Clinical Trial Protocol

Efficacy and safety of pyronaridine/artesunate and pyronaridine/artesunate/praziquantel for treatment of uncomplicated *Schistosoma haematobium* infection in Gabonese adolescents and children

Protocol version 1.0

Trial Sponsor:	Bernhard Nocht Institute for Tropical Medicine (BNITM)
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Trial site:	Centre de Recherches Médicales de Lambaréné (CERMEL)

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Synopsis

Study title	Efficacy and safety of pyronaridine/artesunate and pyronaridine/artesunate/praziquantel for treatment of uncomplicated *Schistosoma haematobium* infection in Gabonese adolescents and children
Study acronym	**CORMA-BIL**
Protocol version	V.1.0
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Clinical Phase	IIa
Trial Centre(s)	Centre de Recherches Médicales de Lambaréné (CERMEL) B.P. 242, Lambaréné Gabon
Rationale	Urogenital schistosomiasis is caused by the parasite *Schistosoma haematobium* and is defined as an important so-called 'neglected tropical disease'. It accounts for a high morbidity particularly among children and adolescents in endemic areas where malaria is often highly co-endemic. Currently, treatment of urogenital schistosomiasis is based on praziquantel [PZQ], which is the only available drug for this indication. Occasionally, specific antimicrobial agents demonstrate simultaneous activity against multiple microorganisms. In this regard, pyronaridine [PY] and artesunate [ART], two important antimalarial drugs, have been shown to exert clinically important activity against *Schistosoma spp*. It is of mention that a currently ongoing study of our research group (i.e. CORMA-MAL study) recently yielded favourable interim results with some evidence of antimalarial activity of PZQ. This introduces the promising possibility of creating a drug regimen to be potentially used for schistosomiasis and malaria. The value of such a multi-disease drug regimen is even further highlighted, as epidemiologic data indicate that malaria and urogenital schistosomiasis affect largely overlapping target populations. This aspect makes such a potential regimen appealing to be operationally implemented as part of schistosomiasis and malaria control campaigns for which mass drug administration is often applied within intermittent preventive treatment programmes. These potential platform synergisms make such a multi-disease drug regimen interesting not only, but particularly in low resource settings. A treatment study evaluating a combination of PZQ with ART in school children with urogenital schistosomiasis demonstrated a favourable safety profile. This favourable safety profile was confirmed by another treatment study evaluating a combination of praziquantel (PZQ) with ART and mefloquine [MFQ] in school children with urogenital schistosomiasis. Given the established safety profile of PY/ART, its availability as fixed-dose paediatric drug formulation, PY/ART may become an attractive option as partner drug combination for antischistosomal therapy with PZQ.

	In the **CORMA-BIL** study, existing preliminary evidence of PY/ART antischistosomal activity will be further investigated. CORMA-BIL is a randomised controlled trial to evaluate the in vivo efficacy, safety and tolerability of PY/ART and PY/ART/PZQ. Adolescent and children participants with uncomplicated *Schistosoma haematobium* infection will be randomly allocated to one of four study arms: A) Placebo, B) pyronaridine/artesunate, C) praziquantel and D) pyronaridine/artesunate/praziquantel. Objectives will be answered each by two study arm pairs, the first pair being 'PY/ART and PLACEBO' and the second pair being 'PY/ART/PZQ and PZQ'.
Aim	To assess the efficacy of pyronaridine/artesunate (PY/ART) and pyronaridine/artesunate/praziquantel (PY/ART/PZQ) in participants with uncomplicated *Schistosoma haematobium* parasites
Primary Objectives	**STUDY ARM PAIR I [PY/ART and PLACEBO]:** - To assess the percentage of egg reduction rate (ERR) between baseline and D42 in the study arm PY/ART compared with PLACEBO **STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:** - To assess the time to re-infection with *Schistosoma haematobium* after treatment with PYR/ART/PZQ compared with PZQ standard treatment assessed by light microscopy
Secondary Objectives	**STUDY ARM PAIR I [PY/ART and PLACEBO]:** 1) To assess the microscopically-determined cure rate in the study arm PY/ART compared with PLACEBO at D42 after administration of study drugs 2) To assess the qPCR-determined cure rate in the study arm PY/ART compared with PLACEBO at D42 after administration of study drugs 3) To compute and compare the areas under the curve (AUC) of *Schistosome* urinary egg excretion in PY/ART versus PLACEBO 4) To assess the serological cure rate (absence of circulating anodic antigen [CAA] in blood samples) in the study arm PY/ART compared with PLACEBO at D42 after administration of study drugs. 5) To compute and compare the areas under the curve (AUC) of parasite-specific glycans (CAA) in PY/ART versus PLACEBO. **STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:** 1) To assess the time to re-infection with *Schistosoma haematobium* after treatment with PY/ART/PZQ compared with PZQ standard treatment assessed by qPCR

	2) To assess the qPCR-determined cure rate in the study arm PY/ART/PZQ compared with PZQ at D98 after administration of study drugs 3) To assess the time to re-infection with *Schistosoma haematobium* defined as presence of parasite-specific glycans (circulating anodic antigen [CAA]) in samples of blood 4) To assess the serological cure rate (absence of CAA in blood samples) in the study arm PY/ART/PZQ compared with PZQ at D98 after administration of study drugs 5) To assess the presence of *Schistosoma haematobium* eggs during week 4 after administration of study drugs (i.e. D21-D28) assessed by microscopy **<u>All study arms:</u>** - To assess the safety and tolerability of study regimens in each study arm during the observation period - To assess the proportion of participants with haematuria in each study arm at D42 after administration of study drugs - To assess the proportion of participants with an incidental *Plasmodium* parasitaemia during the observation period
Exploratory Objectives	- To determine the antibody response to parasite-specific glycans (CAA) over follow-up - To assess and compare the level of specific IgE antibody to Sh22.6 kDa and ShTAL1 antigen before administration of study drugs compared to day 28 (D28) and day 98 (D98) after treatment - To assess ShTAL4 as a direct and indirect marker for early Sh infection (antigen detection, anti-ShTAL4 antibody detection).
Main inclusion/ exclusion criteria	**Inclusion criteria:** • Participants aged between 5 years and below 18 years • Microscopically-determined *Schistosoma haematobium* infection • Uncomplicated *Schistosoma haematobium* infection defined by: presence of microscopically-determined *Schistosoma haematobium* eggs in urine with absence of Katayama fever and absence of clinically significant urinary tract pathology (see exclusion criteria). • Written informed consent must be obtained before any study assessment is performed.

	• Willingness not to take drugs or substances which could have an impact on study drug blood levels (see inclusion/exclusion criteria in study protocol). • Women only of reproductive age: Must agree to practice continuous contraception for the duration of the study. **Exclusion criteria:** • Presence of Katayama fever • Presence of clinically significant urinary tract pathology. The diagnoses of clinically significant urinary tract pathologies are made by the clinical investigator • Pregnancy or breast-feeding • Use of drugs with known antischistosomal activity within 2 months of enrolment into study (including praziquantel and antimalarial treatment with artemisinin-combination therapies) • Contraindications or known allergy to pyronaridine/artesunate or praziquantel • Any other significant disease, disorder or finding which, in the opinion of the investigator, may significantly increase the risk to the participant because of participation in the study (e.g. renal transplantation etc.), affect the ability of the participant to participate in the study or impair interpretation of the study data • Participants unable to be closely followed for social, geographic or psychological reasons • Haemoglobin level below 8 g/dL • Previous participation in the CORMA-BIL study (multiple participation not possible)
Study design	Single-centre, double-blinded, randomised, controlled trial
Study population	Gabonese adolescents and children (5 years to below 18 years) with uncomplicated microscopically-confirmed *Schistosoma haematobium* infection
Subject numbers/study treatment arms	108 volunteers will be randomized to 4 study arms as follows. There is 1:1 randomisation within each study arm pair. **STUDY ARM PAIR I [PY/ART and PLACEBO]:**

	• **PLACEBO Arm**: 30 volunteers • **PY/ART Arm:** 30 volunteers **STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:** • **PZQ Arm**: 24 volunteers • **PY/ART/PZQ Arm:** 24 volunteers Note on sample size: To correct for multiplicity of endpoints the Hochberg procedure will be applied at the analysis stage of the trial.
Route of Administration	Oral
Dose level	**PZQ:** 40mg/kg single dose independent of food. **PY/ART:** Once daily oral dosing for three days independent of food: Paediatric dosing regimen: 5 <-8 kg: 1 sachet daily 8 -<15 kg: 2 sachets daily 15-20 kg: 3 sachets daily 1 sachet contains 20 mg artesunate and 60 mg pyronaridine Adolescent dosing regimen: 20-<24 kg: 1 tablet daily 24-45 kg: 2 tablets daily 45-<65 kg: 3 tablets daily >65 kg: 4 tablets daily 1 tablet contains 60 mg artesunate and 180 mg pyronaridine
Treatment duration	All study groups will receive study drugs on 3 days. **PLACEBO study arm:** Once daily for 3 days. **PY/ART study arm:** Once daily for 3 days. **PZQ study arm:** Single dose on D1 with consecutive placebo doses on D2 and D3 **PY/ART/PZQ study arm:** PY/ART once daily for 3 days. PZQ single dose on D1 with consecutive placebo doses on D2 and D3

Follow-up duration	**Screening:** D0 **Treatment phase:** D1, D2, D3 **Early follow-up phase:** Following the 'Treatment phase', the 'Early follow-up phase' lasts until week 6 of follow-up and applies to both STUDY ARM PAIR I [PY/ART and PLACEBO] and STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. Weekly visits shall be performed once per calendar week. The time-interval between two visits needs to be at minimum 4 days. **Late follow-up phase:** Following the 'Early follow-up phase', the 'Late follow-up phase' lasts until week 14 of follow-up and applies only to STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. Weekly visits shall be performed twice within the same calendar week. The time-interval between two visits needs to be at minimum 2 days. **STUDY ARM PAIR I [PY/ART and PLACEBO]:** For participants in study arm pair I follow-up is completed during week 6 on D42. **STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:** For participants in study arm pair II follow-up is completed during week 14 on D98.
Planned Trial Period	June 2023 – March 2025
Primary Endpoints	**STUDY ARM PAIR I [PY/ART and PLACEBO]:** Log-transformed *Schistosoma haematobium* egg count reduction as determined by urine microscopy on D0 minus egg count on D42 **STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:** Time to re-infection with *Schistosoma haematobium* defined as presence of microscopically-detectable eggs in urine
Secondary Endpoints	**STUDY ARM PAIR I [PY/ART and PLACEBO]:** 1) Absence of *Schistosoma haematobium* eggs in samples of urine assessed by light microscopy on D42 after administration of study drugs (=microscopically-determined cure rate) 2) Absence of *Schistosoma haematobium* DNA in samples of blood assessed by qPCR on D42 after administration of study drugs (=qPCR-determined cure rate) 3) Area under the curve (AUC) of microscopically-detectable egg load on visits between D7 and D42 after administration of study drugs

	4) Absence of circulating anodic antigen (CAA) in blood samples on D42 after administration of study drugs (=serological cure rate) 5) Area under the curve (AUC) of parasite-specific glycans (CAA) on visits between D7 and D42 after administration of study drugs **STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:** 1) Time to re-infection with *Schistosoma haematobium* defined as presence of schistosome DNA in blood samples assessed by qPCR 2) Absence of *Schistosoma haematobium* DNA in samples of blood assessed by qPCR on D98 after administration of study drugs (=qPCR-determined cure rate) 3) Time to re-infection with *Schistosoma haematobium* defined as presence of parasite-specific glycans (circulating anodic antigen [CAA]) in samples of blood 4) Absence of *Schistosoma haematobium* specific glycans (CAA) in samples of blood assessed on D98 after administration of study drugs (=serological cure rate) 5) Absence of *Schistosoma haematobium* eggs during week 4 after administration of study drugs (i.e. D21-D28) assessed by microscopy **ALL STUDY ARMS:** - Occurrence of adverse events (AEs) after study drug administration - Occurrence of haematuria at D42 after administration of study drugs - Occurrence of an incidental *Plasmodium* parasitaemia during the observation period
Exploratory Endpoints	- Determination of IgM and IgG to parasite-specific CAA - Level of specific IgE antibody to Sh22.6 kDa and ShTAL1 antigen before administration of study drugs compared to day 28 (D28) and day 98 (D98) after treatment - Assessing ShTAL4 as a direct and indirect marker for early Sh infection (antigen detection, anti-ShTAL4 antibody detection)
Data and Safety Monitoring Plan	Volunteers will be treated with a standardised dose of PZQ according to national guidelines if schistosomiasis rescue treatment criteria are reached during follow-up. If schistosomiasis rescue treatment criteria are not reached throughout follow-up, antischistosomal treatment will be administered at the end of the study (at D42 in Study arm pair I and at D98 in Study arm pair II). Depending on the participant's convenience, or the individual's participation in other studies of CERMEL the end-of-study antischistosomal treatment may also take place later than at the end of follow-up.

	Should participants contract an episode of malaria during the follow-up an effective dose of atovaquone/proguanil will be administered. Neither atovaquone nor proguanil are known to possess antischistosomal activity and thereby no interference with schistosomiasis-related endpoints is suspected. Also, quinine may be used as treatment for incidental malaria episodes.

Efficacy and safety of pyronaridine/artesunate and pyronaridine/artesunate/praziquantel for treatment of uncomplicated *Schistosoma haematobium* infection in Gabonese adolescents and children

Study code: **CORMA-BIL**

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Abbreviations

ACT	Artemisinin-based combination therapy
AE	Adverse Event
ALT	Alanine aminotransferase
ART	Artesunate
AST	Aspartate aminotransferase
BNITM	Bernhard Nocht Institute for Tropical Medicine
CERMEL	Centre de Recherches Médicales de Lambaréné
CI	Confidence Interval (often 95% CI)
CRF	Case Report Form
EC	Ethics Committee
eCRF	Electronic Case Report Form
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transferase
HCG	Human Chorionic Gonadotropin
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IQR	Interquartile range
IV	Intravenous
LDH	Lactate dehydrogenase
MDA	Mass drug administration
MFQ	Mefloquine
P.	Plasmodium
PCR	Polymerase Chain Reaction (used synonymously in this study protocol with quantitative PCR [qPCR])

PI	Principal Investigator
PY	Pyronaridine
PZQ	Praziquantel
SAE	Serious Adverse Event
S.	Schistosoma
SD	Standard deviation
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper limit of the normal range
WHO	World Health Organization

Background and Rationale

Urogenital schistosomiasis and malaria

Epidemiological overlap of affected target populations

Both malaria and schistosomiasis are parasitic diseases. Malaria is a vector-borne disease caused by parasites of the genus *Plasmodium* (1–3). Urogenital schistosomiasis is caused by the parasite *Schistosoma (S.) haematobium* and is defined as an important so-called 'neglected tropical disease' (4). Malaria is a major cause of childhood morbidity and mortality in highly endemic regions of sub-Saharan Africa (1–3). On the other hand, urogenital schistosomiasis accounts for a high morbidity particularly among children and adolescents in endemic areas where malaria is often highly co-endemic (4). This indicates that malaria and urogenital schistosomiasis are not only often co-endemic but mainly affect children and adolescents i.e. a similar target population (2,4).

