/day
≥34 – <40kg	**1** 100/200mg tablet + **2** 25/50mg tablets/day
≥40 – <47kg	**1** 100/200mg tablet + **3** 25/50mg tablets/day
≥47kg	**2** 100/200mg tablets/day

The participant is followed up for at least 30 minutes after administration of AM and PZQ. If vomiting occurs within 30 minutes of drug administration, the full daily dose should be repeated. If vomiting occurs more than 30 minutes after dosing, half the recommended daily dose should be given. If the repeat dose is vomited, treatment should be discontinued, and the patient is withdrawn from the trial. The administration of AM may be delayed, as long as the three doses are given within a maximum of 5 days. The administration of one of the dose will be cancelled if it is not feasible to organize the administration outside of this period. Children that did not receive a full treatment administration will stay in the study and be followed up as planned.

6.2. Prior and concomitant therapy

Children taking chronic medication for any reason are excluded from the study, as are children with febrile illness or those having received AM or PZQ in the 3 months prior to the study.

Children and parents/guardians are instructed to inform the study members about any previous or planned intake of non-chronic medication during the administration period of the study medication. The investigators will insure that safety is not compromised and evaluate the need to delay administration of the study drug, considering the nature and half-life of the active substance mentioned and its indication.
Praziquantel and Mefloquine are metabolized in the liver and concomitant administration of inducers or inhibitors of the same metabolic pathways should be avoided. Any drug known to prolong the QT interval should be administered cautiously and only if strictly indicated, since mefloquine may have a minimal QT-prolonging effect.

6.3. Packaging

There will be no repackaging nor relabeling of PZQ or AM. The package and label in English or French of the manufacturer will be used as the IMP will be administrated under direct supervision of the study staff.

6.4. Reception, storage, dispensing and return

Upon receipt of the IMP at site, a delegated member of the site staff will review shipment documents and potential damages or temperature excursions. The IMP will then be stored at room temperature in a dedicated area for the study, that will be only accessible by a limited number of delegated study staff members. Both products should not be stored above 30° Celsius. The temperature (min, max, mean) will be monitored each working day.
The medication will be stored centrally, separate from routine medication stock. The study teams will take a part of the stock, as necessary, with them during the field visits.

An inventory log will be kept, monitoring all the received, distributed and expired medication.

PZQ and AM will be administered to the applicable participants by trained and delegated members of the study staff. All intake of all study medication will be done under direct supervision.

After the end of the study, the sponsor will, in agreement with the manufacturer, instruct the site on the further use or destruction of the remaining IMP.

7. SAFETY ASSESSMENT

7.1. Adverse events

Safety and tolerability of the treatments will be evaluated by recording Adverse Events and grading laboratory and vital signs evaluations.

Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a participant or a clinical investigation participant administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An event that is present before the first administration of study medication is only deemed an AE in case the condition of the event worsens after first IMP administration.

In this study, AEs will be collected up to four weeks after the last administration of the study drug. AEs will be questioned and monitored proactively through a pre-established questionnaire of potential adverse events, during the visits with supervised administration of the IMP, during an additional visit on the 7th day after each administration and during the visit at 4 weeks after each drug administration.

Between these field visits, participants (parents and guardians) will be able to use an unrestricted emergency number to contact the study (on-call) doctor for any health problem. In addition, at the level of each village where the study will be conducted, the nurse or nursing assistant at the station or health center will be informed in detail of the study and will be used as a focal point for optimal referral to the doctor for children with adverse reactions. Each health station nurse will be trained by the study team in primary care for adverse events and will have a stock of emergency drugs provided by the study.

In all cases, the study physicians will be responsible for giving specific written instructions for the management of AEs by the nurse, the health center, or possibly even by the hospital staff. He will also be responsible for monitoring the evolution of the AEs until they are fully resolved. The entire procedure for monitoring (S)AEs is detailed in 2 clinical SOPs (participant evaluation and monitoring; collection/management/reporting of adverse events).

All AEs will be reported in the study database.

Serious Adverse Events

A Serious Adverse Event (SAE) is any AE that results in any of the following outcomes:

- Death;
- Life threatening (participant at immediate risk of death);
- Requires in-patient hospitalization or prolongation of existing hospitalization;
- Results in congenital anomaly/birth defect
- Results in a persistent or significant disability or incapacity;
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

  NOTE (1): In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that

would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is an SAE. When in doubt as to whether "hospitalization" occurred or was necessary, the event should be considered an SAE. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an SAE, nor hospitalization for non-medical reasons (e.g., the participant stays at the hospital overnight because (s)he lives too far and/or there is not transport).

NOTE (2): The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

NOTE (3): The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

For the collection of SAEs, the same collection period of four week after the last study drug administration will be applied. SAE will be reported on a dedicated SAE form and sent, whether or not deemed drug related, immediately (or within 24 hours) by e-mail to the Sponsor:

Clinical Trials Unit
Institute of Tropical Medicine
Nationalestraat 155
2000 Antwerp – Belgium
Email: pharmacovigilance@itg.be

There may be situations when an SAE has occurred, and the investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the investigator always assesses causality for every event prior to transmission of the report to the Sponsor. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE-report form accordingly.

The Sponsor will be responsible for reviewing the SAE forms and providing feedback to the Principal Investigator. The sponsor will also immediately discuss each SAE with the DSMB.
The Sponsor will send a line listings of all reported SAE's to the ITM IRB and the EC UZA on a yearly basis. In Senegal, the Principal Investigator will also report (serious) adverse events to the health authorities – through a level 1 public hospital – as required by local law.

Assessment of severity

All (S)AEs will be assessed by the clinician to quantify intensity as follows:

1. Mild: events require minimal or no treatment and do not interfere with the participant's daily activities.
2. Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
3. Severe: events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
4. Life-threatening: Participant at risk for death at the time of the event

Assessment of causality

The investigator is obliged to assess the relationship between investigational product and the occurrence of each AE/SAE. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk

factors, and the temporal relationship of the event to the IMP will be considered and investigated. The investigator will also consult the drug information and the DSMB as needed in the determination of his/her assessment.

The relationship of an adverse event to study drug is to be assessed according to the following definitions and can only be done by the study physician:

1. Definitely unrelated: Reserved for those events which cannot be even remotely related to study participation (e.g. injury caused by a third party).
2. Unlikely related: There is no reasonable temporal association between the study drug and the AE and the event could have been produced by the participant's clinical state or other modes of therapy administered to the participant.
3. Possible related: The suspected AE may or may not follow a reasonable temporal sequence from study drug administration but seems to be the type of reaction that cannot be dismissed as unlikely. The event could have been produced or mimicked by the participant's clinical state or by other modes of therapy concomitantly administered to the participant.
4. Likely related: The suspected adverse event follows a reasonable temporal sequence from study drug administration, abates upon discontinuation of the drug, and cannot be reasonably explained by the known characteristics of the participant's clinical state.
5. Definitely related: Reserved for those events which have no uncertainty in their relationship to test drug administration: this means that a re-challenge was positive.

