Statistical Analysis Plan

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

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1. Introduction

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive description of the main, pre-planned analyses for the study "A proof-of-concept trial to evaluate artesunate-mefloquine as a novel alternative treatment for schistosomiasis in African children (SchistoSAM)". The primary objective of this study is to determine the safety and efficacy of a single course of Artesunate-Mefloquine (AM) compared to the standard Praziquantel (PZQ) regimen. The study conduct is described in the study protocol and in clinicaltrials.gov (NCT03893097).

These planned analyses will be performed by the statistician(s) at the Clinical Trials Unit of the Institute of Tropical Medicine (Antwerp) in collaboration with the research consortium. The analysis results will be described in a statistical analysis report, to be used as the basis of the main research publications according to the study publication plan. This document describes statistical methods for the primary and secondary outcomes of the study as defined by protocol. Additional analyses may be performed but are not covered by the current analysis plan. Statistical methods for these additional analyses will be described together with their respective results.

Analyses will be performed after the final evaluation of clinical efficacy on week 48 is completed. All data points including efficacy, safety, laboratory results, malaria-related results, medical assessment and others will be locked, analyzed and results will be submitted for publication.

This analysis plan will be finalized and approved before the database lock for the final analysis. Major changes in statistical methodology used for the main and pre-planned analyses from this SAP, will require detailed description and justification in the statistical analysis report. The final analysis datasets, programs, and outputs are archived following good clinical practice guidelines (ICH E9).

2. Study design and objectives

2.1. Study design

This is a non-inferiority, multi-center, randomized, two-arm, open label study among schistosomiasis-infected primary school-age children in northern Senegal. Prior to the first drug administration, all children fulfilling the study criteria will undergo a full parasitological and clinical assessment for schistosomiasis and malaria. Subsequently, schistosomiasis-infected children will be recruited and randomized (1:1) stratified by *Schistosoma* species to one of the two following study arms:

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

2.2. Study objectives

Primary objectives

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

Secondary objectives

The secondary objectives of the study are as follows:

a. To evaluate the cumulative efficacy of two additional courses of AM (at 6-week intervals each) for the treatment of schistosomiasis, compared to a single course of AM, and compared to the standard PZQ regimen (control arm).
b. To determine the parasitological efficacy of single and repeated courses of AM by infection intensity.
c. To assess the impact of repeated AM courses on schistosomiasis-related morbidity compared to baseline and to the control arm.
d. To determine the performance of novel schistosomiasis antigen- and DNA-based diagnostic assays as a tool for monitoring anti-schistosomal treatment response.
e. To determine the effect of repeated AM courses on prevalence of *P. falciparum* infection as well as on incidence and morbidity of clinical malaria.
f. To monitor the prevalence of *P. falciparum* molecular markers associated with mefloquine resistance and the potential emergence of reduced artesunate susceptibility.

Safety objectives
The safety objectives in this study are to evaluate the frequency and patterns of adverse events (AEs), serious adverse events (SAEs) and drug-related adverse events up to 4 weeks after both initial treatment administration of AM and PZQ (primary objective) and after the second and third AM administration (secondary objective).

2.3. Study hypothesis

Our primary research hypothesis is that the efficacy of one course of AM is at least similar to, and in case of repeated AM courses, higher than that of a standard PZQ treatment.

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

2.4. Variables of interest

Efficacy
Primary:

Parasitological cure rate (CR), as assessed by microscopy (according to WHO standards), after administration of PZQ and after one AM course (week 4).

Secondary:

1. CR, as assessed by microscopy, after administration of PZQ and after each AM course (at week 4, 10, 16, 24 and 48) per treatment arm, pooled and by *Schistosoma* species, and by infection intensity.
2. Cumulative CR as assessed by microscopy after each additional AM course (at week 10 and 16), pooled and by *Schistosoma* species, and by infection intensity.
3. ERR, as assessed by microscopy, after administration of PZQ and after each AM course (at week 4, 10, 16, 24 and 48) per treatment arm, by *Schistosoma* species and by infection intensity.
4. Prevalence and severity of general and organ-specific schistosomiasis morbidity (as assessed by clinical evaluation, ultrasound, point-of-care morbidity markers and hemoglobin level) at week 24 and week 48 compared to baseline and compared to the control arm. The characteristics that will be assessed are listed in detail in Section 2.5.