Mass treatment approaches in public health disease management and infection control

Treatment of urogenital schistosomiasis is based on praziquantel (PZQ), which to date is the only available drug for this indication. Artemisinin-based combination therapy (ACT) is the combination of an artemisinin derivative with a partner drug and constitutes the to date most successful treatment of malaria (2,5). Besides disease-management approaches that focus on delivering care to individuals there are management approaches aimed at delivering care to whole groups of the population (5–9). Generally, the value of such so-called mass-drug-administration (MDA) campaigns was described as to favourably impact on disease prevalence, as well as, on population morbidity. Its importance was particularly highlighted for settings with generally lower quality of the local health system which are often prevalent in malaria-endemic and schistosomiasis-endemic countries. In such MDA campaigns everybody in a defined target group receives a given treatment for the disease of interest without any prior application of diagnostics.

Anti-malarial mass drug administration programmes

For malaria, MDA campaigns have been known and been occasionally applied for approximately a century (6). However, due to concern of potential development of drug resistance MDAs have until recently been limited to benefit mainly vulnerable groups in endemic populations, namely pregnant women and children (10–12). Thus, it is currently recommended that pregnant women in regions of high malaria transmission receive an effective antimalarial medication monthly (IPTp) (10). Depending on the malaria transmission of specific settings, different recommendations are given by the WHO to benefit the vulnerable population of children (13). The following programmatic approaches are to date known to particularly reduce paediatric morbidity and mortality (11–13):

- Perennial malaria chemoprevention (PMC)
- Seasonal malaria chemoprevention (SMC)
- Intermittent preventive treatment of malaria in school-aged children (IPTsc)

In addition to that, population-wide anti-malarial MDAs have recently received increasing attention and current recommendations of WHO define as many as six MDA strategies (6,13):

- MDA for burden reduction
- MDA for burden reduction in emergency settings
- MDA to reduce transmission of *Plasmodium (P.) falciparum* in very low to low transmission settings
- MDA to reduce transmission of *P. falciparum* in moderate to high transmission settings
- MDA to reduce transmission of *P. vivax*
- Mass relapse prevention (MRP) to reduce transmission of *P. vivax*

Anti-schistosomiasis mass drug administration programmes

On the other hand, for schistosomiasis it is common that MDA campaigns are conducted in regular intervals in regions of high schistosomiasis-endemicity (8). While different medicines may be used within anti-malarial MDAs, solely praziquantel is administered as part of schistosomiasis-related MDA disease control programmes. Traditionally communities have been classified as 'high risk', 'moderate risk' and 'low risk' for schistosomiasis, whereby each of these categories are equivalent to the prevalence of schistosomiasis among school-aged children. Furthermore, most anti-schistosomiasis MDA campaigns target particularly children and adolescents as they carry the brunt of disease burden (4). Drug delivery algorithms are specific to the three afore-mentioned risk categories and determine not only the frequency of the MDA campaigns, but also whether other groups in a population should participate in mass treatment. Table 1 indicates a scheme of MDA based on schistosomiasis prevalence:

Table 1: Recommended treatment strategy for schistosomiasis mass chemotherapy (created after (8,9)*)*

Category	Prevalence among school-aged children	Action to be taken	
High risk community	50% by parasitological methods **OR** 30% by questionnaire for visible haematuria	Treat all school-age children once a year	Also treat adults considered to be at risk (from special groups to entire communities living in endemic areas – e.g. fishermen and irrigation workers)
Moderate risk community	10% to below 50% by parasitological methods **OR** <30% by questionnaire for visible haematuria	Treat all school-age children once every 2 years	Also treat adults considered to be at risk (special risk groups only – e.g. fishermen and irrigation workers)

Low risk community	<10% by parasitological methods	Treat all school-age children twice during their primary schooling	Praziquantel should be available in dispensaries and clinics for treatment of suspected cases

Urogenital schistosomiasis and malaria; two potentially well-suited tropical target diseases for anti-parasite broad-spectrum therapies

Occasionally, specific antimicrobial agents demonstrate simultaneous activity against multiple microorganisms. This introduces the promising possibility of creating drug regimens to be potentially used for more than only one treatment indication. Furthermore, the value of such multi-disease drug regimens rises when designed for important infectious diseases that affect a similar target population; an aspect making such regimens appealing to be operationally implemented not only, but particularly in low resource settings. Epidemiologic data indicate that malaria and schistosomiasis are two infectious diseases affecting largely overlapping target populations (2,4). Furthermore, currently recommended schistosomiasis and malaria control programmes would potentially pose a synergistic programmatic framework whose combined leverage could contribute towards improved control and ultimately elimination of both schistosomiasis and malaria.

CORMA project

This currently unused synergistic potential of malaria and schistosomiasis control programmes is central in the CORMA (Combination Regimen for Mass Drug Administration) project. Currently, it is not recommended to use antimalarial drugs in the treatment schistosomiasis, nor to use PZQ for the treatment of malaria; this is true for administration of single pharmacological agents and of combination regimens. However, over the last decades preliminary evidence has accumulated suggesting that the anti-schistosomal agent PZQ may have anti-plasmodial activity and that anti-plasmodial agents may have anti-schistosomal activity. Therefore, in order to exploit this potential operational disease management synergism of malaria and schistosomiasis control programmes in the future, as well as, finding new anti-malarial and anti-schistosomal drugs the CORMA project was created. CORMA is a project funded by the German Centre for Infection Research (DZIF) that assesses the efficacy of anti-malarial drugs against urogenital schistosomiasis, as well as, the efficacy of the only anti-schistosomal drug PZQ against malaria. The project is conducted sequentially, starting with the efficacy assessment of PZQ against malaria (i.e. CORMA-MAL study) followed by the efficacy assessment of antimalarials against urogenital schistosomiasis (i.e. CORMA-BIL study). The CORMA-MAL study is currently ongoing, while this current document constitutes the study protocol for the CORMA-BIL study.

The following sections will illuminate the evidence on anti-malarial activity of PZQ (i.e. the CORMA-MAL part of the CORMA project) followed by going to the specific parts to be studied by CORMA-BIL.

CORMA-MAL

Praziquantel and current evidence on potential anti-malarial activity

In 1998, a Russian research group assessed the role of PZQ during *Plasmodium* infection in a *P. berghei* mouse model (14). They used a chloroquine-resistant strain (LNK65CHLFR) and a strain with naturally reduced sensitivity to chloroquine (LNK65) and tested the antimalarial efficacy of PZQ, chloroquine, styrylquinazoline and combinations of PZQ/chloroquine and PZQ/styrylquinazoline. PZQ was dosed as 125mg/kg body weight. Study drugs were administered to mice on days 2, 3, 4 after infection; due to no apparent reason an additional dose of study drugs was administered on day 5 after infection only in the LNK65 model. Interestingly, a strong antimalarial potency of PZQ as combination to chloroquine and styrylquinazoline was observed both in the LNK65 and LNK65CHLFR models, while antimalarial activity of single medical agents was comparatively smaller. It is of mention that in the LNK65 mouse model, PZQ reduced parasitaemia as mono-therapy for as long as treatment was administered, however, rising again when PZQ administration was stopped.

Similar evidence comes from a non-randomised clinical study from Pakistan which assessed nine (n=9) and one (n=1) patients (age range: 12-52 years) infected with *P. falciparum* and *P. vivax*, respectively. Patients were administered PZQ 30 mg/kg/day in three divided doses for a maximum of 8 days (15). Although the study is of questionable methodological quality, the Pakistani authors corroborate the Russian preliminary evidence in favour of anti-malarial activity of PZQ by indicating that 8/10 (80%) of patients were blood-smear-negative by day 6 after PZQ treatment initiation. Reportedly, two patients showed response to PZQ treatment, but were given anti-malarial drugs due to development of jaundice. The authors mention that over a three-month follow-up period none of the patients had a re-emergence of parasitaemia, concluding that PZQ might represent an additional drug in the treatment of malaria.

CORMA-MAL preliminary results

The currently ongoing placebo-controlled randomised clinical trial CORMA-MAL (Trial Identifier: PACTR202206584817951) investigates whether PZQ given once daily (40mg/kg/d) for three days as single agent therapy possesses activity against *P. falciparum* in asymptomatic semi-immune Gabonese adults. Recently, an interim analysis (unpublished results) revealed good evidence in favour of antimalarial activity of PZQ. Out of the 15 participants included in the interim analysis 75% (6/8) of participants in the PZQ arm had Plasmodium parasite clearance by D7 after treatment versus 29% (2/7) of participants in the placebo arm (p=0.07). Concordantly, median parasite reduction was 99% by D3 in the PZQ arm versus 19% in the Placebo arm (p=0.039). Similarly, median parasite reduction was 99% by D7 in the PZQ arm versus 71% in the Placebo arm (p=0.13). Linear regression modelling suggested that parasite clearance was twice quicker in the PZQ arm than in the Placebo arm ($Regression\_slope_{PZQ}$: -0.1 $Regression\_slope_{Placebo}$: -0.05; Statistical slope comparison: p=0.02). The trial has now recruited more than half of the target sample size (n=27 out of N=44) and it is highly likely that current preliminary evidence will further corroborate.

In summary, preliminary evidence indicates that PZQ might not only exhibit intrinsic antimalarial properties, but might also be a beneficial partner drug in existing or novel antimalarial drug combination regimens.

Different life-cycle stages of *Schistosoma spp*. and implications for pharmacotherapy

In anti-schistosomal pharmacotherapy drugs can exert activity on various life-cycle stages of *Schistosoma spp*. Therefore, the following sections illuminate different life-cycle stages of *S. haematobium* in detail. Subsequently, anti-schistosomal activity of certain drugs will be presented.

Llife-cycle of Schistosoma spp.

During infection with *Schistosome spp.* various developmental stages occur in the human body (Figure 1).

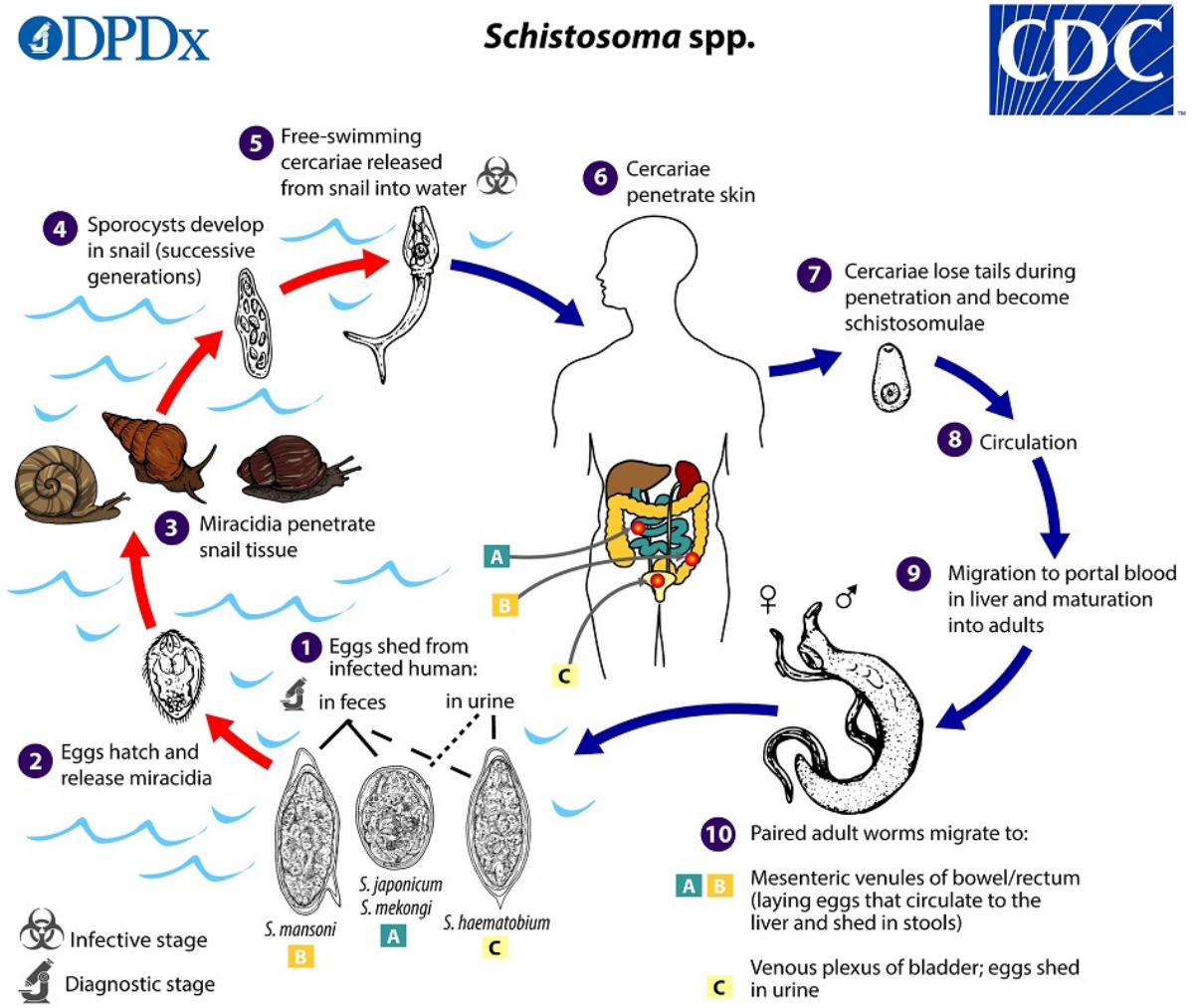


Figure 1: Schistosoma haematobium life-cycle (16)

The following passages stem from a detailed review article by Nation S. et al. on schistosome life cycle stages within the human host (17).

Penetration of skin to passive migration to lungs

Upon exposure with infested fresh-water schistosome cercariae penetrate the epidermis and cause infection. In the skin, the infective schistosome stages are called schistosomula (17). These are often also referred to as juvenile stages. They migrate into and through the dermis, eventually reaching dermal blood vessels. *S. haematobium* schistosomula exit the skin and reach blood vessels by approximately 3 days after infection. Schistosomula in the circulation reach the lungs via the right side of the heart and pulmonary artery. Within the capillary beds of the lungs, the young parasites elongate to facilitate passage through these smaller blood vessels. Radiotracking studies indicate that *S. haematobium* juvenile stages reach the lungs at around day 7 and can be present there up to 25 days post-infection (17).

Lungs to liver

From the lungs, schistosomula can flow with the blood via the pulmonary veins through the left side of the heart and then they can become distributed to the systemic organs (Figure 2) (17). Parasites that pass into the splanchnic arteries (i.e. the celiac or superior/inferior mesenteric arteries) and through these, to the hepatic portal vein. Parasites that do not flow into these arteries get carried in the blood stream elsewhere; they circulate throughout the systemic organs before eventually returning to the lungs to repeat the circuit. This may occur multiple times until schistosomula are lucky enough to be washed into the splanchnic arteries eventually flowing to the liver.