Outcome

The outcome of each (S)AE must be assessed according to the following classification:

1. Recovered: The participant recovered from the event with no residual problems.
2. Not yet recovered: This outcome can only be used for Serious Adverse Events. The event no longer meets a 'Serious' criterion, but medical event is not yet completely cured.
3. Permanent damage: The event has resulted in permanent impairment.
4. Ongoing: The participant is continued to be followed for the event.
5. Death: The participant died.
6. Unknown: The participant cannot be traced and no final outcome for the event could be determined.

A study specific SOP on collecting, handling and reporting AEs and SAEs will be written.

7.2. Data and Safety Monitoring Board

An independent Data and Safety Monitoring board (DSMB), composed of an epidemiologist, a pediatrician and a schistosomiasis expert, will be established for the purpose of providing independent advice on the quality of the data produced and the efficacy and safety of the treatment tested, so contributing to safeguarding the interests of the trial participants. The DSMB will also be provided with each SAE report, and with regular statistical reports on frequency and pattern of (S)AEs before the following AM administration.
In this respect, the DSMB will have the possibility to monitor the safety of the treatment on a continuous basis and advise to stop the study if any major safety concern appears.

A DSMB charter, where the relevant terms of reference are clearly defined, will be signed by each member of the DSMB prior to the start of the trial.

8. STATISTICAL METHODS

The statistical analysis will be described in the statistical analysis plan (SAP), written by the biostatistician, which is binding and will be finalized before database lock or before any other analysis takes place.

8.1. Study hypotheses

The primary hypothesis of this study is that one course of AM is non-inferior in terms of efficacy to the standard PZQ treatment. The secondary hypothesis is that repeated courses of AM are superior in terms of efficacy to the standard PQZ treatment. Furthermore, we hypothesize that novel DNA- and antigen-based diagnostics are more accurate than conventional microscopy in assessing the actual antischistosomal treatment response.

8.2. Variables of interest

Primary

The primary variables to evaluate are:

- The parasitological cure rates (proportion of subjects negative for both *Schistosoma* species assessed by microscopy over total number in each arm) for AM and PZQ at week 4 after inclusion.
- The patient counts with drug-related adverse events and serious adverse events up to 4 weeks after AM and PZQ administration.

Secondary:

The secondary variables to evaluate are:

- The proportion of cured subjects (CR) of the two different arms at week 10, 16, 24 and 48 (see flowchart).
- The count of patients reporting any form of adverse event up to 4 weeks after each study drug administration.
- The egg reduction rate (ERR) at weeks 4, 10, 16, 24 and 48 (see flowchart). The ERR will be expressed as the percentage of eggs present in each subject at each time point over the number of eggs at baseline.
- The cure rate and egg reduction rate at weeks 4, 10 and 16 after first treatment administration by *Schistosoma* species and by intensity of infection.
- The result of the classic and novel diagnostic tests at baseline and at different time points, as well as ~~a~~ composite reference standards.
- Clinical and laboratory characteristics related to schistosomiasis-related morbidity at baseline and at weeks 4, 10, 16, 24 and 48
- Prevalence of malaria infection at baseline and at weeks 24 and 48
- The incidence of clinical malaria during the study until week 48
- Counts of cases of anemia both pooled and by severity levels at baseline, week 24 and week 48
- Binary variables indicating the existence of any or specific types of resistance in the sample size and among the incident malaria cases

8.3. Statistical methods

8.3.1. Analysis populations

For the efficacy analysis, both an intention-to-treat (ITT) and a per-protocol (PP) approach will be adopted, with the per-protocol analysis being the primary approach, as recommended for non-inferiority studies.

In the PP analysis we will include only subjects who attend all treatment administration visits and that are assessed for infections 4 weeks following every treatment administration. In the ITT population we will include subjects who do not complete all AM administration doses either entirely (missed doses) or inside the pre-specified time window.

8.3.2. Baseline characteristics

The number of participants screened and enrolled or excluded will be summarized according to reason for exclusion. Of the enrollees, the number of patients discontinued or lost to follow-up will be recorded by reason and time of discontinuation. These figures will be summarized in a CONSORT flow diagram.

Subjects in each treatment group will be described according to baseline characteristics. The description will be in terms of medians and interquartile ranges for continuous variables and using counts and percentages for categorical variables. Standard statistical tests of significance of imbalance in baseline characteristics will be performed.

8.3.3. Primary analysis

The primary hypothesis for non-inferior efficacy will be assessed by calculating the two-sided 95% confidence interval (CI) for the difference in proportions of cured subjects. The CI will be calculated using Wilson's score method.
Interpretation of the CI will be as follows:

- if the two-sided 95% CI for the difference in cure rates (AM schedule - PZQ schedule) lies entirely above -10% then non-inferiority of the AM schedule is concluded;
- if the 95% CI for the difference in cure rates includes -10%, then non-inferiority cannot be established;
- if the 95% CI for the difference in cure rates lies entirely below -10%, then the AM regimen is clinically inferior to the standard regimen.

8.3.4. Secondary and tertiary analysis

Secondary analyses
The efficacy of the two treatments over time will be assessed both by calculating the two-sided 95% CI for the difference in proportions of cured subjects at different time points as well as using a mixed effects logistic regression model. Schistosomiasis infection at specific time points will be used as the outcome and treatment and visit as independent variables with a random intercept. Subsequent analyses of the cure rate per *Schistosoma* species and by *Schistosoma* infection intensity will be analyzed in a similar way including those characteristics as covariates in the model. The analyses for

ERR will be done using a linear mixed effects model with a random intercept and a logit link function separately for each *Schistosoma species*. All comparison of proportions will be done using the Chi-square or Fisher's exact test and comparison of continuous characteristics will be done using the Wilcoxon rank sum test. The sensitivity of a specific diagnostic test will be calculated as the proportion of children with schistosomiasis (based on the reference standard used) who have a positive result. The specificity of a diagnostic test will be calculated as the proportion of children without schistosomiasis who have a negative result. The positive predictive value of a diagnostic test will be calculated as the proportion of children with schistosomiasis with a positive result. The negative predictive value of a diagnostic test will be calculated as the proportion of people with a negative result who do not have schistosomiasis. These different values of both novel and classic diagnostic tests will be calculated together with 95% confidence intervals. Composite reference standards will be created by the investigators of the study and will be used as a comparison. An alternative reference standard will be calculated using latent class analysis methodology. The prevalence and patterns of resistant mutations between the two treatment groups will be compared using Chi-square or Fisher's exact test.

Safety analyses

All non-serious and serious adverse events will be grouped according to a pre-specified side-effect coding system and tabulated. The number of subjects experiencing any adverse event, any serious adverse event, and any drug-related serious adverse event up to 4 weeks after PZQ and AM administration will be compared between treatment groups using Fisher's exact test. Safety will be analyzed using the all-subjects-treated approach for each time point separately. The safety results during the other AM administration visits will be tabulated in a similar way, but no formal statistical comparison is planned.