5. CR and intensity reduction rate (IRR), as assessed by the different novel diagnostic tests, after administration of PZQ and after each AM course, per treatment arm and by *Schistosoma* species. The diagnostic methods used for this objective as listed in detail in Section 2.5.
6. The diagnostic performance of the novel antigen- and DNA-based diagnostic tests, to monitor anti-schistosomal treatment response, as compared to microscopy.
7. Prevalence of *P. falciparum* infection, as assessed by molecular testing of dried blood spots at baseline and week 24 and 48.
8. Incidence of clinical malaria, as assessed by the number of malaria cases diagnosed through passive case detection during the study period by standard malaria rapid tests.
9. Frequency and severity of anemia, as assessed by determination of hemoglobin levels at baseline and at week 24 and 48 after initial treatment.
10. Prevalence and patterns of mutations in the K13 gene (for artemisinin susceptibility) and increased copy number of the Pfmdr1 gene or other relevant mutations (for mefloquine resistance) observed in the molecular surveys (baseline, week 24 and week 48).
11. Presence and patterns of mutations observed in incident malaria cases.

Safety:

Primary: - SAE's (including serious cases of malaria) up to 4 weeks after initial PZQ and AM administrations
- Drug-related AE's up to 4 weeks after initial PZQ and AM administrations
- All AEs (including malaria) up to 4 weeks after initial PZQ and AM administration

Secondary: - SAE's (including serious cases of malaria) up to 4 weeks after second and third AM administration
- Drug-related AE's up to 4 weeks after second and third AM administration
- All AEs (including malaria) up to 4 weeks after second and third AM administration
- All malaria cases

2.5. Definitions

Complete microscopy samples: At each visit 2 urine samples and 2 stool samples will be collected from each subject. A duplicate slide (slide A and B) will be prepared per stool sample, and one slide per urine sample. Urine and stool samples will provide results on the number of eggs for *Schistosoma haematobium and S. mansoni,* respectively Thus a total of 6 results are expected from each subject at each microscopy visit (2 urine for *S. haematobium,* 2 x 2 stool for *S. mansoni*). Thus, 6 microscopy results per participant will be defined as complete. Exceptionally, *S. haematobium* eggs can be found in stool and *S. mansoni* eggs in urine samples, but these will. will not be considered of primary interest for the analyses NB. The number of participants with ectopic eggs (*S. mansoni* eggs in urine samples and *S. haematobium* in stool samples) will be reported by study visit and site, but will not be included in any of the analyses described in this document.

Number of *Schistosoma* eggs: Two urine samples (10 ml each) and 2 stool samples (2 slides of 25 mg per sample) per subject will be analyzed for the presence of *Schistosoma* eggs. In the urine samples, the total number of *S. haematobium* eggs will be calculated as (number of eggs in sample 1 + number of eggs in sample 2). The final result will be presented as the number of eggs per 10 ml, thus the sum should be divided by 2. In the stool samples, the total number of *S. mansoni* eggs per subject will be calculated as the sum of the 4 slides (2 for each sample). The final result will be presented as number of eggs per gram, thus the sum should be multiplied by 10. Again, ectopic eggs will not be considered in the analyses.

Infection intensity:
The intensity of infection for the microscopy results will be categorized as light (1–99 eggs per gram (epg)), moderate (100–399 epg) and heavy (≥400 epg) for *S. mansoni* and as light (<50 eggs / 10 ml) and heavy (≥ 50 eggs / 10 ml) for *S. haematobium*, according to WHO criteria [1]. The intensity of infection for the POC-CCA results will be categorized as negative (G1), trace (G2-G3), light (G4-G5), moderate (G6-G7) and heavy (G8-G10) [2]. The PCR results will be categorized as undetected, low DNA load (35≤Ct<50), moderate DNA load (30≤Ct<35) and high DNA load (Ct<30) [3]. The UCP-LF-CAA will be categorized as negative (<1.5pg/ml), indecisive (1.5pg/ml ≤ CAA levels < 3pg/ml) and positive (CAA levels ≥ 3 pg/ml) [4].