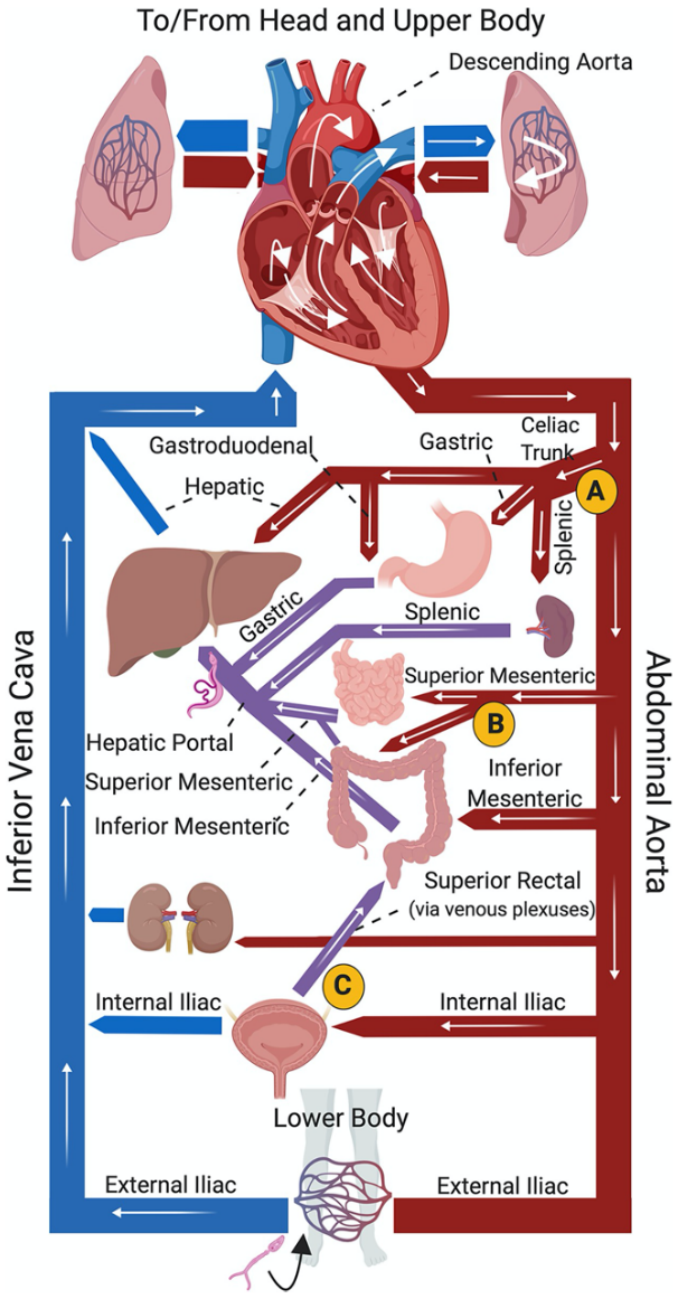


Figure 2. Pathway of schistosomula migration to the liver. Figure taken from (17)

Maturation of schistosomula in liver

Once in the liver, schistosomula are primarily observed in the branches of the hepatic portal vein and are seemingly unable to pass through the sinusoids and back into the systemic blood flow (17). As observed by direct microscopic observation of parasites in situ in the vasculature of anesthetised mice whose abdominal blood vessels have been exposed by surgery, in the portal vein the young worms begin feeding on blood and are noted to be either attached to vessels via the ventral sucker or wedged into a vessel, facing the direction of blood flow. Here, the parasites mature—doubling in weight approximately every 2.3 days in the first 2 weeks. Next, the worms find a mate and the pairs migrate from the liver against the flow of blood to reach their preferred egg laying sites (17).

Migration of adults to venous plexus of bladder and deposition of eggs in target organ

S. haematobium migrates primarily to the veins surrounding the bladder (the vesicle venous plexus) and causes urinary schistosomiasis (Figure 3) (17). The route taken is as follows: first into the splenic vein, then into the inferior mesenteric vein. This vein runs directly into the superior rectal (hemorrhoidal) vein, which leads to a collection of interconnected vessels known as the rectal venous plexus. This plexus connects with another series of interconnected vessels draining the reproductive organs (the uterine and vaginal venous plexuses in females and the prostatic venous plexus in males) which, in turn, connect with the network of vessels draining the bladder (the vesicle venous plexus). The abundant interconnections (anastomoses) between these different venous plexuses in the pelvis facilitate worm movement. These vessels largely lack valves and so offer a network of unrestricted routes for the worms to, and through, the urogenital organ systems (17). Schistosome eggs laid in the vesicle venous plexus can traverse the blood vessel wall, as well as the wall of the bladder, to enter the lumen of the bladder and can then exit the body with urine. In keeping with this, most of the *S. haematobium* adult worms seen at autopsy are found in the blood vessels of the bladder, and the highest percentage of eggs in the organs are likewise found in the bladder (followed by the ureter) (17).

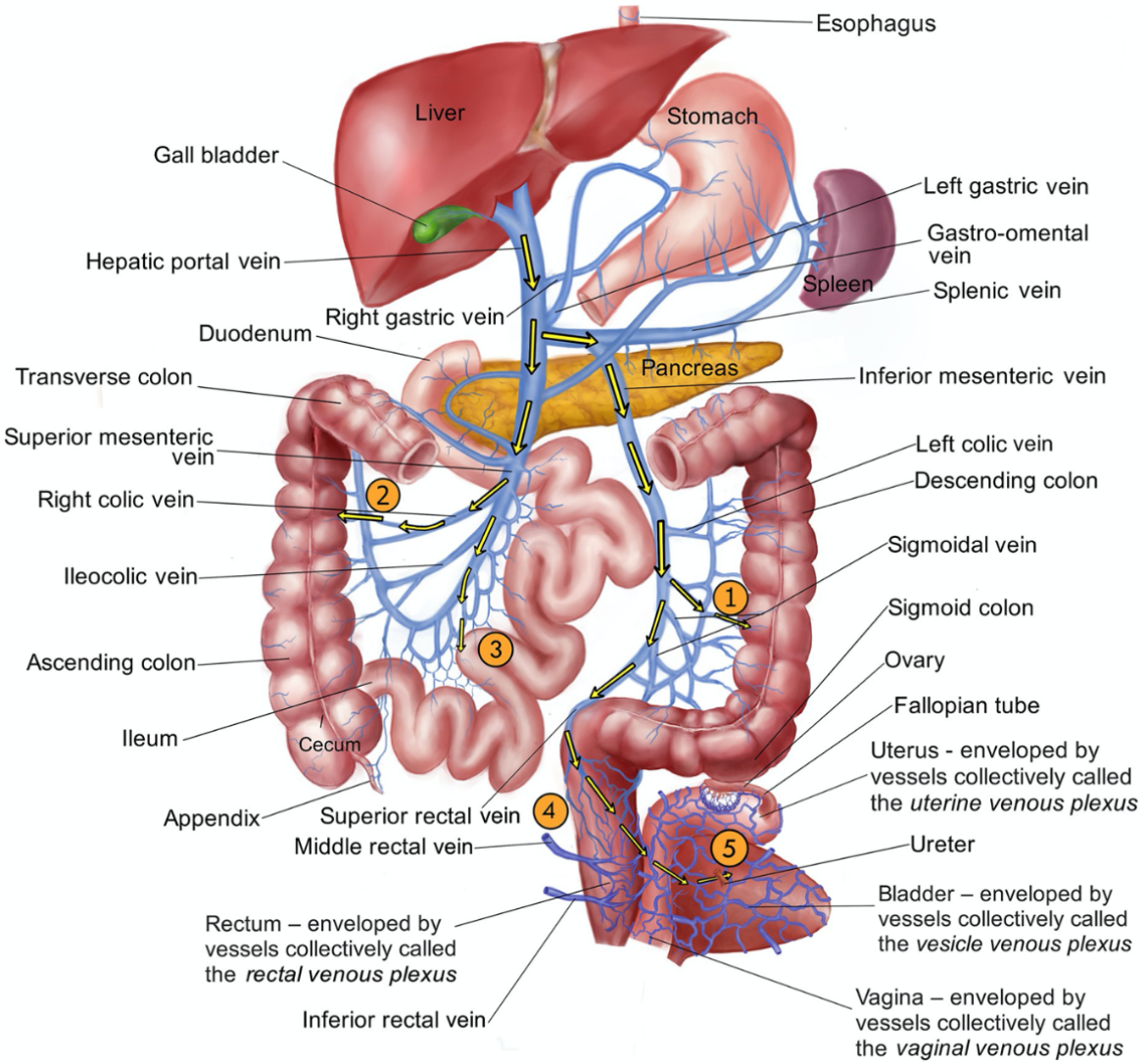


Figure 3: Pathway of adult Schistosome migration to the target organ. Consider numbers 1, 4 and 5 for S. haematobium. Figure taken from (17)

Treatment with praziquantel

PZQ has been used for schistosomiasis control for over 40 years and its efficacy, safety and tolerability has been assessed in a myriad of trials (4,8,18–25). To date, it is the only licensed and officially recommended drug in the treatment of human schistosomiasis.

PZQ is known to exert anti-schistosomal activity exclusively against adult stages of *Schistosoma spp*. Juvenile stages (i.e. schistosomula) are not affected by PZQ therapy. Therefore, it is often necessary to give multiple treatment courses of PZQ which are scheduled several weeks apart from each other. Once that adult worms are killed by the first effective PZQ treatment course, weeks or months later, juvenile stages will have developed into adult stages being then susceptible to the second effective PZQ treatment course (24,26).

Anti-malarial drugs and current evidence on potential anti-schistosomal activity

Some studies conducted within experimental laboratory and clinical research settings have yielded preliminary evidence that pyronaridine (PY), mefloquine (MFQ) and artesunate (ART), three important antimalarial drugs, exert clinically important activity against various life-cycle stages of *Schistosoma spp* (26–29).

Artesunate

According to a meta-analysis published in the Cochrane library, ART given alone or in combination with PZQ are another potential future treatment option against schistosomiasis, but the current evidence base is limited to a few trials with inconsistent results. Artemisinin has the highest activity against immature schistosomes (i.e. schistosomula) (26).

Mefloquine

The following sections are dedicated to summarise the currently available evidence on anti-malarial activity of MFQ.

Experimental, in vitro and mouse model studies

According to a detailed review article by Xiao S. et al. on the in vitro activity of MFQ on *Schistosoma spp*. the evidence can be summarised as follows (27):

> "According to the experimental studies, the deepest impression on the anti-schistosomal properties of mefloquine can be summarized as the following points: (1) single dose of mefloquine possesses a potential effect against three major species of schistosomes (*S. mansoni*, *S. haematobium*, and *S. japonicum*) infecting humans; (2) the drug displays similar effects against developing stages of juvenile and adult schistosomes, which are superior to that of artemisinins and praziquantel; (3) in vitro mefloquine exerts direct killing effect on juvenile and adult schistosomes, while in vivo, the efficacy of the drug is independent to host immune response, (4) mefloquine causes extensive and severe morphological, histopathological, and ultrastructural damage to adult and juvenile schistosomes, particularly, the worm tegument, musculature, gut, and vitelline glands of female worms are the key sites attacked by the drug; (5) combined treatment with mefloquine and praziquantel, or artemisinins shows synergistic effect against schistosome in experimental therapy."

Clinical trials

A randomised, exploratory open-label trial was performed to assess the efficacy and safety of MFQ (25 mg/kg), ART (3 doses of 4 mg/kg), MFQ-ART (3 doses of 100 mg artesunate plus 250 mg mefloquine), and PZQ (40 mg/kg) against *S. haematobium* (30). A total of 83 *S. haematobium*–infected schoolchildren were included in the study. Cure rates of MFQ, ART, MFQ-ART, and PZQ against *S. haematobium* at day 26 after treatment were 21%, 25%, 61%, and 88%, respectively. Both MFQ-ART and PZQ resulted in egg reduction rates of >95%. Significantly lower egg reduction rates were seen in the ART (85%) and MFQ groups (74%). In children coinfected with *S. mansoni*, PZQ and MFQ-ART, but not MFQ and ART alone, resulted in high cure rates and egg reduction rates. Abdominal pain was the most frequent adverse event, with a higher incidence among children treated with MFQ (89%), MFQ-ART (83%), and ART (60%) than among children treated with PZQ (46%).

A nested randomised controlled, assessor-blinded clinical trial recruited pregnant women with *S. haematobium* infection presenting at two antenatal health care centres in rural Gabon (28). The study in which it was nested compared sulfadoxine-pyrimethamine with MFQ as intermittent preventive treatment for malaria in pregnancy (IPTp). Study drugs were administered twice during pregnancy with a 1-month interval after completion of the first trimester. Sixty-five pregnant women were included in this study. *S. haematobium* egg excretion rates showed a median reduction of 98% (IQR: 70%–100%) in the MFQ group compared to an increase of 20% (IQR: −186% to 75%) in the comparator group. More than 80% of patients showed at least 50% reduction of egg excretion and overall cure rate was 47% (IQR: 36%–70%) 6 weeks after the second administration of MFQ IPTp. Even though the study was not powered to detect such an effect, an important reduction of egg excretion in pregnant women was revealed.

In another trial (31) conducted in school-aged children in Côte d'Ivoire the efficacy and tolerability of the following treatments against *S. haematobium* was assessed: (i) PZQ (40 mg/kg; single dose standard treatment); (ii) single dose of MFQ (25 mg/kg) combined with a single dose of PZQ (40 mg/kg); and (iii) MFQ-ART (100 mg artesunate + 250 mg mefloquine) once daily for 3 days combined with single dose of PZQ (40 mg/kg) administered on day 4. Sixty-one children were present on all examination time points and had complete datasets. No difference in efficacy was observed between the three treatment groups on either follow-up. On the 21–22 day posttreatment follow-up, based on available case analysis, cure rates of 33% (95% CI: 11–55%), 29% (95% CI: 8–50%), and 26% (95% CI: 5–48%) were observed for PZQ, MFQ-ART- PZQ, and MFQ- PZQ, respectively. The corresponding egg reduction rates were 94% and above. On the second follow-up, observed cure rates ranged from 19% (PZQ) to 33% (MFQ-ART-PZQ), and egg reduction rates were above 90%. PZQ monotherapy was the best tolerated treatment. In the MFQ-ART-PZQ group, adverse events were reported by 91% of the participants, and in the MFQ- PZQ group, 95% experienced adverse events. With the exception of abdominal pain at moderate severity, adverse events were mild. The authors concluded that the addition of MFQ or MFQ-ART did not increase the efficacy of PZQ against chronic *S. haematobium* infection.

Potentially irreversible side effects of mefloquine

It is the conventional belief that neurological effects of mefloquine are merely due to the long half-life of MFQ and its continued presence in the body. Author R. Nevin proposes that many of the reported lasting adverse neurological effects of mefloquine are consistent with the chronic sequelae of a well characterised but idiosyncratic central nervous system (CNS) toxicity syndrome (or toxidrome) common to certain historical antimalarial and antiparasitic quinolines and associated with a risk of permanent neuronal degeneration within specific CNS regions including the brainstem (32). Therefore, MFQ is currently not regarded top tier in malaria pharmacotherapy.