8.3.5 Subgroup analyses

Besides the subgroup analyses described in the secondary objectives, safety results will be analyzed by weight and age characteristics. Additionally, anemia-related results will be analyzed by Schistosomiasis and malaria co-infection status.

8.3.6 Multiplicity and Missing Data

An interim analysis is planned at week 16 for the efficacy endpoints of cure rate and egg reduction rate after PZQ administration and single and repeated courses of AM. The endpoints of interest will be analyzed and communicated as soon as the data is collected and cleaned. The analysis and results will not influence follow-up or analysis of the rest of the objectives and endpoints in any way.

The primary analysis is a comparison of a single measurement, thus no adjustments for multiplicity are needed.

Subjects who do not attend all treatment administration visits or the follow-up visits will be considered as missing and will be excluded from the per-protocol analysis. No imputation of missing data will be performed.

9. MONITORING AND QUALITY ASSURANCE

This study will be monitored in accordance with regulations applicable to clinical trials, including ICH-GCP and WHO-GCLP, and sponsor-specific SOPs. The PI and involved site research staff will allocate adequate time and resources for such monitoring activities. The investigator will also ensure that the monitor or QA reviewer is given access to all the above noted study-related documents and study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) and has adequate space and resources to conduct monitoring and source data verification.

Monitoring will be performed by the Clinical Research Scientist (CRS) of the ITM Clinical Trial Unit (CTU) and the Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation (IRESSEF). A monitoring plan will be written to detail the frequency, requirements content, percentage of Source Data Verification (SDV) of study monitoring visits. Findings from monitoring visits will be noted in a visit report that will be shared with the study team.
Monitoring visits of the CTU CRS will be combined with supervision visits of other members of the sponsor study team. These visits will also contribute to the quality assurance of the study and will be documented in a visit report.

All laboratory procedure will be carried out according to SOPs developed by the study team and quality control (QC) procedures will be followed for all laboratory techniques in the study site and at ITM.

The sponsor will inform the Investigators concerned immediately upon notification of a pending study inspection by any regulatory authority or funder. Likewise, the investigator will inform the sponsor of any pending inspection.

10. DATA MANAGEMENT

Full details regarding the handling of trial data will be described in the trial Data Management Plan (DMP). The DMP describes the lifecycle for the data to be collected, processed and/or generated and includes information on how data will be collected, processed, shared, curated, preserved and includes the methodologies and standards applied.

Responsibilities

The data manager at the ITM's CTU will be responsible for setup of the eCRF/database, system validation and management, change control, drawing up the data management plan and any other essential data management documentation such as data entry guidelines, validation file, etc.

During the study, the monitor will visit the investigational site to confirm that data are being accurately recorded in the eCRFs from the subject source documents, i.e. source data verification (SDV), as described in chapter 9.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection and quality

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents, or discrepancies should be explained. Only data defined by the study protocol should be captured in the eCRF. In addition to paper source, some data will be entered directly in the electronic CRF.

Clinical data (including adverse events (AEs) and concomitant medications) and clinical laboratory data will be entered into eCRFs using a ICH-GCP compliant data capture system, REDCap. For practical reasons, data collection on site will be done with the REDCap Mobile App using tablets, while data management will be done using the web application. The data system includes password protection and internal validation checks to identify data that appear inconsistent, incomplete, or inaccurate. Data modifications will be automatically tracked by an audit trail detailing the date and time of the correction and the name of the person making the correction. The system will be validated before moved to production and taken into use. A data dictionary containing metadata and SOP for using the system will be maintained.

Confidentiality and security

All participant related information will be kept strictly confidential. All records will be kept in a secure location and only research staff will have access to the records.

All personal data collected in the eCRF and study database will be pseudonymized, by which a unique trial specific code will be assigned to each study participant. Any information that could lead to the identification of the participant will not be included in the study data electronic file.

The eCRF is only accessible via a login with personal username and password. Trial team members will be given user rights based on their study role and a list of authorized users will be kept at the CTU and updated regularly. Data backups will be maintained on the ITM server.

Record retention and archiving

Data management documentation (DMP, Validation File, metadata, SOP for data entry, user list, training log) will be stored electronically in an access-controlled study-specific folder on the ITM server; paper documents will be kept in a study-specific binder at the CTU.

The eCRF system and the database will be hosted at the ITM. After the trial database is locked and released to the statistician, a final copy of the database will be stored at the Sponsor. The IRESSEF will also receive a copy of the trial site's final and locked data as write-protected PDF-files. The PDF-files will be stored on a USB flash drive and will be provided to the Investigator. The Investigator should retain all source documents for each participant in the study, as well as all other essential trial documents.

11. ETHICAL ISSUES

11.1. Ethical and regulatory review

Ethical approval: The initial protocol and informed consent documents, as well as any further substantial amendments, will be submitted for ethical approval in the sponsor country, as well as the study country. The concerned Ethics Committees (ECs) are:

- The Institutional Review Board (IRB) of the ITM, Antwerp, Belgium
- The EC of the Antwerp University Hospital, Belgium
- The National Ethics Council for Research in Health (CNERS) in Senegal

Regulatory approval: The initial protocol and informed consent documents, as well as any further study documents and substantial amendments, will be submitted in French for regulatory approval to the competent authorities in the study country, the Ministry of Health in Senegal.
No study-specific interventions will take place before written approval by all Ethics Committees above has been obtained and the local regulatory requirements have been complied with, and the signature of the clinical study protocol of each contractual party involved have been obtained.
The study will be carried out according to the principles stated in the Declaration of Helsinki, all applicable regulations and according to the most recent GCP and GCLP guidelines.
The study will also be included in the Clinicaltrials.gov public registry prior to the start of participant recruitment.

11.2. Protocol amendments

Once the final clinical study protocol has been issued and signed by the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the appropriate steps before being implemented. Any substantial change must be approved by all the bodies and EC's that have approved the initial protocol, prior to being implemented, unless it is due to participant's safety concerns (in which case the immediate implementation can be necessary for the sake of participant's protection). In case modifications to the protocol or amendment are requested by any local EC/CA during the review process, these must be discussed and agreed upon with the Sponsor prior to any resubmission incorporating those changes.

11.3. Informed consent

No participant may be admitted into the study until the Investigator or designee has obtained the written informed consent of the parents/guardian, and verbal consent (assent) from the participating child.
Details for obtaining informed consent and assent are described in detail in chapter 5.2.
Participation in this trial is entirely voluntary. Any participant (children or his/her parent(s) or legal guardian) may withdraw from the study at any time without this decision affect the medical advice and care provided.