Cure rate (CR): The proportion of egg-positive individuals, as assessed by urine and stool microscopy, who become egg-negative after treatment. A person will be considered negative for *S. mansoni* if no eggs are discovered in any of the 2 stool samples. Likewise, a person will be considered negative for *S. haematobium* if no eggs are discovered in any of the 2 urine samples. A pooled cure rate will be calculated as the proportion of persons who are negative for both *Schistosoma* species.
A best and a worst-case scenario will be considered in case of missing samples, as described later in the document. In the best-case scenario, all missing samples will be considered negative, while in the worst-case scenario, all missing samples will be considered positive.
NOTE: Missing samples at the different time points will be removed from the numerator and the denominator in the CR calculations.

Cumulative cure rate (CCR): The proportion of egg-positive individuals, as assessed by urine and stool microscopy, who become egg-negative after repeated treatment administrations. For each additional treatment round i (i= 2 or 3) the CCR will be:

$$CCR_i = \frac{\#\ cured\ at\ round\ 1 + \cdots + \#\ cured\ at\ round\ i}{\#\ schisto\ positive\ at\ baseline}$$

Subjects who became positive for schistosomiasis will be excluded both from the numerator and the denominator of the CCR formula for all the time they become positive and beyond. Likewise, subjects with missing microscopy results will be also excluded from the calculations at the time points where results are missing.

Egg-reduction rate (ERR) and Intensity reduction rate (IRR): The ERR will be calculated using the following formula:

$$ERR_i = 100\ x\ (1 - \frac{mean\ egg\ counts\ at\ visit\ i}{mean\ egg\ counts\ at\ baseline})$$

$$\text{IRR(POC CCA)}_i = 100\ x\ (1 - \frac{mean\ G\ score\ at\ visit\ i}{mean\ G\ score\ at\ baseline})$$

$$\text{IRR(UCP LF CAA)}_i = 100\ x\ (1 - \frac{mean\ CAA\ levels\ at\ visit\ i}{mean\ CAA\ levels\ at\ baseline})$$

The ERR calculations will be done separately for each *Schistosoma* species, POC-CCA for *S.mansoni* only and UCP LF CAA and PCR for any *Schistosoma* species. The formula for calculating the IRR for PCR needs to be further investigated and will be included in the statistical analysis report. In the last years there has been an increasing number of published papers reporting and comparing both arithmetic and geometric means [5–10]. Accordingly, all ERR-related results will be calculated using both the arithmetic and the geometric means. In case of many incomplete microscopy samples, two versions of ERR estimates will be calculated and reported; the first version will only include subjects with complete samples in the calculations, and the second, will include all subjects using all available samples in the calculations.

Malaria:

P. falciparum infection prevalence (baseline, week 24, week 48) will be calculated using the results of the qPCR on the collected dried-blood spot samples.

Positive results in standard malaria rapid tests in the health post in the village together with reporting of fever as a symptom will be considered as clinical malaria cases. In cases where a participant has reported taking a diagnostic test for malaria, but the test result is not reported or is unknown, then those cases will be included in the denominator of the prevalence calculation but not as positive cases. A worst-case-scenario for malaria prevalence will be calculated considering those cases as positive.

Timing of visits:

Treatment administration visits: According to the protocol the three days of the AM administration should be completed within 5 calendar days.

Parasitological evaluation visits: A time window of 7 days will be allowed for all evaluation visits (week 4, week 10, week 16, week 24 and week 48) using the baseline or the first day (Day 0) of the previous treatment administration round as a reference.

Parasitological sample collection: A time window of 7 days will be allowed between collection of 2 respective stool or urine samples at each visit (week 4, week 10, week 16, week 24 and week 48). The preparation dates for stool and urine samples will be used for this comparison.

Schistosomiasis-related morbidity markers:

Morbidity predictors have been identified for either *Schistosoma* species according to the guidelines described in [11,12]. All markers listed below will be presented as they were recorded in the study's CRFs, but alternative presentations (recoding, apply cut-off points, etc) might also be used to present the results.