Pyronaridine

In vitro and in vivo studies

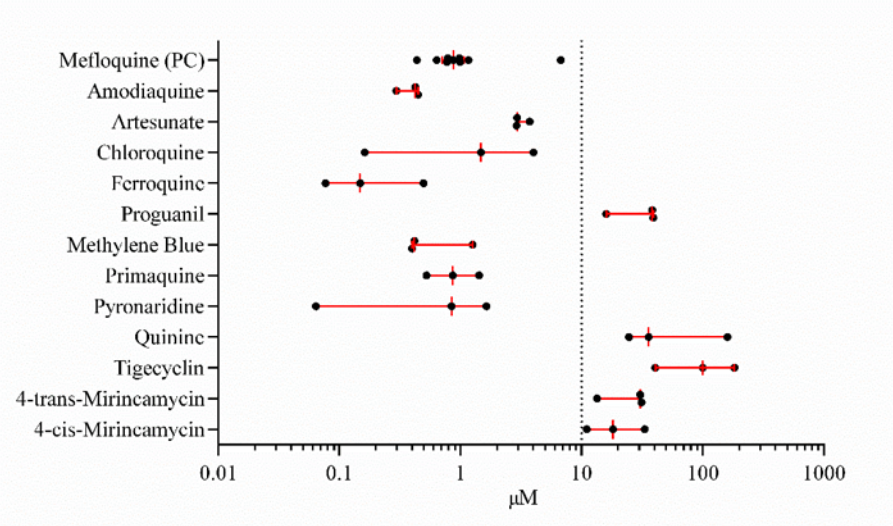
Activity against only adult life cycle stages

Researchers in China assessed the anti-schistosomal activity induced by MFQ and PY via measuring the inhibition of hemozoin formation (33). Using MFQ hydrochloride and PY phosphate at 25 micromol/L concentration, an assay of inhibition of beta-hematin formation was established in 1 mol/L sodium acetate buffers containing hematin with various pH of 4.0, 4.2, 4.4, 4.6, 4.8, and 5.0. The medium of RPMI 1640 supplemented by 10% calf serum was used to maintain the adult *S. japonicum*, and the 50% and 95% lethal concentrations (LC50 and LC95) to kill the adult worms of each drug were then determined. Meanwhile, the interaction of quinine, PY and chloroquine combined with hemin against adult schistosomes was also assessed.

In the acidic acetate-hematin solution, 25 micromol/L PY showed significant inhibition of beta-hematin formation at pH 4.4-5.0 with inhibition rates of 81.3%-97.0%. At pH 4.6, the inhibition rates of beta-hematin formation in acetate-hematin solution induced by MFQ at concentration of 25 beta mol/L were 79.7% and the beta-hematin formation was continually inhibited by MFQ at pH 4.8 and 5.0 with inhibition rates of 83.1%-90.6%. The LC50 and LC95 of MFQ and PY were 4.93 and 6.123 microg/ml and 37.278 and 75.703 microg/ml, respectively. Unexpectedly, in schistosomes exposed to PY at a toxic concentration of 50 micromol/L (46 microg/ml) in combination with 153.4 mol/L (100 microg/ml) hemin for 72 h, all of the worms were protected from the toxic action induced by PY, which was revealed in normal motor activity and normal appearance of morphology in the majority of the worms. Furthermore, in an in vivo sub-study, mice infected with adult schistosomes were treated orally with PY at a daily dose of 400 mg/kg for 3 days, or intraperitoneally with PY at a daily dose of 100 mg/kg for 2 or 3 days. No apparent efficacy was seen. When MFQ, was administered orally to infected mice at a single dose 200 mg/kg all groups of mice treated showed moderate or higher efficacy with worm burden reductions of 61.1%-98.1%.

Activity against juvenile and adult life cycle stages

A research group from Tübingen, Germany assessed the activity of antiplasmodial compounds against juvenile and adult life cycle stages of *S. mansoni* (29). Compounds were progressively tested in vitro against schistosomula (Supplementary Figure 1 and Supplementary Table 1) and against ex vivo adult *S. mansoni* (Supplementary Figure 2 and Supplementary Tables 2-4). Active compounds were further tested in mice infected with *S. mansoni* in the prepatent phase and in the adult stage. MFQ and PY had an IC50 against schistosomula below 10 µM , while MFQ is more rapidly acting as PY(Supplementary Figure 1 and Supplementary Table 1). In in vitro schistosomula assays, the lactate assay identified compounds with rapid onset of anti-schistosomal activity.

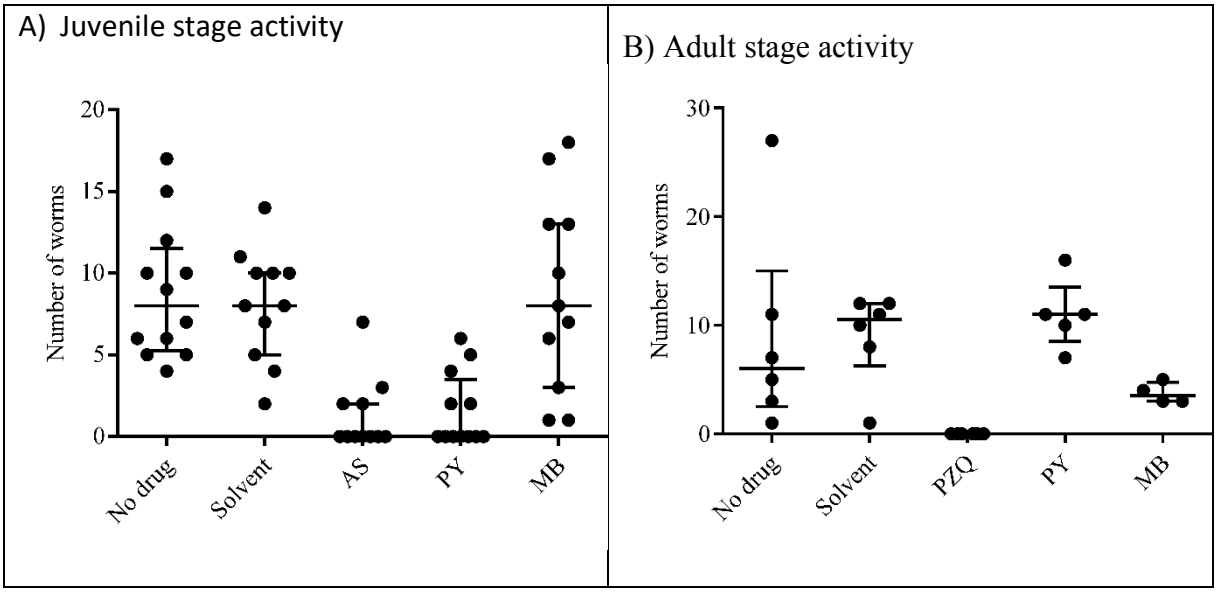


Supplementary Figure 1: Dot plot of individual IC50 values (step 1). Each compound was tested in 3 independent in vitro schistosomula experiments (except for MFQ, N = 9) and individual IC50s (filled circles) are shown. Viability of schistosomula after 7 days drug exposure was assessed by microscopy. The IC50 is given in µM. Red bar and lines indicate median IC50 and IQR. Dotted line: Threshold for drug activity is an IC50 < 10 µM. IQR: Interquartile range.

Supplementary Table 1: Assay-dependent IC50s of compounds against schistosomula (step 1). In vitro viability of *S. mansoni* schistosomula was assessed by microscopy after 72 h and at day 7 after drug exposure, respectively, and by resazurin assay and lactate assay after 7 days only. Median (IQR) in µM is reported. Each drug was independently tested at minimum 3 times. # could not be measured due to drug colour interference with reader.

Drug	**Microscopy at 72 h**	**Microscopy day 7**	**Resazurin day 7**	**Lactate day 7**
Mefloquine	4.9 (2.7-7.8)	0.8 (0.7-1.1)	0.7 (0.6-1.9)	1.7 (0.7-4.1)
Pyronaridine	71.6 (41.3-99.6)	0.8 (0.1-1.6)	no inhibition	no inhibition

In subsequent in vivo testing, 58% of mice infected with *S. mansoni* at the juvenile stage were cured by PY; MFQ was not considered in this type of experiment.



Supplementary Figure 2: Worm number reduction in vivo. Mice were treated with respective compounds after A) 14 days (juvenile worms) and B) 9 weeks (adult worms) of *S. mansoni* infection, respectively. Mice were killed and adult worms were recovered and counted. MB: methylene blue, PY: pyronaridine, AS: artesunate, PZQ: praziquantel. Each data point

represents one mouse. Thick bar: median, whiskers: IQR (interquartile range). A statistically significant difference ($P < 0.05$) to no drug control was detected for A) AS, PY and MB, and for B) PZQ, respectively.

Supplementary Table 2: Drug activity against ex vivo adult worms (step 2). Worms were exposed in vitro for 7 days to methylene blue, pyronaridine, and praziquantel (positive control) at respective concentrations. 'No drug' and DMSO were the negative controls. Activity of methylene blue and pyronaridine at 30 µM were also evaluated after 24 hours (*). Pooled data obtained from 3 experiments per drug are displayed.

Drug	Concentration in µM	No. of worms tested	% worms affected	% worms dead
No drug	NA	12	0	0
DMSO	0.1%	10	0	0
Praziquantel	1	12	0	100
Methylene blue	5	17	35	59
	10	12	0	100
	30	12	0	100
	30*	6	0	100
Pyronaridine	5	15	0	60
	10	11	45	55
	30	12	0	100
	30*	6	0	100

Supplementary Table 3: In vivo activity against juvenile *S. mansoni* in mice (step 3). Mice infected with *S. mansoni* for 2 weeks (worms are in the juvenile stage) were treated with methylene blue or pyronaridine. Negative control treatment: No drug and solvent, positive control treatment: artesunate. WBR: Worm burden reduction, LGS: Liver granulation score. * 1 mouse died during the treatment week. IQR: Interquartile range. NA: Not applicable.

***In vivo* juvenile worms (2 weeks old)**								
Drug	**Dose in mg/kg**	**No. of mice**	**No. of worms/mouse recovered Median (IQR)**			**Cure in %**	**WBR in %**	**LGS Median (IQR[a])**
			Total	Male	Female			
No drug	NA	12	8.0 (5.3-11.5)	5.5 (4.0-9.0)	2.0 (1.0-4.5)	NA	0	2.5 (2.0-3.0)
Solvent	NA	12	9.0 (5.5-10.8)	8.0 (4.0-9.8)	1.5 (0-2.8)	NA	0	3.0 (2.3-3.0)
Artesunate	300	12	0.0 (0.0-2.8)	0.0 (0.0-2.8)	0.0 (0-0.8)	58	79	0.0 (0.0-0.8)
Pyronaridine	500	12	0.0 (0.0-3.5)	0.0 (0-1.8)	0.0 (0-1.0)	58	82	0.0 (0.0-0.8)

Supplementary Table 4: In vivo activity against adult *S. mansoni* in mice. Mice infected with *S. mansoni* for 9 weeks (worms are in the adult stage) were treated with methylene blue or pyronaridine. Negative control treatment: No drug and solvent, positive control treatment: Praziquantel. WBR: Worm burden reduction, LGS: Liver granulation score. * 1 mouse died during the treatment week. IQR: Interquartile range. NA: Not applicable.

***In vivo* adult worms (9 weeks old)**

Drug	Dose in mg/kg	No. of mice	No. of worms/mouse recovered Median (IQR)			Cure in (%)	WBR in %	LGS Median (IQR[a])
			Total	Males	Females			
No drug	NA	6	6.0 (2.5-15.0)	4.0 (1.8-8.8)	2.0 (0.8-6.3)	NA	0	3.0 (3.0-3.0)
Solvent	NA	6	10.5 (6.3-12.0)	5.5 (4.0-6.3)	5.0 (2.3-5.3)	NA	0	3.0 (2.8-3.0)
Praziquantel	500	6	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	100	100	3.0 (2.8-3.0)
Pyronaridine	500	6*	11.0 (8.5-13.5)	6.0 (5.5-7.0)	4.0 (3.0-7.0)	0	0	3.0 (3.0-3.0)

The authors concluded that PY warrants further investigations as candidate for schistosomiasis treatment. The compound is approved for human use and evidence for its potential as anti-schistosomal compounds can be obtained from clinical testing. Particularly, the anti-juvenile stage activity of PY is a promising finding that warrants further studies.

Clinical trials

A nested exploratory study from Gabon assessed the efficacy of conventional artemisinin-based combination therapy and investigational second-generation ACTs when given for treatment of uncomplicated malaria on concomitant urogenital schistosomiasis (34). Urine samples were collected from patients confirmed with uncomplicated malaria for diagnosis of urogenital schistosomiasis by microscopy before malaria treatment. Egg excretion rate and cure rate were determined 4- and 6-weeks after treatment with either ART-PY. Urinalysis by test strip was performed on each urine sample for detection of microhaematuria before and after treatment. 21 malaria patients with *S. haematobium* co-infection were analysed. *S. haematobium* egg excretion rates showed a median reduction of 97% (interquartile range (IQR), 65% to 100%) 4 weeks post treatment and 83% (IQR, -38% to 98%) 6 weeks post treatment in the ART-PY group. Cure rate was 47% (IQR, 36%–70%) 6 weeks post treatment with ART-PY. 6 weeks posttreatment, microhaematuria decreased from 4+ (1+ to 4+) to 3+ (0 to 3+) after treatment with ART-PY.

In this trial antimalarial treatment with ART-PY was efficacious in reducing egg excretion of *S. hematobium* adding a benefit for urogenital schistosomiasis control.

Using pyronaridine, artesunate, and praziquantel in a multi-drug, anti-schistosomal regimen

As illuminated above, several studies conducted within experimental laboratory and clinical research settings have yielded favourable preliminary evidence that PY, MFQ and ART, exert a clinically important activity against *Schistosoma spp*. However, currently existing preliminary evidence requires further corroboration, particularly by adequately powered randomised (potentially placebo-) controlled trials. Also, consideration is warranted on what single drugs to further select into a pharmacological combination regimen.

The antimalarial combination regimen of PY/ART (Pyramax) is commercially available as fixed-dose combination and paediatric drug formulation. On the other hand, ART and MFQ are not available as fixed-dose combination or paediatric drug formulation; moreover, oral ART tablets are at present not even commercially available as single formulation. Furthermore, there is concern on the potentially irreversible neurological side effects of MFQ, while a large study has yielded robust evidence in favour of good safety and tolerability of PY/ART (35).

Therefore, the differential safety profile between PY/ART and ART/MFQ paired with the procurement difficulties of both MFQ and ART (as single agent formulation) predisposes PY/ART to be studied in a first step on its efficacy against *Schistosoma spp*.

Tolerability and safety profile of Pyramax (pyronaridine/artesunate)

Pyramax is a fixed-dose combination treatment of PY/ART. A single-arm, open-label, cohort event monitoring study was conducted at 6 health centres in Cameroon, Democratic Republic of Congo, Gabon, Ivory Coast, and Republic of Congo between June 2017 and April 2019 to assess the safety and tolerability of Pyramax (35). The trial protocol as closely as possible resembled real-world clinical practice for the treatment of malaria at the centres. Eligible patients were adults or children of either sex, weighing at least 5 kg, with acute uncomplicated malaria who did not have contraindications for PY-ART treatment as per the summary of product characteristics. Patients received fixed-dose PY-ART once daily for 3 days, dosed by body weight, without regard to food intake. A tablet formulation was used in adults and adolescents and a paediatric granule formulation in children and infants under 20 kg body weight. The primary outcome was the hepatic event incidence, defined as the appearance of the clinical signs and symptoms of hepatotoxicity confirmed by a >2× rise in alanine aminotransferase/aspartate aminotransferase (ALT/AST) versus baseline in patients with baseline ALT/AST >2× the upper limit of normal (ULN). As a secondary outcome, this was assessed in patients with ALT/AST >2× ULN prior to treatment versus a matched cohort of patients with normal baseline ALT/AST. The safety population comprised 7,154 patients, of mean age 13.9 years (standard deviation (SD) 14.6), around half of whom were male (3,569 [49.9%]). Patients experienced 8,560 malaria episodes; 158 occurred in patients with baseline ALT/AST elevations >2×ULN. No protocol-defined hepatic events occurred following PY-ART treatment of malaria patients with or without baseline hepatic dysfunction. Thus, no cohort comparison could be undertaken. Also, as postbaseline clinical chemistry was only performed where clinically indicated, postbaseline ALT/AST levels were not systematically assessed for all patients. Adverse events of any cause occurred in 20.8% (1,490/7,154) of patients, most frequently pyrexia (5.1% [366/7,154]) and vomiting (4.2% [303/7,154]). Adjusting for *Plasmodium falciparum* reinfection, clinical effectiveness at day 28 was 98.6% ([7,369/7,746] 95% CI: 98.3 to 98.9) in the per-protocol population. There was no indication that comorbidities or malnutrition adversely affected outcomes. The key study limitation was that postbaseline clinical biochemistry was only evaluated when clinically indicated.