11.4. Confidentiality and data protection

Personal and medical information from the trial will be kept confidential, in line with the requirements of the European General Data Protection Regulation 2016/679, and with applicable requirements in Senegal. Each study participant will be given a unique study-specific participant code and personal identifiers will not appear on the Case Report Forms, the database, or the biological samples, which

will be pseudonymized from the beginning of the collection process. Access to all paper and electronic study files will be restricted to authorized study staff. Study computers and databases will be user-name and password-protected. Electronic datasets during the study will be shared on secure platforms. All documents that may identify the participants, e.g., signed informed consent forms, original hospital and laboratory records, identification logs, etc., will only be stored at the study sites and will not be sent to ITM.

This project involves collection and processing of personal data including biomedical, biometric, and demographic data. However, data on sexual lifestyle, political opinion, religious or philosophical conviction will not be collected. Furthermore, genetic information will not be collected.
Prior data collection and processing, authorization for data and sample transfer will be asked from applicable ethics and/or regulation committees of both Senegal and Belgium.

11.5. Community involvement

The first step will consist in informing administrative, medical authorities as well as community leaders about the project purpose and the design of the study. Before starting the study, a communication plan will be designed to inform the population, and most importantly the school directors and teachers, on the study procedures. Verbal permission of the head of households and school directors will be sought. Also, the health centers closest to the study schools will be invited to the kick-off meeting, to inform them about the study and train them in monitoring for expected side effects of the study medication and how to keep the study team informed about any study participant that comes to the health center, so that potential (S)AEs may be collected, and patients will be immediately and adequately treated. These local health facilities will also be regularly visited during the study to keep them focused and motivated for listing participants who have visited them

11.6 Risks and benefits

11.6.1. Risks

Minor participants
This project is built around a randomized clinical trial involving human participants who are considered as vulnerable, for at least two reasons: (1) children of school age are minors and, therefore, not entitled to make an autonomous decision on trial participation; (2) children in this trial mainly belong to socially vulnerable groups (where poor access to health care may affect voluntarism in the informed consent process, since trial participation may be primarily seen as a strategic choice to secure trial-related health and non-health benefits).

To ensure full protection of the trial participants, the trial is conducted according to strict protocols and regulations and a written informed consent will be obtained from parents or guardian (as well as assent from the children) as described in section 5.2.

Medical interventions including invasive techniques
The clinical trial involves medical interventions in human participants, i.e., a full clinical examination, anthropometric measurements, a search for signs and symptoms of chronic schistosomiasis or malaria and an ultrasound examination of organ-specific schistosomiasis morbidity. The laboratory assessments will require the collection of blood by finger prick (considered as invasive technique that might cause pain), urine and stool samples, which will be clearly mentioned and detailed in the Informed Consent Form. No other invasive or dangerous techniques are required.

All medical examinations and collections of biological samples will be carried out by adequately qualified staff in a dedicated room, ensuring the privacy and confidentiality of all participants.

Tolerance of the investigational Medical Product

The side-effects of the treatments will be discussed with the parents/guardian. Long-term experience with AM combination for malaria treatment has shown that the side effect profile is manageable, and usually better tolerated by children than by adults. Oral artesunate does not pose problems in terms of safety and tolerability. The tolerability of mefloquine is widely considered to be less than artesunate; however, it has been used as treatment in tens of thousands of malaria patients in various countries and as chemoprophylaxis for the last 30 years in travelers and pregnant women with no major problems, provided counselling is adequate and specific contra-indications are respected [39,40]. This drug is usually better tolerated by children than by adults [39]. Particular attention will be paid during screening to the classic contra-indications of mefloquine (i.e. history of epilepsy and psychiatric disturbances). Primary school children will be closely monitored for side effects, particularly during the AM courses. In case of severe intolerance (digestive or neurological), treatment will be discontinued, and the child concerned will be excluded from the trial and treated and followed up until adverse symptoms subside.

A safe interval of 6 weeks between each AM administration is proposed because some data in Asia suggest that the frequency of side effects may increase if mefloquine is reused for treatment within four weeks after a first administration [40].

Nevertheless, a robust safety monitoring system has been set up to pro-actively scrutinize the tolerance and safety profile of (artesunate)-mefloquine. Firstly, each dose of AM will be given under direct medical observation during the three first days, with assessment of tolerance since the previous administration and possibility of immediate discontinuation based on medical decision. Toxicity of mefloquine usually appears early and is rather acute (vomiting, neurological manifestations). Second, another contact with the participant either by phone call or home visit will be organized systematically by the study team one week after the first day of AM administration to proactively capture any "delayed" side effect.

Thirdly, parents of all enrolled children will be advised to bring the study ICF whenever their children need to attend the well identified study health centers at any moment during the study period, (in particular after the first and until the next AM administration) making them easily identifiable by all local health care workers. The local staff will be involved in the study, adequately informed and specifically trained to manage and report these adverse events during the preparatory period. The local investigator (medical doctor based in Richard Toll) will be immediately contacted by phone to be informed about, and provide further advice, for any problem potentially related to the study during the whole study period. Finally, medical assessment and specific queries regarding tolerance of previous administration will take place just before each subsequent AM administration. During the preparatory visits, all practicalities of the safety monitoring procedure will be established in a detailed SOP, after discussion with all local health staff.

Side effects related to PZQ are rare but may include headache, dizziness, stomach pain, nausea, tiredness, weakness, joint/muscle pain, loss of appetite, vomiting and sweating. These side effects are usually mild and temporary and may be related to some inflammatory reactions to the dying parasites. In very rare cases, more serious side effects occur such as bloody diarrhea, fever, irregular/slow heartbeat, seizures. Those side effects are short lasting and often disappear with 48h after drug administration.

Risk of favoring malaria resistance

The risk of selecting resistant parasites is extremely unlikely (or even close to non-existing) for the following reasons:

- So far, no artemisinin or partner drug resistance has been reported in large community studies conducted in high endemic areas that have evaluated ACTs for intermittent preventive treatment or seasonal malaria chemoprophylaxis [90–92].
- Malaria incidence in this area is the lowest in Senegal with less than 5 cases per 1000 inhabitants, which makes it highly unlikely that the drug can be administered to a malaria patient.
- Children with clear signs of malaria or with any sign of the disease will not be eligible, making selection pressure for AM – that could lead to resistance – impossible, because the drug will not come into contact with plasmodium in this situation.

Loss of confidentiality

Delegated study team members will be responsible for reporting the patient's personal details and identification code in a subject identification code list (pseudonymization). To ensure confidentiality, this list will be kept in a separate, locked cupboard together with the signed ICFs and only authorized members of the study team will have access to it. All participant information will be stored in locked filing cabinets in areas with access limited to study site staff. All participant information and laboratory specimens, including stored specimens, reports, study data collection, process, and administrative forms will be identified by the study code, date of sample collection and type of study visit. Also, clinical data that is directly captured electronically in the field will only use the patient-specific study code. Names will not be used in the electronic database, nor in any written or oral report. The database will be secured with password-protected access systems (see chapter 10 for details). Participant's study information will not be released to anybody outside the medical team, except as necessary (and under confidentiality agreement) for the independent monitoring, auditing and for inspection by competent authorities.