1. Abdominal examination: Hepatomegaly (Absent/Present)
2. Abdominal examination: Splenomegaly (Absent/Present)
3. Abdominal examination: Collateral veins (Absent/Present)
4. Abdominal examination: Ascites (Absent/Present)
5. Hemoglobin measurement (both as continuous variable and categorized as anemia: Hemoglobin result < 11,5 mg/dL for children aged 6-11years old or < 12 mg/dL for children aged 12-14 years old)
6. Ultrasound: Visualized colon (Yes/No)
7. Ultrasounds: Colon walls > 5 mm (Yes/No)
8. Ultrasound: Collateral vessels (Yes/No)
9. Ultrasound: Ascites (Yes/No)

10. Ultrasound: Spleen length (continuous in cm)
11. Ultrasound: Spleen thickness (continuous in cm)
12. Ultrasound: Spleen echogenicity (Normal/Hypoechogene/Hyperechogene)
13. Ultrasound: Liver PSL (Left parasternal line) (continuous in cm)
14. Ultrasound: Liver MCL (Right midclavicular line) (continuous in cm)
15. Ultrasound: Liver portal vein diameter (continuous in mm)
16. Ultrasound: Liver preferential image pattern (A,B0, B1, B2, C, D, Dc, E, Ec, F)
17. Ultrasound: Liver alternative image pattern (A,B0, B1, B2, C, D, Dc, E, Ec, F)
18. Ultrasound: Liver: other hepatic pathologies (cirrhotic appearance, steatotic appearance, other hepatic pathologies)
19. Ultrasound: Gallbladder wall size (<4mm, ≥ 4mm)
20. Ultrasound: Gallbladder wall protrusions (Yes/No)
21. Ultrasound: Gallbladder sludge content/stones (Yes/No)
22. Ultrasound: Gallbladder bed fibrosis (Yes/No)
23. Ultrasound: Gallbladder pain on palpation (Yes/No)
24. Hemoglobin (both as continuous variable and categorized as anemia: Hemoglobin result < 11,5 mg/dL for children aged 6-11years old or < 12 mg/dL for children aged 12-14 years old)
25. Ultrasound: Bladder shape (normal/abnormal)
26. Ultrasound: Bladder wall irregularities (No/Focal/Multifocal)
27. Ultrasound: Bladder wall thickness (Normal/Focal/Multifocal)
28. Ultrasound: Bladder masses > 10 mm (Absent/One/Multiple)
29. Ultrasound: Bladder number of masses (continuous)
30. Ultrasound: Bladder pseudo-polyps (Absent/Present)
31. Ultrasound: Bladder number of pseudo-polyps (continuous)
32. Ultrasound: Bladder contents (Absent/Present)
33. Ultrasound: Bladder type (open field)
34. Ultrasound: Left ureter (Non visualized/partially dilated/completely dilated/irregularities)
35. Ultrasound: Left renal pelvis (Without fractures/Fracture 2-5mm, Fracture 5-10 mm, Parenchyma > 1 cm, hydronephrosis)
36. Ultrasound: Right ureter (Non visualized/partially dilated/completely dilated/irregularities)
37. Ultrasound: Right renal pelvis (Without fractures/Fracture 2-5mm, Fracture 5-10 mm, Parenchyma > 1 cm, hydronephrosis)
38. Urine strips: Blood (0, 1+, 2+, 3+, 4+)
39. Urine strips: Proteins (0, 1+, 2+, 3+)
40. Urine strips: Nitrites (0, +)
41. Urine strips: Leucocytes (0, 1+, 2+, 3+)
42. Urine strips: Glucose (0, 1+, 2+, 3+, 4+)
43. Fecal occult blood test result (Positive/Trace/Negative)
44. Symptoms: Abdominal pain (No/Light/Moderate/Severe)
45. Symptoms: Blood in stool (No/Light/Moderate/Severe)
46. Symptoms: Fatigue (No/Light/Moderate/Severe)
47. Symptoms: Dysuria (No/Light/Moderate/Severe)
48. Symptoms: Hematemesis (No/Light/Moderate/Severe)
49. Symptoms: Hematuria (No/Light/Moderate/Severe)
50. Symptoms: Urinary urgency (No/Light/Moderate/Severe)
51. Symptoms: Urinary incontinence (No/Light/Moderate/Severe)
52. Symptoms: Perineal pain (No/Light/Moderate/Severe)
53. Symptoms: Backache (No/Light/Moderate/Severe)

Additional/novel diagnostic tests:
The diagnostic tests that will be used for schistosomiasis diagnosis according to the protocol, including a cut-off for determination of a positive schistosomiasis case, are described below.