The authors concluded that PY-ART had good safety, tolerability and effectiveness in a representative African population under conditions similar to everyday clinical practice.

Tolerability and safety profile of praziquantel

PZQ has been used for schistosomiasis control for over 40 years and its safety and tolerability has been assessed in a myriad of trials (4,8,18–25). Side effects are mild and mostly comprise nausea, vomiting, malaise and abdominal pain. From individuals with heavy schistosome infections less frequent side effects were reported occurring shortly after treatment, such as acute cholic with bloody diarrhoea, which is hypothesised to be due to a massive worm shift and antigen release (4).

The recommended standard regimen in MDA approaches is 40mg/kg bodyweight as a single dose (4,8). However, for individual case management higher doses up to 60mg/kg can be administered for up to three days. This is for instance reflected by the recommended treatment guidelines of schistosomiasis by the German Society for Tropical Medicine and International Health (DTG). Such guidelines recommend dosages of 40mg/kg/day for three days, as well as, 60mg/kg/day for three days depending on the infective *Schistosoma* species (24). A favourable toxicity profile was described for PZQ with reportedly very low toxicity in animals

and no important long-term safety issues in humans (4,36). Traditionally, PZQ was administered to children below 4 years of age as off-label use due to missing data in this age group. However, in the last decade many studies were conducted that demonstrated safety in pre-school aged children (18–21,23). PZQ is also considered safe for treatment of pregnant women (37). Furthermore, in analogy to many existing antimalarial drugs efforts are currently undertaken to make PZQ available also as paediatric drug formulation which would facilitate favourable administrability to the target population of children and adolescents (38,39).

Tolerability and safety profile of combination treatments containing praziquantel and antimalarials

To date, there is not a single licensed or marketed pharmacological combination treatment regimens that contains antimalarial drugs, as well as, PZQ. Therefore, data on the safety and tolerability on such combination treatments stem from experimental research studies.

Two treatment studies evaluating a combination of PZQ with ART in school children with urogenital schistosomiasis demonstrated a favourable safety profile (30,43). This favourable safety profile was confirmed by another treatment study evaluating a combination of PZQ with ART and MFQ in African school children with urogenital schistosomiasis (31).

Requirements of multi-drug, multi-disease regimen PY/ART/PZQ in the treatment of urogenital schistosomiasis in endemic settings

As described above, PZQ possesses efficacy only against adult life-cycle stages of *Schistosoma spp* often necessitating additional treatment courses several weeks after the initial treatment. Therefore, as part of pharmacotherapeutic trials on urogenital schistosomiasis it is important to assess efficacy against all life-cycle stages of *S. haematobium*: not only against the adult life cycle stages, but also against juvenile life cycle stages. In a best-case scenario, assuming combined drug activity against all life cycle stages, this would confer radical cure after administration of one treatment course. This would be particularly useful in endemic settings, where institutions and individuals often also face limitations of resources.

The CORMA-BIL study is a randomised controlled clinical study assessing the efficacy of multidrug combination therapies against both juvenile and adult life-cycle stages in two study arm pairs, respectively:

Study arm pair I:
This study arm pair is dedicated to assess the efficacy of pyronaridine/artesunate against adult life-cycle stages of *S. haematobium*.

Study arm pair II:
This study arm pair is dedicated to assess the efficacy of pyronaridine/artesunate/praziquantel against juvenile life-cycle stages of *S. haematobium*.

Study overview

Trial Design

Single-centre, assessor-blinded, randomised, controlled trial

Population

Gabonese adolescents and children (5 years to below 18 years) with uncomplicated microscopically-confirmed *Schistosoma haematobium* infection

Intervention/Control

All study groups will receive study drugs on 3 consecutive days. The following passages show the intervention and control groups stratified by study arm pair.

STUDY ARM PAIR I [PY/ART and PLACEBO]:

Intervention$_{\text{study arm pair I}}$**:**
PY/ART study arm:
Oral treatment with PY/ART administered as once-daily dose for 3 consecutive days (D1, D2, D3). Dosing is independent of food.

Paediatric dosing regimen:
5 <-8 kg: 1 sachet daily
8 -<15 kg: 2 sachets daily
15-20 kg: 3 sachets daily

1 sachet contains 20 mg artesunate and 60 mg pyronaridine

Adolescent dosing regimen:
20-<24 kg: 1 tablet daily
24-45 kg: 2 tablets daily
45-<65 kg: 3 tablets daily
>65 kg: 4 tablets daily

1 tablet contains 60 mg artesunate and 180 mg pyronaridine

Control$_{\text{study arm pair I}}$**:**
PLACEBO:
Oral treatment with PLACEBO administered as once-daily dose for 3 consecutive days (D1, D2, D3). Dosing is independent of food.

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:

Intervention$_{\text{study arm pair II}}$**:**
PY/ART/PZQ study arm:

Oral treatment for 3 days. PY/ART once daily for 3 days. PZQ single dose on D1 with consecutive placebo doses on D2 and D3. Dosing is independent of food.

Paediatric dosing regimen:
5 <-8 kg: 1 sachet daily
8 -<15 kg: 2 sachets daily
15-20 kg: 3 sachets daily

1 sachet contains 20 mg artesunate and 60 mg pyronaridine.

Adolescent dosing regimen:
20-<24 kg: 1 tablet daily
24-45 kg: 2 tablets daily
45-<65 kg: 3 tablets daily
>65 kg: 4 tablets daily

1 tablet contains 60 mg artesunate and 180 mg pyronaridine

Control$_{\text{study arm pair II}}$**:**
PZQ study arm:
40mg/kg single dose of PZQ on D1 with consecutive PLACEBO doses on D2 and D3. Dosing is independent of food.

Outcome

The following passages show the primary outcomes stratified by study arm pair.

STUDY ARM PAIR I [PY/ART and PLACEBO]:
Percentage of egg reduction rate (ERR) between baseline and D42

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:
Time to re-infection with *Schistosoma haematobium* after treatment with study drugs.

Aims

To assess the efficacy of pyronaridine/artesunate (PY/ART) and pyronaridine/artesunate/praziquantel (PY/ART/PZQ) in participants with uncomplicated *Schistosoma haematobium* parasites.

Objectives

The following passages show the objectives stratified by study arm pair.

Primary Objective:

STUDY ARM PAIR I [PY/ART and PLACEBO]:

- To assess the percentage of egg reduction rate (ERR) assessed by light microscopy between baseline and D42 in the study arm PY/ART compared with PLACEBO

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:
- To assess the time to re-infection with *Schistosoma haematobium* after treatment with PYR/ART/PZQ compared with PZQ standard treatment assessed by light microscopy.

Secondary Objectives:

STUDY ARM PAIR I [PY/ART and PLACEBO]:
1) To assess the microscopically-determined cure rate in the study arm PY/ART compared with PLACEBO at D42 after administration of study drugs.

2) To assess the qPCR-determined cure rate in the study arm PY/ART compared with PLACEBO at D42 after administration of study drugs.

3) To compute and compare the areas under the curve (AUC) of *Schistosome* urinary egg excretion assessed by light microscopy in PY/ART versus PLACEBO.

4) To assess the serological cure rate (absence of circulating anodic antigen [CAA] in blood samples) in the study arm PY/ART compared with PLACEBO at D42 after administration of study drugs.

5) To compute and compare the areas under the curve (AUC) of parasite-specific glycans (CAA) in PY/ART versus PLACEBO.

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:
1) To assess the time to re-infection with *Schistosoma haematobium* after treatment with PY/ART/PZQ compared with PZQ standard treatment assessed by qPCR.

2) To assess the qPCR-determined cure rate in the study arm PY/ART/PZQ compared with PZQ at D98 after administration of study drugs.

3) To assess the time to re-infection with *Schistosoma haematobium* defined as presence of parasite-specific glycans (circulating anodic antigen [CAA]) in samples of blood.

4) To assess the serological cure rate (absence of CAA in blood samples) in the study arm PY/ART/PZQ compared with PZQ at D98 after administration of study drugs.

5) To assess the presence of *Schistosoma haematobium* eggs during week 4 after administration of study drugs (i.e. D21-D28) assessed by microscopy.

All study arms:
- To assess the safety and tolerability of study regimens in each study arm during the observation period.

- To assess the proportion of participants with haematuria in each study arm at D42 after administration of study drugs.

- To assess the proportion of participants with an incidental *Plasmodium* parasitaemia during the observation period

Exploratory Objectives:

- To determine the antibody response to parasite-specific glycans (CAA) over follow-up

- To assess and compare the level of specific IgE antibody to Sh22.6 kDa and ShTAL1 antigen before administration of study drugs compared to day 28 (D28) and day 98 (D98) after treatment

- To assess ShTAL4 as a direct and indirect marker for early Sh infection (antigen detection, anti-ShTAL4 antibody detection).

Sample size

The sample size rationale will be described stratified by study arm pair:

STUDY ARM PAIR I [PY/ART and PLACEBO]:

To calculate the sample size, we estimated that participants in the placebo group will have a 10% egg load reduction (i.e. egg reduction rate [ERR]) on day 42 after treatment versus 65% in the intervention group. With a power of 80% and an alpha error of 5% and considering a ratio 1:1 (treatment versus placebo group), we need 25 subjects in the placebo group and 25 subjects in intervention group. Accounting for a possible dropout rate of 20% in each study arm the final sample size will require recruitment of a total of 60 subjects.

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:

To calculate the sample size, we estimated that participants in the placebo group will on average have a re-infection at 54 days after treatment versus 68 days in the intervention group. With a power of 80% and an alpha error of 5% and considering a ratio 1:1 (treatment versus placebo group), we need 20 subjects in the placebo group and 20 subjects in intervention group. Accounting for a possible dropout rate of 20% in each study arm the final sample size will require recruitment of a total of 48 subjects.

Summary on sample size of overall trial:
108 volunteers will be randomized to 4 study arms as follows. There is 1:1 randomization within each study arm pair.

STUDY ARM PAIR I [PY/ART and PLACEBO]:

- **PLACEBO Arm**: 30 volunteers
- **PY/ART Arm:** 30 volunteers

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:

- **PZQ Arm**: 24 volunteers
- **PY/ART/PZQ Arm:** 24 volunteers

Note on sample size considerations:
To correct for multiplicity of endpoints the Hochberg procedure will be applied at the analysis stage of the trial.

Endpoints and assessment methods

The following passages describe the endpoints stratified by study arm pair.

Primary endpoints:

STUDY ARM PAIR I [PY/ART and PLACEBO]:
Log-transformed *Schistosoma haematobium* egg count reduction as determined by urine microscopy on D0 minus egg count on D42
Assessment method: Light microscopy

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:
Time to re-infection with *Schistosoma haematobium* defined as presence of microscopically-detectable eggs in urine
Assessment method: Light microscopy

Secondary endpoints:

STUDY ARM PAIR I [PY/ART and PLACEBO]:
1) Absence of *Schistosoma haematobium* eggs in samples of urine assessed by light microscopy on D42 after administration of study drugs (=microscopically-determined cure rate)
Assessment method: Light microscopy

2) Absence of *Schistosoma haematobium* DNA in samples of blood assessed by qPCR on D42 after administration of study drugs (=qPCR-determined cure rate)
Assessment method: Quantitative PCR

3) Area under the curve (AUC) of microscopically-detectable egg load on visits between D7 and D42 after administration of study drugs
Assessment method: Light microscopy

4) Absence of circulating anodic antigen (CAA) in blood samples on D42 after administration of study drugs (=serological cure rate).
Assessment method: Phosphor lateral flow (UCP-LF CAA) assay

5) Area under the curve (AUC) of parasite-specific glycans (CAA) on visits between D7 and D42 after administration of study drugs.
Assessment method: Phosphor lateral flow (UCP-LF CAA) assay

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:
1) Time to re-infection with *Schistosoma haematobium* defined as presence of Schistosome DNA in blood samples assessed by qPCR
Assessment method: Quantitative PCR

2) Absence of *Schistosoma haematobium* DNA in samples of blood assessed by qPCR on D98 after administration of study drugs (=qPCR-determined cure rate)
Assessment method: Quantitative PCR

3) Time to re-infection with *Schistosoma haematobium* defined as presence of parasite-specific glycans (circulating anodic antigen [CAA]) in samples of blood.
Assessment method: Phosphor lateral flow (UCP-LF CAA) assay

4) Absence of *Schistosoma haematobium* specific glycans (CAA) in samples of blood assessed on D98 after administration of study drugs (=serological cure rate)
Assessment method: Phosphor lateral flow (UCP-LF CAA) assay

5) Absence of *Schistosoma haematobium* eggs during week 4 after administration of study drugs (i.e. D21-D28) assessed by microscopy

Assessment method: Light microscopy

ALL STUDY ARMS:
- Occurrence of adverse events (AEs) after study drug administration
Assessment method: Clinical examination and laboratory analysis

- Occurrence of haematuria at D42 after administration of study drugs
Assessment method: Urine dipstick

- To assess the proportion of participants with an incidental *Plasmodium* parasitaemia during the observation period
Assessment method: Light microscopy of blood films and qPCR analysis of dried blood spots

Exploratory endpoints:

- Determination of IgM and IgG to parasite-specific circulating anodic antigen (CAA) and circulating cathodic antigen (CCA)
Assessment method: ELISA *(Note: Assessment method may change depending on the laboratory where analyses will finally be performed)*

- Level of specific IgE antibody to Sh22.6 kDa and ShTAL1 antigen before administration of study drugs compared to day 28 (D28) and day 98 (D98) after treatment
Assessment method: ELISA *(Note: Assessment method may change depending on the laboratory where analyses will finally be performed)*

- Assessing ShTAL4 as a direct and indirect marker for early Sh infection (antigen detection, anti-ShTAL4 antibody detection).
Assessment method: ELISA *(Note: Assessment method may change depending on the laboratory where analyses will finally be performed)*

Duration of the study

The duration of the study will be categorised into the following three parts: **Screening** (D0), **Treatment phase** (D1, D2, D3) and **follow-up phase** (with its subcategories **'Early follow-up phase'** and **'Late follow-up phase'**).

- During the 'Treatment phase', participants will be hospitalised for 3 days.

- Following the 'Treatment phase', the 'Early follow-up phase' lasts until week 6 of follow-up and applies to both STUDY ARM PAIR I [PY/ART and PLACEBO] and STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. Weekly visits shall be performed once per calendar week. The time-interval between two visits needs to be at minimum 4 days.

- Following the 'Early follow-up phase', the 'Late follow-up phase' lasts until week 14 of follow-up and applies only to STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. Weekly visits shall be performed twice within the same calendar week. The time-interval between two visits needs to be at minimum 2 days.

Follow-up has a different duration depending on the respective study arm pair: For STUDY ARM PAIR I [PY/ART and PLACEBO] follow-up ends 6 weeks after administration of study drugs (i.e. on D42), while for STUDY ARM PAIR II [PY/ART/PZQ and PZQ] follow-up ends 14 weeks after administration of study drugs (i.e. on D98).