During or after final publication of results, individual patient data might be shared for secondary research in an access-controlled way and on an anonymized basis, in line with the requirements that are currently being set up.

11.6.2. Benefits

The study will allow children to be diagnosed with schistosomiasis. They have a 50% change to receive the standard treatment and 50% chance to be in the experimental arm, but both arms contain medication with a very well described safety profile. The clinical and biological follow-up of the children in the study who are infected with schistosomiasis will be very strict. Any side effects or incidental finding, will be discovered in an early stage. Those presenting any severe pathology will be referred to health facilities for appropriate care and follow-up.

At week 48 post-inclusion, PZQ will be administered for free (at the study cost) to all enrolled schoolchildren and all infected children that were not eligible for the study. In addition, under the coordination and with the funding of the national control program, MDA with PZQ will be possibly conducted to the rest of schools according to WHO guidelines, based on the prevalence of schistosomiasis infection that we will observe in the study school-age children (74,75). At the community level, mass treatment with praziquantel will also eventually be given to all villagers, again based on observed prevalence figures and in accordance with WHO and Senegalese Ministry of Health guidelines, thus contributing to the efforts of the national schistosomiasis control program.

Another benefit of the study for the community is the regular presence of a medical team during the study. If necessary and feasible, it may be consulted by persons who are not part of the study, provided that this does not interfere with the conduct of the study. Study team members can provide medical advice during field visits, which is often done in Senegal, and is highly appreciated by the community. However, this is not the responsibility of this study.

At the local level, a potential benefit of this study will be the implementation of operating procedures that can be used by Senegalese scientists and health personnel. In addition, the skills and experience that will be acquired during this trial will strengthen the capacity of local scientists to conduct complementary research in similar contexts. The scientific production resulting from this project will also be important elements for the career development of local scientists.

11.7. Compensation

Study participants will receive no financial compensation. However, all examinations that are described in the trial are performed free of charge. Children that are diagnosed with schistosomiasis but are not eligible for the trial because of the presence of exclusion criteria or because their parents or guardian did not consent (or the children themselves did not assent) will be treated with PZQ according to WHO guidelines. AEs and SAEs will be managed according to specific SOP's. In case of severe intolerance (digestive or neurological), treatment will be discontinued, and the child concerned will be withdrawn from the trial and treated and followed up through regular health care facilities until adverse symptoms subside.
Moreover, the trial budget will cover the costs of transportation to the hospital if the children would be referred to the reference hospital for further examination and case management. In some cases, the trial budget may contribute to fees for hospital care and medication for study participants.

11.8. Insurance

As required by the Belgian law on experiments on the human person of May 7th, 2004, the sponsor has obtained a no-fault liability insurance covering any harm, injury or (material) damage which may occur to study participants and which may be directly or indirectly caused by their participation in the trial. The insurance provisions will also be mentioned during the informed consent discussion.

11.9. Shipment, storage and use of samples

The samples will only be used for the research objectives described in the trial protocol. If any additional analyses, not initially planned, are considered as relevant for the project (i.e. new diagnostics) and agreed upon by all partners, an amendment will be submitted to the relevant ECs. The maximum storage duration and the eventual disposal of the samples will also be defined in the protocol and mentioned in the inform consent forms.
Stool and urine samples, as well as dried blood spots on filter paper from Senegal will be shipped to the ITM. Parents/guardians will be requested to provide consent for the long-term storage and shipment of samples abroad. Material transfer agreements will be put in place before shipments are carried out.
Any secondary use will need to be approved by the applicable ethical review boards, as well as by the researchers involved in this study. These samples will be stored at ITM and the IRESSEF for a maximum of 50 years.

11.10. Environment & Health and Safety

Our research does neither involve use of elements that may cause harm to the environment, to animals or plants nor deal with endangered fauna and/or flora and/or protected areas. However, standards for a safe working environment that are already established will be maintained in all study sites.

11.11. Dual use

Our research does not provide any knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agriculture, plants, animals, the environment, or material.

11.12. Exclusive focus on civil applications

Our study is exclusively focused on civil applications. Thus, the research itself and its outcome do neither intend to be used in military application nor aim to serve military purposes.

12. DISSEMINATION OF RESULTS, INTELLECTUAL PROPERTY

All study documents are provided by the Sponsor to the Investigators and his/her appointed staff in confidence. None of this material may be disclosed to any party not directly involved with the study, without written permission from the Sponsor.

Data sharing has become increasingly important to enable further analyses and broaden scientific collaborations beyond the initial collaborators. This is especially true for neglected tropical diseases, such as schistosomiasis, where robust data from well-structured clinical trials are limited. This process of data sharing involves ensuring the harmonization of data quality, the protection of the confidentiality of participants and communities, and fair scientific credit to the country where the data originated. The project will evaluate the possible repositories or collaborative platforms where anonymized final data can be shared, according to the general policies of the parties involved in this study.

The dissemination and exploitation activities in SchistoSAM will be coordinated by the project management team (composed of CTU experts, ITM supervisors and IRESSEF investigators), based on a central Dissemination and Communication plan, and guided by the results of the trial, and the additional laboratory workup.
The main scientific output will be an **independent evaluation of AM's safety and efficacy** (both of single and repeated courses) against schistosomiasis compared to that of PZQ. As already underlined, if the efficacy is found equal (at least non-inferior) to that of PZQ, it could be considered a valuable second-line treatment for schistosomiasis (considering its 3-day duration), taking into account the specificity of each region. This should lead to a high impact publication in a major Infectious Diseases Journal and open the way to additional confirmative/complementary clinical studies in other settings.
In case the trial results are negative, it will also be important to publish the data, which would then probably discard AM as an alternative therapeutic option and allow researchers to fully focus on other novel treatments.
An additional set of scientific publications will report on the **in-depth evaluation of novel (ultrasensitive) antigen- and DNA-based assays** for assessing the response to antischistosomal treatment (in comparison to conventional microscopy). Also, this study will determine the value of point-of-care diagnostics (POC-CAA) and morbidity markers (hematuria and fecal occult blood by POC assays) in the diagnosis of schistosomiasis infection and morbidity, as well as monitoring after antischistosomal treatment.
Other publications include an update of the current status of schistosomiasis and malaria infection and morbidity in school-age children in the study region and the impact of repeated AM courses on malaria infection and morbidity in Schistosoma-infected children (non-exhaustive list).

Other communication activities will include research meetings with local researchers and program managers and scientific presentation during international congresses dedicated to infectious and tropical diseases

Reporting and publication will be done in accordance with the CONSORT statement.

13. ARCHIVING

The sponsor and Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be verified. The relevant (essential) documents are those documents which individually and collectively permit to assess the conduct of the trial, the quality of the data produced and the compliance with GCP standards and applicable regulatory requirements. The Investigator's File (IF) should contain all the (essential) documents as listed ICH-GCP guidelines. A copy of all source data and Case Report Forms must always be kept on site at IRESSEF.