Table 1: Table of Schistosoma diagnostic tests used in the study

Test	Species	Sample	Units	Schistosomiasis positive if
POC-CCA	*S. mansoni*	Urine	G1 - G10	G4-G10 (G2-G3 = trace)
UCP-LF CAA	*S. mansoni* *S. haematobium* combined	Urine	t/FC line ratio	This will be filled in at a later stage
ITS2-based PCR	*S. mansoni* *S. haematobium*	Urine/Stool	cycle-threshold (C_t) value	≤ 50

The results of microscopy for all urine and stool samples will be coded as a binary variable for positive/negative result as described above. For the other novel diagnostic tests described in the Table 1, binary variables will be created as described above, as well as variables for the infection intensity. For the POC-CCA, two indicator variables will be used, treating the trace results as negative and as positive respectively.

Patterns of mutation:

Among the varATS qPCR positive samples obtained by qPCR on the dried blood spot of the participants, genetic mutations described to be associated with artemisinin and/or mefloquine resistance will be investigated.
The pfmdr1 gene, a marker for mefloquine/piperaquine resistance, will be PCR amplified and sanger sequenced to analyze the presence of SNPs at amino acid positions 86, 184 and 1246. Sample isolates are reported as mutated if the presence of mutations 86Y or 184F or 1246Y are found in single or mixed clone infections. Haplotypes are defined as NFD or NFY or NYY or YYD or YFD or YFY or YYY.

Mutations in the kelch propeller domain (k13 gene) are associated with artemisinin resistance. Kelch propeller domain of the k13 gene will be sanger sequenced and single nucleotide polymorphism (SNP) mutations will be reported if they are found in single or mixed clone infections.

2.6. Data review meeting

During the different visits of the study the weight of subjects is measured to calculate the correct dose for treatment administration. A data review meeting will be held before database lock to review, discuss and decide whether special cases should be included in the final dataset and the analyses, based on a review of the patient data, blinded to treatment allocation. Any other discrepancies in study outcomes will also be discussed during the data review meeting, such as, but not limited to missing samples, ectopic eggs, time window between visits or tests, etc.

3. Description of study population

3.1. Participant accounting

The number of participants screened, those who meet the study inclusion criteria and enrolled (randomized) or excluded will be summarized according to reason for exclusion. Of the enrollees, the number of participants discontinued or lost to follow-up will be recorded by reason and time of discontinuation. These figures will be summarized in a CONSORT flow diagram. A table summarizing the enrolment process by site and treatment arm will also be produced (Example Table 1a and 1b).

3.2. Description of study population

Participants in each treatment group and overall will be described with respect to selected baseline characteristics. The description will be in terms of medians and interquartile ranges for continuous characteristics and using counts and percentages for categorical characteristics. Differences in each characteristic will be noted, but no formal statistical tests will be used. (Example Table 2)

4. Description of analysis populations

4.1. Analysis populations

For the efficacy analysis, both an intention-to-treat (ITT) and a per-protocol (PP) approach will be adopted. The PP approach assesses the non-inferiority of the efficacy of the different treatment arms, including only the subjects who comply with the procedures as described in the study protocol. The PP analysis will be the primary analysis approach, as recommended for non-inferiority studies. An ITT analysis will also be performed, in accordance with ICH guidelines and to assess if both approaches lead to similar conclusions. For the safety analyses the as-treated approach will be used.

4.1.1. Intention to treat (ITT) analysis

The intention-to-treat provides a pragmatic comparison of the two treatments, taking into account the effects of non-compliance to protocol guidelines, wrong treatment allocation, low adherence, treatment interruption, etc. These analyses will be conducted on all patients assigned to the treatment groups as randomized, regardless of the study treatment or non-study treatment received. Randomized subjects who are not found to be schistosomiasis-positive at recruitment due to delays in the microscopy results or other reasons will be excluded from the ITT analysis and will be classified as screening failures.

4.1.2. Per protocol (PP) analysis

An analysis based on a "per protocol" approach will be conducted to assess the non-inferiority of treatments, as recommended for non-inferiority studies. Thus, analyses based on the subjects following the study protocol and will be the main strategy of analysis adopted for the primary and secondary endpoints. In Table 2 the protocol violations are classified as minor and major; minor violations will be included in the PP analysis population and major violations are excluded. The participant accounting for the analysis populations is described in Example Table 3.