For more detailed information see chapter "Follow-up visits".

The start of the trial is defined as the date of the first visit (i.e. screening visit) of the first participant. The end of the trial is the date of the last visit of the last participant.

Potential risks for participants

Phlebotomy

The maximum volume of blood drawn is approximately 150 ml over a maximum observation period of 98 days. Additional blood samples could be required for safety reasons. However, this volume should not compromise these otherwise healthy participants. There may be minor bruising, local tenderness or presyncopal symptoms associated with venepuncture, which will not be documented as AEs if they occur. Rare side effects are infections, thrombophlebitis and nerval lesions.

Pyronaridine/Artesunate (Pyramax)

The safety of pyronaridine and artesunate has been evaluated in clinical trials of approximately 12,200 patients (44). Side effects are stratified in the table below.

System Organ Class	Common (>1/100 to <1/10)	Uncommon (>1/1000 to <1/100)	Rare (<1/1000)
Blood and lymphatic system disorders	Anaemia, eosinophilia, neutropenia, increased platelet count*	Basophilia, leukocytosis, leukopenia, lymphocytosis, monocytosis, splenomegaly, thrombocytopenia	Lymphopenia, pancytopenia
Cardiac disorders	Bradycardia	Palpitations, ventricular extrasystoles	Arrhythmia, atrioventricular block first degree, sinus arrhythmia
Ear and labyrinth disorders		Vertigo	Ear pain, hearing impaired, tinnitus
Eye disorders			Conjunctivitis
Gastrointestinal disorders	Abdominal pain, vomiting	Constipation, diarrhoea, dyspepsia, gastritis, nausea	Abdominal tenderness, aphthous stomatitis, stomach discomfort, tongue ulceration
General disorders and administration site conditions		Asthenia, fatigue	Chest pain, chills, hypothermia, pyrexia
Hepatobiliary disorders		Hepatomegaly	Hepatosplenomegaly, liver tenderness
Immune system disorders			Hypersensitivity
Infections and infestations		Gastroenteritis, malaria, oral herpes, respiratory tract infection, tinea capitis, upper respiratory tract infection, urinary tract infection	Bronchitis, bronchopneumonia, infection parasitic, pharyngitis, pharyngotonsilitis, Plasmodium falciparum infection, pneumonia, rhinitis, subcutaneous abscess, tracheobronchitis
Investigations	Transaminases increased	Blood albumin decreased, blood alkaline phosphatase increased, blood creatine phosphokinase increased, blood creatinine decreased, blood sodium increased, electrocardiogram abnormal, electrocardiogram QT prolonged, liver function test abnormal	Blood albumin increased, blood bilirubin decreased, blood bilirubin increased, blood creatinine increased, blood potassium decreased, haematocrit increased, red blood cell count increased, white blood cells urine
Metabolism and nutrition disorders	Hypoglycaemia	Anorexia, hyperkalaemia	Decreased appetite, hyperglycaemia
Musculoskeletal and connective tissue disorders		Myalgia	Arthralgia, back pain

Nervous system disorders	Headache	Dizziness, dysgeusia, paraesthesia	Somnolence
Pregnancy, puerperium and perinatal conditions			Abortion complete
Psychiatric disorders		Insomnia	Sleep talking
Renal and urinary disorders		Haematuria, proteinuria	Ketonuria
Reproductive system and breast disorders			Vulvovaginal pruritus
Respiratory, thoracic and mediastinal disorders		Cough	Asthma, epistaxis, haemoptysis, rhinorrhoea
Skin and subcutaneous tissue disorders		Hyperhidrosis, pruritus, rash	Blister, dermatitis, urticaria papular
Vascular disorders			Hypertension, hypotension

* A rise in platelets generally from a low to normal level was commonly reported

Praziquantel

Side effects of praziquantel include nausea (≥10%), vomiting (≥10%), gastrointestinal and abdominal pain (≥10%), headache (≥10%), drowsiness (≥10%), urticaria (≥10%), diarrhoea (<10%), anorexia (<10%), dizziness (<10%), somnolence (<10%), fever (<10%), rash (<10%), myalgia (<10%), unspecific arrhythmia (<0.0001%), pruritus (<0.0001%), seizure (<0.0001%), allergic reaction (<0.0001%), eosinophilia (<0.0001%) (41).

Potential benefits for participants

Participants will not benefit directly from participation in this study. The only benefits for the participants will be information about their general health status and the treatment from the uncomplicated *Schistosoma haematobium* infection at the end of the follow up. Potential incidental malaria episodes during follow-up will be cured. It is hoped that the information gained from this study will contribute to the development of safe and effective drug with combined activity against schistosomiasis and malaria. Compensation for missed working day if any and transportation fee for follow-up visit will be reimbursed.

Recruitment and withdrawal of trial participants

Participants

The study is designed as a randomised, controlled, assessor-blinded clinical trial to assess the efficacy of Pyramax-containing regimens to treat uncomplicated *Schistosoma haematobium* infections in subjects in an endemic area of Gabon. Adolescent and children participants (5 years to below 18 years) living in the catchment area of the Centre de Recherches Médicales de Lambaréné (CERMEL) catchment areas will be screened for microscopically-confirmed *Schistosoma haematobium* infection.

Informed Consent

All participants will sign and date the informed consent form before any study specific procedure is performed. At the screening visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasised:

• Participation in the study is entirely voluntary
• Refusal to participate involves no penalty or loss of medical benefits
• The participant may withdraw from the study at any time
• The participant is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial and will then have time to consider whether or not to participate. Potential participants will be asked to sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the Investigator`s File. These forms will also be signed and dated by the Investigator.

Inclusion Criteria

The subject must satisfy all the following criteria to be eligible for the study:

- Participants aged between 5 years and below 18 years
- Microscopically-determined *Schistosoma haematobium* infection
- Uncomplicated *Schistosoma haematobium* infection defined by: presence of microscopically-determined *Schistosoma haematobium* eggs in urine with absence of Katayama fever and absence of clinically significant urinary tract pathology (see exclusion criteria).
- Written informed consent must be obtained before any study assessment is performed

- Willingness not to take drugs or substances which could have an impact on study drug blood levels. The timeframe for the abstinence of these drugs should be throughout the duration of the study or will be individually calculated by the study team, if necessary
    - The following is a selection of drugs/substances to be avoided unless explicitly given as study medication or rescue medication (both in the period prior to Screening and during the patient's participation):
        - 4-Aminoquinolines chloroquine and piperaquine Arylaminoalcohols quinidine, 8-Aminoquinolines primaquine, 9-phenanthrene methanol halofantrine, Abametapir, Alfentanil, Alprazolam, Amiodaron, Amitriptylin, Amlodipin, Aprepitant, Aripiprazol, Atorvastatin, Azythromycin, Buspiron, Carbamazepin, Chloramphenicol, Chloroquine, Chlorproguanil, Ciclosporin, Cimetidin, Citalopram, Clarithromycin, Clindamycin, Clofazimin, Clomipramin, Clonazepam, Clotrimazol, Codein, Combinations of sulfadoxine pyrimethamine, Cyclophosphamid, Dapsone, Deferasirox, Dexamethason, Diltiazem, Doxorubicin, Doxycycline, Erdafitinib, Erlotinib, Erythromycin, Escitalopram, Etoposid, Felodipin, Fentanyl, Fluconazol, Flunitrazepam, Fluoxetin, Fosaprepitant, Fusidic Acid, Gefitinib, Ginseng, Grapefruit, Haloperidol, Hypericum ("Johanniskraut"), Imipramin, Indinavir, Itraconazol, Ivabradin, Ivosidenib, Ketoconazol, Liquorice, Lovastatin, Mefloquine, Methadon, Methotrexate and other folate antagonists, Metoclopramide (period prior to screening to Day 5 post-dose), Miconazole, Midazolam, Modafinil, Nefazodon, Nelfinavir, Nifedipin, Norfluoxetin, Oxcarbazepin, Pentamidine, Phenobarbital, Phenytoin, Pimozid, Primidon, Pyrimethamine, Quinine, Quinolones, Rifampicin, Rifampin, Risperidon, Ritonavir, Sarilumab, Sertralin, Sildenafil, Siltuximab, Simeprevir, Simvastatin, Sirolimus, Stiripentol, Sulfadiazine, Sulfadoxine, Sulfalene, Sulfasalazine, Sulfisoxazole, Sulfonamides, Tacrolimus, Tadalafil, Tafenoquine, Tamoxifen, Telithromycin, Tocilizumab, Triazolam, Trimethoprim sulfamethoxazole, Turmeric ("Gelbwurzel"), Valeriana ("Baldrian"), Venlafaxin, Verapamil, Vinblastin, Vindesin, Ziprasidon

    - Traditional and herbal remedies are not permitted from 7 days prior to dosing and during the study.
- Women only of reproductive age: Must agree to practice continuous contraception for the duration of the study. Those methods include: combined (estrogen and progestogen containing) hormonal contraception, associated with inhibition of ovulation, oral, intravaginal or transdermal progestogen-only hormonal contraception associated with inhibition of ovulation, oral, injectable, implantable intrauterine device (IUD), intrauterine hormonereleasingsystem (IUS), bilateral tubal occlusion, vasectomised partner, sexual abstinence and use of condoms.

Exclusion Criteria

The subject may not enter the study if any of the following criteria apply:

- Presence of Katayama fever
- Presence of clinically significant urinary tract pathology. The diagnoses of clinically significant urinary tract pathologies are made by the clinical investigator
- Pregnancy or breast-feeding
- Use of drugs with known antischistosomal activity within 2 months of enrolment into study (including praziquantel and antimalarial treatment with artemisinin-combination therapies)
- Contraindications or known allergy to pyronaridine/artesunate or praziquantel
- Any other significant disease, disorder or finding which, in the opinion of the investigator, may significantly increase the risk to the participant because of participation in the study (e.g. renal transplantation etc.), affect the ability of the participant to participate in the study or impair interpretation of the study data
- Participants unable to be closely followed for social, geographic or psychological reasons
- Haemoglobin level below 8 g/dL
- Previous participation in the CORMA-BIL study (multiple participation not possible)

Withdrawal of Participants

In accordance with the principles of the current revision of the Declaration of Helsinki (updated 2013) and any other applicable regulations, a participant has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for

doing so. The Investigator may withdraw the participant at any time in the interests of the participant's health and well-being. In addition, the participant may withdraw/be withdrawn for any of the following reasons:

• Administrative decision by the Investigator
• Ineligibility (either arising during the study or retrospectively, having been overlooked at screening)
• Significant protocol deviation
• Participant non-compliance with study requirements
• An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures

The reason for withdrawal will be recorded in the case report form (CRF). If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the participant, until the AE has resolved, stabilised or a non-trial related causality has been assigned. Any participant who is withdrawn from the study may be replaced, if that is possible within the specified time frame. The medical monitor may recommend withdrawal of participants. If a participant withdraws/is withdrawn from the study before reaching the criterion for malaria diagnosis, a complete, appropriate, curative course of anti-malarial therapy must be completed. The importance of this will be emphasised to study participants at screening. If a participant withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the participant specifically requests otherwise.

Pregnancy

Pyronaridine/artesunate should not be administered during the first trimester of pregnancy, however, is considered safe during second and third trimester (44). Praziquantel is considered safe for treatment of pregnant women (37). However, based on potentially differential parasite kinetics between pregnant and non-pregnant participants, pregnant women should not be included in this current Phase II trial. Should a study participant become pregnant during the trial, she will be followed up as other participants and in addition will be followed until pregnancy outcome. We will not routinely perform nonessential venepuncture on such participants. All initial reports of pregnancy must be reported to the sponsor by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, stillbirth, and congenital anomaly) are considered serious adverse events and must be reported using the Serious Adverse Event (SAE) Form. Any subject who becomes pregnant during the study must discontinue further study treatment and be withdrawn from the study.

Treatment of trial participants

Allocation of the groups

Participants fulfilling all inclusion/exclusion criteria will be randomly allocated to one of the study arms within each study arm pair (A] PLACEBO, B] PY/ART and C] PZQ, D] PY/ART/PZQ). The allocation ratio is 1:1 and 1:1 within each study arm pair. For administrative reasons recruitment into study arm pairs may also be conducted sequentially

(STUDY ARM PAIR I [PY/ART and PLACEBO] first, followed by STUDY ARM PAIR II [PY/ART/PZQ and PZQ], or vice versa); this will be determined before recruitment of the first participant. Randomisation lists will be created using permuted blocks. Treatment allocation codes will be stored in opaque-sealed envelopes on whose surface is a pre-defined allocation sequence number. Each time a participant fulfils the trial's eligibility criteria the opaque-sealed envelope with the next allocation sequence number is opened by the pharmacist or his delegate. During the treatment phase all participants will be hospitalised for three days. Study procedures during the treatment phase and follow-up phase will be the same in the two study arm pairs, however, follow-up comprises 6 weeks for STUDY ARM PAIR I [PY/ART and PLACEBO] and 14 weeks for STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. The unblinded pharmacist or his delegate will tell the blinded investigators whether the subject participates in STUDY ARM PAIR I or STUDY ARM PAIR II, so that follow-up schedules may be planned accordingly.

Study procedures

Blinding

Laboratory investigators processing and analysing biological samples will be blinded to group allocation. The unblinded pharmacist or designate will prepare the treatment assigned by the randomisation system and complete the corresponding treatment record form. Subsequently, verum and/or placebo will be administered by a qualified person who is not involved in diagnostic and analytic processes of the study. Other study physicians and study participants will be unaware of group allocation. Only the pharmacists and the qualified person administering study drugs know the group allocation. The study drugs are not identical and will be administered to each participant separately out of the original packaging without changing labels.

Ascertainment of basic demographic information, clinical and past medical history

- Full medical history
- Prior concomitant medication
-Demographic data (sex, age)
- Clinical examination (including measurement of height and weight at baseline)

This should be ascertained during the screening visit (See Table 2).

Urine sampling and laboratory diagnostics

Urine samples shall be prepared at every visit during screening, treatment phase and follow-up (both early and late follow-up phases; Table 2).

Urine light microscopy

Urine light microscopy will be used to detect and quantify *S. haematobium* eggs. A minimum urine quantity of 10 ml should be used, although it is desirable to use much higher quantities (i.e. >100 ml). Participants should be encouraged to collect urine of a given visit day, the help ensure that a sufficient urine quantity is available at the visit. Note that in case that a participant cannot produce 10 ml at a given visit the available volume of urine should still be used and analysed. The screened urine volume will be documented on the paper-based source

document to ensure between-participant and within-participant comparability of parasitological results.

Urine dipstick analysis

Urine dip stick analysis shall be used to detect and quantify the possible presence of haematuria. In addition to that, the clinical investigator should inspect the urine for macroscopic presence of haematuria; this will be recorded in the paper-based source document.

Urine β-HCG test in female participants

This is performed at screening to rule out any potential pregnancy at the beginning of the trial (Table 2).

Blood sampling and laboratory diagnostics

Schistosomiasis diagnostic blood sampling

A series of blood samples will be drawn to perform different analytical assays to detect and quantify *S. haematobium*.

Serum sampling (withdrawal of venous blood)

Serum samples shall be prepared at every visit during screening, the treatment phase and the early follow-up phase. During the late follow-up phase they should only be performed during the 'clinical visit' and not during the 'field visit' (for more detailed guidance see Table 2).