All the relevant study documentation present should be retained for a minimum of twenty (20) years and according to applicable local regulations. The Sponsor should be informed prior to destruction of the files.
After the study, the IF will remain available for internal audits and/or inspections of regulatory authorities for a period of twenty years, unless differently requested by national authorities.

14. REFERENCES

1. Colley, D. G., Bustinduy, A. L., Secor, W. E. & King, C. H. Human schistosomiasis. *Lancet (London, England)* **383,** 2253–64 (2014).
2. McManus, D. P. *et al.* Schistosomiasis. *Nat. Rev. Dis. Prim.* **4,** 13 (2018).
3. King, C. H., Dickman, K. & Tisch, D. J. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet (London, England)* **365,** 1561–9 (2005).
4. Abajobir, A. A. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **390,** 1211–1259 (2017).
5. Abajobir, A. A. *et al.* Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **390,** 1151–1210 (2017).
6. Murray, C. J. L. *et al.* Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380,** 2197–2223 (2012).
7. Meurs, L. *et al.* Epidemiology of mixed Schistosoma mansoni and Schistosoma haematobium infections in northern Senegal. *Int. J. Parasitol.* **42,** 305–311 (2012).
8. Zwang, J. & Olliaro, P. L. Clinical efficacy and tolerability of praziquantel for intestinal and urinary schistosomiasis-a meta-analysis of comparative and non-comparative clinical trials. *PLoS Negl. Trop. Dis.* **8,** e3286 (2014).
9. Zwang, J. & Olliaro, P. Efficacy and safety of praziquantel 40 mg / kg in preschool-aged and school-aged children : a meta-analysis. *Parasit. Vectors* 1–16 (2017). doi:10.1186/s13071-016-1958-7
10. World Health Organization. Assessing the efficacy of anthelminthic drugs against schistosomiasis and soil-transmitted helminthiases. (2013).
11. King, C. H. *et al.* Utility of repeated praziquantel dosing in the treatment of schistosomiasis in high-risk communities in Africa: a systematic review. *PLoS Negl. Trop. Dis.* **5,** e1321 (2011).
12. World Health Organization. Preventive chemotherapy in human helminthiasis. (2006).
13. World Health Organization. Schistosomiasis and soil-transmitted helminthiases: number of people treated in 2015. *Wkly. Epidemiol. Rec.* **91,** 585–95 (2016).
14. Coulibaly, J. T. *et al.* Efficacy and safety of praziquantel in preschool-aged and school-aged children infected with Schistosoma mansoni : a randomised controlled , parallel-group , dose-ranging , phase 2 trial. *Lancet Glob Heal.* **5,** 688–698 (2017).
15. Cioli, D., Pica-mattoccia, L., Basso, A. & Guidi, A. Molecular & Biochemical Parasitology Schistosomiasis control : praziquantel forever ? *Mol. Biochem. Parasitol.* **195,** 23–29 (2014).
16. Bergquist, R., Utzinger, J. & Keiser, J. Controlling schistosomiasis with praziquantel: How much longer without a viable alternative? *Infect. Dis. poverty* **6,** 74 (2017).
17. Wu, W., Wang, W. & Huang, Y. X. New insight into praziquantel against various developmental stages of schistosomes. *Parasitol. Res.* **109,** 1501–1507 (2011).
18. Siqueira, L. da P. *et al.* Schistosomiasis: Drugs used and treatment strategies. *Acta Trop.* **176,** 179–187 (2017).
19. Webster, B. L. *et al.* Praziquantel treatment of school children from single and mixed infection foci of intestinal and urogenital schistosomiasis along the Senegal River Basin: monitoring treatment success and re-infection patterns. *Acta Trop.* **128,** 292–302 (2013).
20. Wang, W., Wang, L. & Liang, Y.-S. Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitol. Res.* **111,** 1871–1877 (2012).
21. Pinto-almeida, A., Mendes, T. & Oliveira, R. N. De. Morphological Characteristics of Schistosoma mansoni PZQ-Resistant and -Susceptible Strains Are Different in Presence of Praziquantel. *Front Microbiol* **7,** 1–11 (2016).
22. Vale, N. *et al.* Praziquantel for Schistosomiasis: Single- Drug Metabolism Revisited, Mode of Action, and Resistance. *Antimicrob Agents Chemother* **61,** 1–16 (2017).
23. Geerts, S. & Gryseels, B. Anthelmintic resistance in human helminths: a review. *Trop Med Int Heal.* **6,** 915–21 (2001).
24. Melman, S. D. *et al.* Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of Schistosoma mansoni. *PLoS Negl. Trop. Dis.* **3,** (2009).
25. Crellen, T. *et al.* Reduced Efficacy of Praziquantel Against Schistosoma mansoni Is Associated With Multiple Rounds of Mass Drug Administration. *Clin. Infect. Dis.* **63,** 1151–1159 (2016).
26. Gouveia, M. J., Brindley, P. J., Gärtner, F., Costa, J. M. C. da & Vale, N. Drug Repurposing for Schistosomiasis: Combinations of Drugs or Biomolecules. *Pharmaceuticals (Basel).* **11,** (2018).
27. Liu, R., Dong, H.-F., Guo, Y., Zhao, Q.-P. & Jiang, M.-S. Efficacy of praziquantel and artemisinin derivatives for the treatment and prevention of human schistosomiasis: a systematic review and meta-analysis. *Parasit. Vectors* **4,** 201 (2011).
28. Xiao, S. Mefloquine , a new type of compound against schistosomes and other helminthes in experimental studies. *Parasitol. Res.* 3723–3740 (2013). doi:10.1007/s00436-013-3559-0
29. Keiser, J; Utzinger, J. Antimalarials in the Treatment of Schistosomiasis. *Curr Pharm. Des* **18,** 3531–8 (2012).
30. Utzinger, J., Shu-Hua, X., Tanner, M. & Keiser, J. Artemisinins for schistosomiasis and beyond. *Curr Op Investig Drugs* **8,** 105–116 (2007).
31. Utzinger, J., Tanner, M. & Keiser, J. ACTs for schistosomiasis: Do they act? *Lancet Infect. Dis.* **10,** 579–581 (2010).