Table 2: The protocol deviations classified as minor or major deviation

Protocol Violation	Major/Minor Deviation	Comments

Inclusion criteria		
1. Age: 6 – 14 years	Major	
2. Enrolled in one of the selected primary schools in the district of Richard Toll	Major	
3. Infected with schistosomiasis (i.e., presence of *Schistosoma* spp. eggs in urine and/or stool)	Major	
4. Informed consent from parents/guardians signed	Major	
Exclusion criteria		
1. Planned travel of more than 1 month within the first 4 months after enrolment	Major	
2. Ongoing epilepsy or history of epilepsy or repeated non-febrile seizures	Major	
3. History of psychiatric illness (depression, generalized anxiety, psychosis, schizophrenia or other major psychiatric disorders)	Major	
4. History of known allergy to any of the three study drugs (praziquantel, mefloquine, artesunate / artemether)	Major	
5. Chronic medication for any reason	Major	
6. Severe malnutrition (BMI <3DS of WHO standards	Major	
7. Clinical signs of severe portal hypertension (ascites and / or collateral circulation)	Major	
8. Any underlying serious illness based on clinical judgment	Major	
9. Hemoglobin level <7 g / dl (HemoCue)	Major	
10. Any febrile illness documented in the previous 2 days or during the assessment	Major	
11. Exposure to PZQ (praziquantel) or ACT (artemisinin combination therapy, ie, antimalarial treatment, within the last 3 months)	Major	
Treatment deviations		
1. Missing at least a full day of treatment (day 0, 1, or 2) at any of the 3 treatment administration visits	Major/Minor	Major if at least 2 out of 3 treatment administrations are missed. Minor, if only one of the 3 days (day 0, 1 or 2) is missed
2. Not taken the randomized treatment.	Major	
3. Intake of PZQ or ACT between the baseline visit and study visit on week 48.	Major	A physician will review all the treatments in order to identify potential schistosomiasis-related treatments.
4. Repeated vomiting of allocated treatment	Major	If vomiting occurs on the first AM administration, the subject will only be included in the ITT analysis. If the vomiting occurs in subsequent AM administrations, then all results prior to that time point will be used in the PP analysis and all the ones following that time point will be included in the ITT analysis.
5. Discontinued treatment	Major	
6. Treatment administration completed in more than 5 days	Major	

7. Dosage miscalculation	Minor/Major	Major if administered dose was lower, minor if administered dose was larger than target dose
Follow-up deviations		
1. Missing stool/urine samples completely or partially	Major	
2. Evaluation visits done outside a ± 7-day time window.	Major	Time between evaluation visits (week 4, week 10, week 16, week 24 and week 48) will be calculated using the baseline or the first day (Day 0) of the previous treatment administration round as a reference.
3. Parasitological samples analyzed in more than 7 days	Minor/Major	Major if there is a difference of 15 days or more between the two samples of urine and stool for all visits except recruitment.

4.1.3. Safety (As-treated) analysis

For the analysis of safety outcomes, all participants who effectively received any study drug (i.e. at least one treatment dose) are included in the safety analysis of the treatment group that corresponds with the treatment received ('as-treated' approach). This means that patients having vomited the first dose (day 0 of first treatment administration) and having had their treatment discontinued will not be included in this analysis.