For the following diagnostic methods, sufficiently many serum collection tubes should be filled so that each 1 ml of serum aliquots can be pipetted into Eppendorf tubes. A withdrawal of approximately 6.5 ml of blood should ensure that the target serum quantities are reached. Eppendorf tubes filled with serum should be frozen at -80°C until analysis.

Molecular diagnostics
Target quantity to be frozen: 1 ml serum. This will be used for determination of *S. haematobium* DNA using quantitative PCR.

Glycan diagnostics
Target quantity to be frozen: 1 ml serum. This will be used for determination of CAA using upconverting phosphor particle lateral flow (UCP-LF CAA) assay.

Anti-glycan antibody diagnostics
Target quantity to be frozen: 1 ml serum. This will be used for determination of IgM and IgG against circulating anodic antigen (CAA) and circulating cathodic antigen (CCA) using ELISA.

Thick and thin blood smears & dried blood spots

Thick and thin blood smear samples will be prepared and analysed with light microscopy for the detection of *Plasmodium* parasites according to local SOPs. They will be performed routinely at certain time points (see Table 2). Furthermore, they should be prepared if an

incidental episode of malaria is suspected; when to test for malaria depends on the judgement of the clinical investigator. Whenever blood smears are prepared, dried blood spots shall be prepared as well.

Haematology and biochemistry tests

Full blood count analysis (i.e. haematological assessment) and biochemistry (i.e. clinical chemistry) will be performed at certain time points (see Table 2) to monitor laboratory safety.

The following parameters will be analysed throughout the study:

Haematology:
- Red blood cells (RBCs)
- Haemoglobin
- Haematocrit
- Platelets
- White blood cells (WBCs)
- Neutrophils
- Eosinophils
- Basophiles
- Lymphocytes
- Monocytes

Biochemistry:
- Total bilirubin
- Direct bilirubin
- Albumin
- ALT
- AST
- Alkaline phosphatase
- LDH
- Creatine kinase
- Urea
- Creatinine
- Haptoglobin
- Sodium
- Potassium
- Glucose

Further parameters may be determined if judged medically necessary clinical investigator or the medical monitor. Also, the frequency of haematology and/or biochemistry assessments may be increased if medically required.

Administration of study drugs

Once that the presence of all inclusion criteria and the absence of all exclusion criteria are secured a randomisation envelope may be opened by the unblinded pharmacist or his delegate. The respective dosing regimens for each study arm are listed below:

STUDY ARM PAIR I [PY/ART and PLACEBO]:

Intervention study arm pair I:
PY/ART study arm:
Oral treatment with PY/ART administered as once-daily dose for 3 consecutive days (D1, D2, D3). Dosing is independent of food.

Paediatric dosing regimen:
5 <-8 kg: 1 sachet daily
8 -<15 kg: 2 sachets daily
15-20 kg: 3 sachets daily

1 sachet contains 20 mg artesunate and 60 mg pyronaridine

Adolescent dosing regimen:
20-<24 kg: 1 tablet daily
24-45 kg: 2 tablets daily
45-<65 kg: 3 tablets daily
>65 kg: 4 tablets daily

1 tablet contains 60 mg artesunate and 180 mg pyronaridine

Control study arm pair I:
PLACEBO:
Oral treatment with PLACEBO administered as once-daily dose for 3 consecutive days (D1, D2, D3). Dosing is independent of food. The same number of placebo tablets shall be given on each given dosing day, as Pyramax tablets would be necessary (based on the participant's body weight). In case the participant weighs less then 20 kg then 1 (one) placebo tablet shall be administered per dosing time point.

STUDY ARM PAIR II [PY/ART/PZQ and PZQ]:

Intervention study arm pair II:
PY/ART/PZQ study arm:
Oral treatment for 3 days. PY/ART once daily for 3 days. PZQ single dose on D1 with consecutive placebo doses on D2 and D3. Dosing is independent of food. The same number of placebo tablets shall be given on D2 and D3, as PZQ tablets were given on D1.

Paediatric dosing regimen:
5 <-8 kg: 1 sachet daily
8 -<15 kg: 2 sachets daily
15-20 kg: 3 sachets daily

1 sachet contains 20 mg artesunate and 60 mg pyronaridine

Adolescent dosing regimen:
20-<24 kg: 1 tablet daily
24-45 kg: 2 tablets daily
45-<65 kg: 3 tablets daily
>65 kg: 4 tablets daily

1 tablet contains 60 mg artesunate and 180 mg pyronaridine

Control$_{\text{study arm pair II}}$:
PZQ study arm:
40mg/kg single dose of PZQ on D1 with consecutive PLACEBO doses on D2 and D3. Dosing is independent of food. The same number of placebo tablets shall be given on D2 and D3, as PZQ tablets were given on D1.

Administration of standardised praziquantel rescue treatment

Volunteers will be treated with a standardised dose of PZQ according to national guidelines if schistosomiasis rescue treatment criteria are reached during follow-up.

PZQ rescue treatment criteria are, as follows:

- 1) Presence of *Schistosoma haematobium* eggs in urine

  AND

- 2a) Presence of Katayama fever or clinically significant urinary tract pathology
  OR
- 2b) Presence of severe anaemia (i.e. Haemoglobin level below 8 g/dL)

The diagnosis of Katayama fever and acute 'clinically significant bladder disorder' are made clinically by the investigator. The following signs, symptoms or conditions are associated with Katayama fever and *S. haematobium*-related bladder pathologies, respectively:

- Katayama fever:
  - Fever: axillary temperature ≤ 37.5°C or oral / tympanic temperature ≤ 38°C
  - Urticaria
  - Eosinophilia
  - Diarrhoea
  - Hepatomegaly
  - Splenomegaly
  - Cough and wheeze
  - Cachexia

Note: Katayama fever is a systemic disease with immune complexes caused by growing schistosome parasites (usually schistosomula) (24,45). *Since PZQ is not active against schistosomula (i.e. juvenile parasite stages) it is generally not recommended to administer PZQ for the treatment of Katayama fever in the confirmed absence of parasite eggs in urine. Therefore, solely symptomatic treatment is recommended for such participants.*

- Clinically significant urinary tract pathology:
  - Symptomatic haematuria
  - Dysuria
  - Cystitis
  - Urethritis
  - Prostatitis
  - Salpingitis
  - Epididymitis
  - Obstructive uropathy
  - Hydronephrosis

Note: Such signs, symptoms and pathologies are usually caused by adult schistosomes against which PZQ is active (24).

If the clinical investigator believes that it is in the best interest of the participant to receive the standardised PZQ rescue treatment then it may also be administered in the absence of the above-mentioned criteria.

Timing of standardised PZQ rescue treatment administration:
If schistosomiasis rescue treatment criteria are not reached throughout follow-up, antischistosomal treatment will be administered at the end of follow-up. Follow-up ends on D42 in STUDY ARM PAIR I [PY/ART and PLACEBO] and on D98 in STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. Depending on participant convenience, or the individual's participation in other studies the end-of-study antischistosomal treatment may also take place later than on D98.

Treatment of incidental malaria episodes

Should participants contract an episode of malaria during the follow-up an effective dose of atovaquone/proguanil (i.e. Malarone) will be administered. Neither atovaquone nor proguanil are known to possess antischistosomal activity and thereby no interference with schistosomiasis-related endpoints is suspected. Also, quinine may be used as treatment for incidental malaria episodes.

If applicable: Medication according to local guidelines will be used for severe malaria.

Follow-up visits

The study recognises a **'Screening visit'**, a **'Treatment phase'**, as well as, an '**Early follow-up phase'** and a **'Late follow-up phase'**. Participants must be seen during all scheduled visits within the acceptable time frame. Missed or rescheduled visits should not lead to automatic discontinuation.

Screening visit

All potential participants will have a screening visit, which should take place on the same day as the administration of study drugs on visit of D1 (Note: Time interval between SCR and dosing of D1 shall not exceed 24 hours). Informed consent will be obtained from those eligible as described above. Once the informed consent is signed, the following screening procedures indicated in Table 2 will be undertaken:

- Full medical history, clinical examination (including measurement of height and weight) and ascertainment of demographic details
- The inclusion and exclusion criteria for the study will be assessed
- Urine light microscopy
- Urine dipstick analysis
- urine β-HCG test in female participants;
- Full blood count (haematology assessment)
- Biochemistry
- Thick and thin smear samples & dried blood spots

AEs will be recorded from the administration of the first dose of study drugs onwards. Abnormal clinical findings from the medical history, vital signs assessment or blood tests at any point in the study will be assessed using established reference intervals of CERMEL laboratory. If a laboratory test is out of range it may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the participant will be informed and appropriate medical care arranged with the permission of the participant.

Treatment phase; study drug administration (D1-D3)

Medication will take place at CERMEL and participants will be hospitalised for 3 days. However, based on participant convenience the subject may also return home after the given treatment visit if the clinical investigator believes that this will not compromise the probability that the participant misses the consecutive days' treatment visits within the acceptable time frame. Before the medication is administered the following measures will be performed on all participants:

- Urine light microscopy
- Urine dipstick analysis

> *Note: Screening results for urine light microscopy and urine dipstick analysis may be used for D1 visit if the time interval between SCR and D1 did not exceed 24 hours.*

- Schistosomiasis diagnostic blood sampling
- Potential adverse events (AEs)
- Potential concomitant medication is ascertained
- Clinical examination

Afterwards study medication is administered.

Note on acceptable time frame of visits during the 'Treatment phase':
During the treatment phase (D1-D3) dosing of PZQ on day 1 (i.e. D1) marks the reference time point for dosing on D2, which is to be scheduled 24 hours after D1. Concordantly, dosing on D2 shall be conducted 24 hours after D1. Each visit is assigned a time point and a window period of ± 1 hour within which the visit will be conducted.

Early follow-up phase (First 6 weeks; applies to study arm pair I and study arm pair II)

After hospitalisation and administration of the full dose of study treatments there shall be a further follow-up visit on day 6 (± 1 day) during week 1. After week 1 (W1), follow-up visits will be performed once per calendar week for 6 weeks (i.e. 'Early follow-up phase'). Early follow-up visits should be conducted at the clinical research centre.

The following assessments will be performed per visit:

- Urine light microscopy
- Urine dipstick analysis
- Schistosomiasis diagnostic blood sampling
- Potential adverse events (AEs)
- Potential concomitant medication is ascertained
- Clinical examination

Other assessments (e.g. haematology, biochemistry, blood films and dried blood spots) will be performed less frequently (see Table 2).

At the end of the 'Early follow-up phase' (i.e. D42) participants in STUDY ARM PAIR I [PY/ART and PLACEBO] reach the "End of study" (see below) and receive standardised PZQ treatment, while participants in STUDY ARM PAIR II [PY/ART/PZQ and PZQ] do NOT receive standardised PZQ treatment and continue follow-up in the "Late follow-up phase" (see below).

Note on acceptable time frame of visits during the 'Early follow-up phase':
The time-interval between two visits needs to be at minimum 4 days.
Example: If the visit of week 2 (W2) was conducted on a Friday, then the earliest the visit of W3 can be performed is on the following Wednesday. Then, the visit of W4 can be conducted earliest on a Monday.

Late follow-up phase (Further follow-up until week 14; applies only to study arm pair II)

The 'Late follow-up phase' applies only to participants in STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. During the 'Late follow-up phase' follow-up visits will be performed twice per calendar week until week 14.

Once per calendar week there will be a **'clinical visit'** during which the following assessments will be performed (Note that these are exactly the same assessments as during the early follow-up phase):

- Urine light microscopy
- Urine dipstick analysis
- Schistosomiasis diagnostic blood sampling
- Potential adverse events (AEs)
- Potential concomitant medication is ascertained
- Clinical examination

In addition to the 'clinical visit', a **'field visit'** shall be performed within the same calendar week. During this field visit urine shall be collected for schistosome egg screening. For the participant's convenience this field visit can be performed at the participant's home. The following assessments will be performed per 'field visit':

- Urine light microscopy
- Urine dipstick analysis
- Potential adverse events (AEs)
- Potential concomitant medication is ascertained

Other assessments (e.g. haematology, biochemistry, blood films and dried blood spots) will be performed less frequently (see Table 2).

It is recommended to start the late follow-up phase with a 'field visit' (W7.1) followed by a 'clinical visit' (W7.2). From then on it is suggested to continue with 'field visits' and 'clinical visits' in alternating manner (e.g. field visit --> clinical visit --> field visit --> clinical visit etc.) until the end of study is reached.

Note on acceptable time frame of visits during the 'Late follow-up phase':
Within a given calendar week the time-interval between two visits needs to be at minimum 2 days. *Example: If the visit of week 7.1 (W7.1) was conducted on a Monday then the earliest the visit of W7.2 can be performed is on the following Thursday.*

End of study

The following assessments will be performed at the 'End of study' visit:

- Urine light microscopy
- Urine dipstick analysis
- Schistosomiasis diagnostic blood sampling
- Potential adverse events (AEs)
- Potential concomitant medication is ascertained
- Full blood count (haematology assessment)
- Biochemistry
- Thick and thin smear samples & dried blood spots
- Clinical examination
- Administration of PZQ (see below)

After the performance of all assessments at the end of the study, the rescue treatment (PZQ) will be given to all study participants irrespective of parasitological results in urine light microscopy. This ensures that all study participants irrespective of study arm allocation will reach complete parasite clearance. In accordance with national guidelines, a single weight-

adjusted dose of 40mg/kg PZQ will be given to study participants including an explanation on how to self-administer the medication at home.

Follow-up ends on D42 in STUDY ARM PAIR I [PY/ART and PLACEBO] and on D98 in STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. Depending on participant convenience, or the individual's participation in other studies the end-of-study antischistosomal treatment may also take place later than on D98.

Note on acceptable time frame for 'End of study' visit:
For STUDY ARM PAIR I [PY/ART and PLACEBO]: Within the above-described sequence of once-per-week follow-up visits the 'End of study visit' shall be performed at day 42 ± 3 days of follow-up.

For STUDY ARM PAIR II [PY/ART/PZQ and PZQ]: Within the above-described sequence of twice-per-week follow-up visits the 'End of study visit' shall be performed on day 98 ± 3 days of follow-up.

Table 2.1: Study procedures (to be continued below). Screening, Treatment phase and Early follow-up phase.

STUDY ARM PAIR I [PY/ART and PLACEBO] & STUDY ARM PAIR II [PY/ART/PZQ and PZQ]											
		Treatment phase			**Early follow-up phase**						
Visits	**SCR#**	**D1**	**D2 (24 ± 1 hours later)**	**D3 (24 ± 1 hours later)**	**D6 (± 1 day)**	**W2**	**W3**	**W4**	**W5**	**W6***	**UNS**
Demographics, medical history	x										
Clinical examination	x	x	x	x	x	x	x	x	x	x	(x)
Incl./Excl. criteria	x										
Urine ß-HCG	x										(x)
Urine light microscopy	x	x	x	x	x	x	x	x	x	x	(x)
Urine dipstick analysis	x	x	x	x	x	x	x	x	x	x	(x)
Schistosomiasis diagnostic blood sampling (6.5 ml)		x	x	x	x	x	x	x	x	x	(x)
Adverse event assessment			x	x	x	x	x	x	x	x	(x)
Concomitant medication assessment		x	x	x	x	x	x	x	x	x	(x)
Study drug administration		x	x	x							
Standardised PZQ treatment										**x***	(x)
Full blood count (1.2ml)	x						x			x	(x)
Biochemistry (2.7ml)	x									x	(x)
Thick and thin blood smears & dried blood spots (0.2ml)	x						x			x	(x)
Blood volume (total = 129.7 ml)	4.1 ml	6.5 ml	6.5 ml	6.5 ml	6.5 ml	6.5 ml	7.9 ml	6.5 ml	6.5 ml	10.6 ml	

(x) = if judged necessary by clinical investigator at unscheduled visit (UNS); Note that additional assessments may be performed at any time point, if judged medically necessary by the clinical investigator. Safety related procedures may always be added if the clinical investigator, or the local safety monitor consider it necessary.
The time interval between the Screening visit and D1 shall not exceed 24 hours.
***At week 6 (W6; i.e. D42± 3 days), follow-up is finalised for participants in STUDY ARM PAIR I [PY/ART and PLACEBO]. Only participants in study arm pair I should receive standardised PZQ rescue treatment at this time point.**

Table 3.2: Study procedures (continued). Late follow-up phase.