32. Pérez del Villar, L., Burguillo, F. J., López-Abán, J. & Muro, A. Systematic review and meta-analysis of artemisinin based therapies for the treatment and prevention of schistosomiasis. *PLoS One* **7,** e45867 (2012).
33. Elmorshedy, H., Tanner, M., Bergquist, R. N., Sharaf, S. & Barakat, R. Acta Tropica Prophylactic effect of artemether on human schistosomiasis mansoni among Egyptian children : A randomized controlled trial. *Acta Trop.* **158,** 52–58 (2016).
34. Keiser, J. *et al.* Mefloquine — An Aminoalcohol with Promising Antischistosomal Properties in Mice. *PLoS Negl. Trop. Dis.* **3,** (2009).
35. Abou-shady, O. M. Therapeutic effect of mefloquine on Schistosoma mansoni in experimental infection in mice. *J. Parasit. Dis.* **40,** 259–267 (2016).
36. Keiser, J. *et al.* Efficacy and Safety of Mefloquine , Artesunate , Mefloquine-Artesunate , and Praziquantel against Schistosoma haematobium : *Clin. Infect. Dis.* **50,** 1205–1213 (2010).
37. Basra, A. *et al.* Efficacy of Mefloquine Intermittent Preventive Treatment in Pregnancy Against Schistosoma haematobium Infection in Gabon : A Nested Randomized Controlled Assessor-Blinded Clinical Trial. *Clin. Infect. Dis.* **56,** 68–75 (2013).
38. Keiser, J. *et al.* Praziquantel, Mefloquine-Praziquantel, and Mefloquine-Artesunate-Praziquantel against Schistosoma haematobium : A Randomized, Exploratory, Open-Label Trial. *PLoS Negl. Trop. Dis.* **8,** (2014).
39. Schlagenhauf, P. *et al.* Use of mefloquine in children - a review of dosage, pharmacokinetics and tolerability data. *Malar. J.* **10,** 292 (2011).
40. Lee, S. J., Ter Kuile, F. O., Price, R. N., Luxemburger, C. & Nosten, F. Adverse effects of Mefloquine for the treatment of uncomplicated malaria in Thailand: A pooled analysis of 19, 850 individual patients. *PLoS One* **12,** 1–16 (2017).
41. Sirima, S. B. *et al.* Comparison of artesunate–mefloquine and artemether–lumefantrine fixed-dose combinations for treatment of uncomplicated Plasmodium falciparum malaria in children younger than 5 years in sub-Saharan Africa: A randomised, multicentre, phase 4 trial. *Lancet Infect. Dis.* **16,** 1123–1133 (2016).
42. Adedoja, A. *et al.* Co-endemicity of Plasmodium falciparum and intestinal helminths infection in school age children in rural communities of Kwara state Nigeria. *PLoS Negl. Trop. Dis.* **9,** 1–13 (2015).
43. Kinung'hi, S. M., Magnussen, P., Kaatano, G. M., Kishamawe, C. & Vennervald, B. J. Malaria and helminth co-infections in school and preschool children: A cross-sectional study in Magu district, North-Western Tanzania. *PLoS One* **9,** (2014).
44. Yapi, R. B. *et al.* Infection and Co-infection with Helminths and Plasmodium among School Children in Côte d'Ivoire: Results from a National Cross-Sectional Survey. *PLoS Negl. Trop. Dis.* **8,** (2014).
45. Florey, L. S. *et al.* Partnering parasites: Evidence of synergism between heavy schistosoma haematobium and plasmodium species infections in Kenyan children. *PLoS Negl. Trop. Dis.* **6,** 1–11 (2012).
46. Morenikeji, O. A., Eleng, I. E., Atanda, O. S. & Oyeyemi, O. T. Renal related disorders in concomitant Schistosoma haematobium-Plasmodium falciparum infection among children in a rural community of Nigeria. *J. Infect. Public Health* **9,** 136–142 (2016).
47. Sokhna, C. *et al.* Increase of malaria attacks among children presenting concomitant infection by Schistosoma mansoni in Senegal. *Malar. J.* **3,** 43 (2004).
48. de Vlas, S. Underestimation of Schistosoma mansoni pravalences. *Parasitol Today* **8,** 274–277 (1992).
49. Engels, D., Sinzinkayo, E., De Vlas, S. J. & Gryseels, B. Intraspecimen fecal egg count variation in Schistosoma mansoni infection. *Am. J. Trop. Med. Hyg.* **57,** 571–577 (1997).
50. Knopp, S., Becker, S. L., Ingram, K. J., Keiser, J. & Utzinger, J. Diagnosis and treatment of schistosomiasis in children in the era of intensified control. *Expert Rev. Anti. Infect. Ther.* **11,** 1237–1258 (2013).
51. Utzinger, J., Becker, S. L., van Lieshout, L., van Dam, G. J. & Knopp, S. New diagnostic tools in schistosomiasis. *Clin. Microbiol. Infect.* **21,** 529–542 (2015).
52. Verweij, J. J. & Rune Stensvold, C. Molecular testing for clinical diagnosis and epidemiological investigations of intestinal parasitic infections. *Clin. Microbiol. Rev.* **27,** 371–418 (2014).
53. ten Hove, R. J. *et al.* Multiplex real-time PCR for the detection and quantification of Schistosoma mansoni and S. haematobium infection in stool samples collected in northern Senegal. *Trans. R. Soc. Trop. Med. Hyg.* **102,** 179–185 (2008).
54. Meurs, L. *et al.* Is PCR the next reference standard for the diagnosis of schistosoma in stool? A comparison with microscopy in Senegal and Kenya. *PLoS Negl. Trop. Dis.* **9,** 1–16 (2015).
55. Vinkeles Melchers, N. V. S. *et al.* Diagnostic Performance of Schistosoma Real-Time PCR in Urine Samples from Kenyan Children Infected with Schistosoma haematobium: Day-to-day Variation and Follow-up after Praziquantel Treatment. *PLoS Negl. Trop. Dis.* **8,** (2014).
56. van Lieshout, L. & Roestenberg, M. Clinical consequences of new diagnostic tools for intestinal parasites. *Clin. Microbiol. Infect.* **21,** 520–528 (2015).
57. Fernández-Soto, P. *et al.* A Loop-Mediated Isothermal Amplification (LAMP) Assay for Early Detection of Schistosoma mansoni in Stool Samples: A Diagnostic Approach in a Murine Model. *PLoS Negl. Trop. Dis.* **8,** (2014).
58. Javier Gandasegui, Pedro Fernández-Soto , Antonio Muro , Constança Simões Barbosa, Fabio Lopes de Melo, Rodrigo Loyo, E. C. de S. G. A field survey using LAMP assay for detection of Schistosoma mansoni in a low. *PLoS Negl. Trop. Dis.* **12,** 1–16 (2018).
59. Lodh, N. *et al.* Point of care diagnosis of multiple schistosome parasites: Species-specific DNA detection in urine by