Only STUDY ARM PAIR II [PY/ART/PZQ and PZQ]																	
	Late Follow-up phase																
Visits	**W7.1**	**W7.2**	**W8.1**	**W8.2**	**W9.1**	**W9.2**	**W10.1**	**W10.2**	**W11.1**	**W11.2**	**W12.1**	**W12.2**	**W13.1**	**W13.2**	**W14.1**	**W14.2**	**UNS**
Demographics, medical history																	
Clinical examination		x		x		x		x		x		x		x		x	(x)
Incl./Excl. criteria																	
Urine ß-HCG																	(x)
Urine light microscopy	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)
Urine dipstick analysis	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)
Schistosomiasis diagnostic blood sampling (6.5 ml)		x		x		x		x		x		x		x		x	(x)
Adverse event assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)
Concomitant medication assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	(x)
Study drug administration																	
Standardised PZQ treatment																**x****	(x)
Full blood count (1.2ml)						x						x				x	(x)
Biochemistry (2.7ml)												x				x	(x)
Thick and thin blood smears & dried blood spots (0.2ml)						x						x				x	(x)
Blood volume (total = 129.7 ml)	0	6.5 ml	0 ml	6.5 ml	0 ml	7.9 ml	0 ml	6.5 ml	0 ml	6.5 ml	0 ml	10.6 ml	0 ml	6.5 ml	0 ml	10.6 ml	

****At week 14.2 (W14.2; i.e. D98± 3 days), follow-up is finalised also for participants in STUDY ARM PAIR II [PY/ART/PZQ and PZQ]. At W14.2 (i.e. D98± 3 days) also participants in study arm pair II should receive standardised PZQ treatment.**

Assessment of safety

The safety and tolerability of PY/ART and PY/ART/PZQ treatments will be assessed by analysing the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

Definitions

Adverse Event (AE)

An Adverse Event (AE) is any new unfavourable and unintended sign, symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each AE has to be documented in order of appearance, given by a number. In addition, the start and end time of the AE has to be noted precisely. The investigator must enter the information within 24 hours in the paper-based source document and eCRF using the AE section.

The term 'new' unfavourable and unintended sign/symptom/disease implies that this 'new' sign/symptom/disease was not present at baseline or was not part of the past medical history of the participant.
Example: In case a patient reports sleeping problems at baseline and further reports 'sleeping problems' during follow up, then 'sleeping problems' will not be counted as adverse events.

Exceptions from this rule can be made if the problem, if a clinician is convinced the respective sign/symptom/disease has become much different in severity/intensity, quality etc.

Grading of adverse events

The investigator will assess the **severity/intensity** of the adverse event using the following terms:

Table 4: Severity of Adverse Events

Grade 1	**Mild**	Awareness of sign or symptom, but easily tolerated; Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
Grade 2	**Moderate**	Enough discomfort to cause interference with usual activity; Mild to moderate limitation in activity* - some assistance may be needed; no or minimal medical intervention/therapy required
Grade 3	**Severe**	Incapacitating with inability to work or do usual activity; Marked limitation in activity**, some assistance usually required; medical intervention/therapy required, hospitalisations possible
Grade 4	**Life-threatening**	Note: this must also be reported as Serious Adverse Event; Extreme limitation in activity, significant assistance

		required; significant medical intervention/therapy required, hospitalisation or hospice care probable
Grade 5	**Death**	Note: this must also be reported as Serious Adverse Event

***Includes limitation in instrumental activities of life: preparing meals, shopping for groceries or clothes etc**
****Includes limitation in self-care activities of life: bathing/showering, dressing/undressing, feeding self, using the toilet etc**

Serious or Life-Threatening AEs
ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

Causality assessment between study medication and AE

Afterwards the investigator has to decide a term for describing the relationship to the study drug:

Table 5: Relationship to Study Drug (Causality assessment)

Definite	Clear-cut temporal association, with a positive re-challenge test or laboratory confirmation; Event or laboratory test abnormality, with plausible time relationship to drug intake; Cannot be explained by disease or other drugs; Response to withdrawal plausible (pharmacologically, pathologically) event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon).
Probable	Clear-cut temporal association, with improvement upon drug withdrawal and not reasonably explained by the participant's known clinical state. Event or laboratory test abnormality, with reasonable time relationship to drug intake. Unlikely to be attributed to disease or other drugs response to withdrawal clinically reasonable.
Possible	Event or laboratory test abnormality, with reasonable time relationship to drug intake. Less clear temporal association, other aetiologies are possible. Could also be explained by disease or other drugs, other possible aetiologies should be recorded on the Source Document Information on drug withdrawal may be lacking or unclear.
None	No temporal association with the study drug; related to other aetiologies such as concomitant medications or conditions or subject's known clinical state. Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) Disease or other drugs provide plausible explanations.

The investigator will document the **taken action(s)** following identification of the adverse event:

- No action taken
- Study drug discontinued
- Patient withdrawn from study
- Concomitant medication required

- Hospitalisation required or prolonged (this should also be reported as a SAE)
- Other

The investigator will follow-up the adverse event until resolution or until no further medically relevant information can be expected. **Adverse event outcome** will be classified as follows:

- Resolved
- Resolved with sequelae
- Continuing (at the end of the study)
- Death
- Unknown (if patient is lost-to-follow-up and AE outcome is unknown)

Serious Adverse Event (SAE)

A SAE is an adverse event which:
• causes death or
• is life-threatening or
• necessitates or prolongs hospitalisation or
• results in persistent or significant disability/incapacity or
• is a congenital defect or malformation
• is another medically important event

A decision on medical and scientific grounds is required to assess whether an immediate notification of an event is warranted in other situations, such as medically important events which are not life-threatening, fatal or cause hospitalisation, but could endanger the patient or required an intervention to prevent one of the above conditions developing.

Remark: Examples of such events are intensive care in the emergency room or at home to treat a bronchospasm; convulsions not causing hospitalisation, or the development of drug addiction or drug abuse.

Monitoring of AEs (including SAEs)

The Site Principal Investigator (PI) will make a causality assessment. He should be informed about any new data on a participant for whom a causality link had already been established to reconsider its analysis and if necessary reassess the causality. Data shall only be modified following an official query procedure. Source Document review meetings shall be organised regularly, during which the site PI and the local study team shall review AE data (intensity, causality, and date of event manifestation in relation to the start of treatment). Safety data should be entered immediately in the eCRF.

A Medical Monitor will be available for the trial site to resolve arising safety related questions and uncertainties. He will oversee the medical aspects of the clinical trial and will actively collaborate with the sites. The medical monitor will have access to the eCRF and can address a request to the site PI concerned to add any further information needed for the safety analysis. The medical monitor will conduct the final review of the eCRF for any participants who have had an adverse event.

Obligation of AE and SAE notification

Adverse Events

The AEs, regardless of their seriousness and causal relationship to the study drug, arising between the first administration of study medication and the last study visit (as per the protocol), must all be recorded on the participant's Source Document (AE recording section). When possible, the symptoms must be regrouped within a single syndrome or diagnosis. The healthcare personnel shall have to specify the date of manifestation of the event, its intensity, final evolution, the measures taken and the treatment undertaken (if any).

Serious Adverse Events (SAEs)

In case of SAEs, the healthcare personnel must immediately contact the Investigator for validation of the seriousness and determination of the causality. Subsequently, the procedure described below must be followed, independent of causality:

- Send (within 24 hours of knowledge) the signed and dated copy of the "Adverse Event form" and the "SAE form" electronically to the medical monitor, sponsor, clinical monitor and the international study coordinator
- Contact immediately (the same day) the medical monitor, responsible for safety in case of death or life-threatening events.
- Inform the Ethics Committees of the occurrence of any SAE or if applicable as per local regulation.
- The follow-up of each fatal or life-threatening AE must be provided to the medical monitor, sponsor-coordinating PI, clinical monitor and the international study coordinator within the same timeline as the initial report (within 24 hours of knowledge and preferably by email).
- Attach to the Source Document the photocopy of all available results and examinations which were undertaken (and their date). Analysis results must be accompanied by the laboratory normal ranges. Special consideration shall be taken to ensure participant anonymity, and to the correct completion of the participant's study specific identifier in the copies of the source documents provided to the sponsor.

Emergency procedures

The site PI is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the study.

Follow-up of Adverse Events

The healthcare personnel must take all appropriate measures to protect the safety of the participants. Personnel must ensure to document follow-up of the evolution of each AE (clinical, biological or other) until resolution or until the stabilisation of the participant's status.
In case of a SAE the participant must be followed until complete resolution and normalization of all analysis results, or until chronicity of the participant's status. This can imply that the follow-up of the participant may continue beyond the period of follow-up per protocol, and that additional investigations could be requested by the sponsor.

All new relevant information concerning the initial SAE shall be recorded on the "SAE form" by the local study team, and shall be validated by the site PI/co-PI who shall transfer the form to the medical monitor, sponsor-coordinating PI, clinical monitor and the international study coordinator.

Procedures for unblinding in case of safety issues

Unblinding should not be performed carelessly, as it introduces bias. However, if it is medically imperative to know which trial medication the subject is receiving, the (blinded) principal investigator or blinded authorised person should unblind the treatment allocation. The (blinded) principal investigator or blinded authorised person should contact the unblinded pharmacist (or his delegate) and request information on the allocated treatment. Date, time and reason for unblinding need to be documented in the paper-based source document of the participant and the sponsor should be informed within 24 hours of unblinding.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

Upon receiving the SAE form, the sponsor and medical monitor will discuss and decide on the expectedness of the SAE. Any AE that is mentioned in the investigator brochure or summary of product characteristics (SmPCs) is considered 'expected'. An AE not mentioned in the investigator brochure or SmPC is considered 'unexpected'. A SUSAR is an SAE that is both 'unexpected' and at least 'possibly related' to the study medication (i.e. if there is no association to the study medication it is no SUSAR).

The Principal Investigator (PI) will report all SUSARs to the Ethics Committee (EC) within 7 calendar days in case of Fatal and life-threatening SUSARs and within 15 calendar days in case of SUSARs that are not fatal or life threatening.
The PI will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

Laboratory safety

When grading abnormal laboratory values the investigator needs to first judge if the abnormal lab value entails clinical significance. If this is the case (i.e. **abnormal lab value & clinical significance**) then routine grading shall be performed as explained above for clinical symptoms (by primarily using 'Table 3 Severity of Adverse Events'). In case the laboratory value is outside of local laboratory ranges, but **not clinically significant** then this shall not be documented as an adverse event.

Statistics

Data analysis will consist of descriptive summaries for treatment groups. The primary endpoint is log-transformed *S. haematobium egg* count reduction on D0 minus D42 (STUDY ARM PAIR I [PY/ART and PLACEBO]), as well as, time to re-infection with *S. haematobium* (STUDY ARM PAIR II [PY/ART/PZQ and PZQ]).

In STUDY ARM PAIR I [PY/ART and PLACEBO] this will be assessed for each participant and summarised as geometric mean including 95% Confidence Intervals. In case of non-parametric

data distribution medians and interquartile ranges (or ranges) will be used to summarise the primary endpoint distribution. T-test and Wilcoxon-rank sum test will be for hypothesis testing of parametric and non-parametric data, respectively.

In STUDY ARM PAIR II [PY/ART/PZQ and PZQ] this will be assessed for each participant and summarised as median time to re-infection including interquartile ranges (or ranges). In case of parametric data distribution, the mean time to re-infection including its 95% Confidence Intervals will be used to summarise the primary endpoint distribution. Kaplan-Meier plots may be used for visualisation of data. T-test, log-rank test, or Wilcoxon-rank sum test will be for hypothesis testing of parametric and non-parametric data, respectively. Poisson regression may be performed, if applicable.

To correct for multiplicity of endpoints the Hochberg procedure will be applied at the analysis stage of the trial.

For secondary endpoints, descriptive summaries and plots over the time course for both individual patient results and groups will be presented. Where appropriate numbers and percent of participants achieving the given endpoint will be presented per group. Where appropriate highly skewed data will be log-transformed and presented as geometric means with 95% confidence intervals. Time to event data will be described using the Kaplan-Meier method and if appropriate by Poisson regression models. Area under the curve (AUC) will be computed using the trapezoidal rule.

Quality control and quality assurance procedures

Monitoring

Monitoring will be performed according to ICH Good Clinical Practice (GCP) by a monitor who is independent from the study. The monitor will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigators will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

Medical monitor

A medical monitor, i.e. a clinician with experience in clinical trials who is permanently on site and is independent of the clinical trial team, will oversee participant safety.

Ethics

This study will be performed in accordance with the current revision of the Declaration of Helsinki and the latest versions of the ICH guidelines for Good Clinical Practice (GCP) and guidelines for Good Clinical Laboratory Practice (GCLP).

Informed Consent

Written, informed consent will be obtained from all study subjects prior to inclusion, as described above. This consent may be withdrawn by the study subject at any time, without being required to provide a reason.

Research Ethics Committee

This trial will be submitted for ethical review to the Institutional Review Board (Comité d'Ethique institutionel, CEI) of the Centre de Recherches Médicales de Lambaréné (CERMEL), which is registered with the Registration Office for Human Research Protections (OHRP) with the numbers: IORG0007336 / IRB00008812.

Participant Confidentiality

All data will be pseudonymised. Participant data will be identified by a unique study number in the database. Separate confidential files containing identifiable information will be stored in secured locations. Only the Sponsor representative, investigators, the clinical monitor, and the Institutional Review Board will have access to the records.

Data handling and record keeping

Data Handling

The PI or his designee will be the data manager with responsibility for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. All data will be recorded in case record forms. This includes safety data, laboratory data and outcome data.

Record Keeping

All files and source documents will be kept confidentially in locked safety cabinets. The PI, co-investigators and clinical research nurses will have access to records. The investigators will permit authorised representatives of the sponsor, regulatory agencies and the monitors to examine clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

Source Data and Case Report Forms (CRFs)

All protocol-required information will be collected in CRFs designed by the investigator. Source documents will be in paper form, and the data will be entered into the clinical trial software (e.g. RedCap). Source documents are original documents, data, and records from which the participant's CRF data are obtained. For this study these will include, but are not limited to; participant consent form, blood results, laboratory records and correspondence. In this study this will include, but is not limited to medical history, medication records, vital signs, physical

examination records, blood results, adverse event data and details of study interventions. All source data and participant CRFs will be stored securely.

Data Protection

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the sponsor.

Procedures for the collection, storage and future use of biological sample

All of the stored study research samples are labelled by a code that only the trial site can link to the subject. Samples are stored at the trial site in secure facilities with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data. Samples may be transferred to another facility in the purpose of analysis if the site is not able to analyse the sample for logistical reasons. Data will be archived in compliance with national and international guidelines.

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