loop-mediated isothermal amplification (LAMP). *Acta Trop.* **173,** 125–129 (2017).
60. Adriko, M. *et al.* Evaluation of circulating cathodic antigen (CCA) urine-cassette assay as a survey tool for Schistosoma mansoni in different transmission settings within Bugiri District, Uganda. *Acta Trop.* **136,** 50–57 (2014).
61. Lamberton, P. H. L., Kabatereine, N. B., Oguttu, D. W., Fenwick, A. & Webster, J. P. Sensitivity and Specificity of Multiple Kato-Katz Thick Smears and a Circulating Cathodic Antigen Test for Schistosoma mansoni Diagnosis Pre- and Post-repeated-Praziquantel Treatment. *PLoS Negl. Trop. Dis.* **8,** (2014).
62. Colley, D. G., Andros, T. S., Campbell, C. H. & Jr. Schistosomiasis is more prevalent than previously thought: what does it mean for public health goals, policies, strategies, guidelines and intervention programs? *Infect. Dis. poverty* **6,** 63 (2017).
63. Colley, D. G. *et al.* A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of Schistosoma mansoni. *Am. J. Trop. Med. Hyg.* **88,** 426–432 (2013).
64. Corstjens, P. L. A. M. *et al.* Up-converting phosphor technology-based lateral flow assay for detection of Schistosoma circulating anodic antigen in serum. *J. Clin. Microbiol.* **46,** 171–176 (2008).
65. van Dam, GJ. Bogitsh, R.J.M. van Zeyl, J.P Rotmans, A. M. D. Schistosoma mansoni/In vitro and in vivo excretion of CAA and CCA by developing schistosomula and adult worms. *J Parasitol* **82,** 557–564 (1996).
66. Polman, K. *et al.* Specificity of circulating antigen detection for schistosomiasis mansoni in Senegal and Burundi. *Trop. Med. Int. Heal.* **5,** 534–537 (2000).
67. Knopp, S. *et al.* Sensitivity and Specificity of a Urine Circulating Anodic Antigen Test for the Diagnosis of Schistosoma haematobium in Low Endemic Settings. *PLoS Negl. Trop. Dis.* **9,** 1–19 (2015).
68. CF, H. The use of ultasound in schistosomiasis. *Adv Parasitol* **48,** 225–284 (2001).
69. Webster, J. P., Koukounari, A., Lamberton, P. H. L., Stothard, J. R. & Fenwick, A. Evaluation and application of potential schistosome-associated morbidity markers within large-scale mass chemotherapy programmes. *Parasitology* **136,** 1789–1799 (2009).
70. Barda, B., Coulibaly, J. T., Hatz, C. & Keiser, J. Ultrasonographic evaluation of urinary tract morbidity in school-aged and preschool-aged children infected with Schistosoma haematobium and its evolution after praziquantel treatment : A randomized controlled trial. *PLoS Negl. Trop. Dis.* 1–13 (2017). doi:10.1371/journal.pntd.0005400
71. Ochodo, E. A. *et al.* Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas. *Cochrane database Syst. Rev.* CD009579 (2015). doi:10.1002/14651858.CD009579.pub2
72. Emukah, E. *et al.* Urine heme dipsticks are useful in monitoring the impact of Praziquantel treatment on Schistosoma haematobium in sentinel communities of Delta State, Nigeria. *Acta Trop.* **122,** 126–131 (2012).
73. Bustinduy, A. L. *et al.* Fecal Occult Blood and Fecal Calprotectin as Point-of-Care Markers of Intestinal Morbidity in Ugandan Children with Schistosoma mansoni Infection. *PLoS Negl. Trop. Dis.* **7,** 1–9 (2013).
74. WHO Expert Committee on the Control of Schistosomiasis. *Prevention and control of schistosomiasis and soil-transmitted helminthiasis : report of a WHO expert committee.* (World Health Organization, 2002).
75. WHO | Prevention and control of schistosomiasis and soil-transmitted helminthiasis: WHO Technical Report Series N° 912. *WHO* (2016).
76. Seck, M. C. *et al.* Malaria prevalence, prevention and treatment seeking practices among nomadic pastoralists in northern Senegal. *Malar. J.* **16,** 1–11 (2017).
77. Niang, M. *et al.* Substantial asymptomatic submicroscopic Plasmodium carriage during dry season in low transmission areas in Senegal: Implications for malaria control and elimination. *PLoS One* **12,** 1–13 (2017).
78. WHO | Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. *WHO* (2011).
79. Richter, J., Campange, G., Hatz, G., Berquist, N. R. & Jenkins, J. M. ULTRASOUND IN SCHISTOSOMIASIS A Practical Guide to the Standardized Use of Ultrasonography for the Assessment of Schistosomiasis-related Morbidity. *World Heal. Organ.* 55 (1996). doi:10.1111/j.1365-2893.2009.01252.x
80. van Grootveld, R. *et al.* Improved diagnosis of active Schistosoma infection in travellers and migrants using the ultra-sensitive in-house lateral flow test for detection of circulating anodic antigen (CAA) in serum. *Eur. J. Clin. Microbiol. Infect. Dis.* **37,** 1709–1716 (2018).
81. van Dam, G. J. *et al.* A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of Schistosoma circulating anodic antigen. *Exp. Parasitol.* **135,** 274–82 (2013).
82. Corstjens, P. L. A. M. *et al.* Tools for diagnosis, monitoring and screening of Schistosoma infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology* **141,** 1841–55 (2014).
83. Pillay, P. *et al.* Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital Schistosomiasis in primary school girls in KwaZulu Natal, South Africa. *Am. J. Trop. Med. Hyg.* **90,** 546–52 (2014).
84. Obeng, B. B. *et al.* Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. *Ann. Trop. Med. Parasitol.* **102,** 625–633 (2008).
85. Oyebola, K. M. *et al.* A barcode of multilocus nuclear DNA identifies genetic relatedness in pre- and post-Artemether/Lumefantrine treated Plasmodium falciparum in Nigeria. *BMC Infect. Dis.* **18,** 392 (2018).
86. Hofmann, N. *et al.* Ultra-Sensitive Detection of Plasmodium falciparum by Amplification of Multi-Copy Subtelomeric Targets. *PLOS Med.* **12,** e1001788 (2015).
87. Witkowski, B. *et al.* A surrogate marker of piperaquine-resistant Plasmodium falciparum malaria: a phenotype–

genotype association study. *Lancet Infect. Dis.* **17,** 174–183 (2017).
88. Ariey, F. *et al.* A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. *Nature* **505,** 50–55 (2014).
89. Vachot-Ganée, L. *et al.* A novel field-based molecular assay to detect validated artemisinin-resistant k13 mutants. *Malar. J.* **17,** 175 (2018).
90. Somé, A. F. *et al.* Selection of drug resistance-mediating Plasmodium falciparum genetic polymorphisms by seasonal malaria chemoprevention in Burkina Faso. *Antimicrob. Agents Chemother.* **58,** 3660–3665 (2014).
91. Zongo, I. *et al.* Randomized noninferiority trial of dihydroartemisinin-piperaquine compared with sulfadoxine-pyrimethamine plus amodiaquine for seasonal malaria chemoprevention in Burkina Faso. *Antimicrob. Agents Chemother.* **59,** 4387–4396 (2015).
92. Tumwebaze, P. *et al.* Impact of antimalarial treatment and chemoprevention on the drug sensitivity of malaria parasites isolated from Ugandan children. *Antimicrob. Agents Chemother.* **59,** 3018–3030 (2015).