5. Statistical Methods

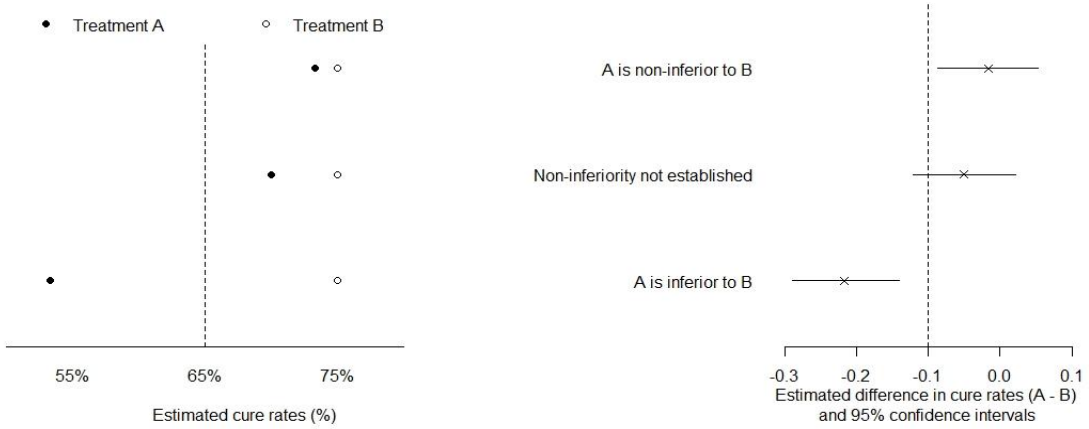
5.1. Primary efficacy analysis

The primary analysis of the study will be the assessment of clinical non-inferiority of a single AM treatment for the treatment of schistosomiasis compared to the standard PZQ regimen at week 4 of the study. The primary hypothesis will be assessed using the difference in cure rates (proportion of cured subjects) between the two treatment arms. If the difference in cure rates is less than 10 percentage points, the two treatments are therapeutically equivalent (i.e.: the single-course AM treatment is clinically non-inferior to the standard-of-care PZQ treatment). If the difference in cure rates is 10 percentage points or larger, then one treatment is clinically inferior. This analysis will be done for the two *Schistosoma* species separately and pooled (both species together), since *Schistosoma* species was used as a stratum in the randomization. All three results (pooled and per species comparisons) will be considered primary analysis results (Example Table 4).

Assessment of the difference in cure rates will be performed by calculating the two-sided 95% confidence interval (CI), using the Wilson's score method, for the difference in proportions of cured subjects (AM schedule - PZQ schedule) using the following decision rule (see also figure 1):

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

Figure 1: Graphical presentation of decision rule for clinical non-inferiority.



The decision rules use two-sided 95% confidence intervals (or equivalently one-sided 97.5% confidence intervals) to be consistent with the amount of evidence required in superiority trials as recommended in the Consort statement on non-inferiority trials (CONSORT - Reporting of Non-inferiority and Equivalence Randomized Trials: www.consort-statement.org) and ICH guidelines.
In the PP analysis only subjects with complete samples will be included for the calculation of the CR. In the ITT analysis, CR will be calculated using subjects with complete samples only (1) and subjects with either complete or incomplete samples (2). These two versions of the estimates will be compared to the estimate from the PP analysis.

5.2. Secondary objectives

Efficacy over time
The cumulative efficacy of two additional courses of AM (at 6-week intervals each) for the treatment of schistosomiasis, compared to a single course of AM, and compared to the standard PZQ regimen (control arm) will be assessed by calculating the two-sided 95% CI for the CR and for the CR difference at different time points (at week 10, 16, 24 and 48). The comparisons at each time point will be performed in a similar way as the primary endpoint (per species and pooled), but no pre-specified margin will be used for formal inference. Estimation of the CR for the PP analysis population (complete samples only) and for the ITT population (subjects with complete and subjects with any available samples) will be done similar to the primary efficacy analysis (Example Table 4 and Example Table 5).

Additionally, mixed-effects logistic regression models will be fitted, one for each *Schistosoma* species, using schistosomiasis infection at the specific time points as the outcome, treatment and visit as independent variables and a random intercept for subjects. An interaction term between treatment and visit will be examined. Subsequent analyses of the cure rate by *Schistosoma* infection intensity will be analyzed in a similar way including infection intensity as a categorical predictor in the model. Possible statistically significant interaction terms between treatment arm, infection intensity and visit will be examined. Results of these regression models will be presented as in Example Table 7.

Estimation of the ERR will be done using the formula described in Section 2.5 and the confidence intervals (CIs) will be calculated by using a bootstrap resampling method (with replacement) over 1,000 replicates and expressed as a univariate calculation of the 2.5th and 97.5th percentiles. As for the CR, only subjects with complete samples will be used in the calculation in the PP analysis. In the ITT analysis both subjects with complete samples only and subjects with either complete or incomplete samples will be included in the ITT analysis (2 separate estimations) (Example Table 6). The determination of ERR after single and repeated courses of AM compared to the standard PZQ regimen will be done for each *Schistosoma* species separately, using generalized linear mixed effects models with the average number of eggs as an outcome and a random intercept. Treatment group and visit will be the categorical fixed-effects covariates in the model, and a potential interaction effect between treatment and visit will be examined. Subsequent analyses of the ERR by *Schistosoma* infection intensity will be analyzed in a similar way including infection intensity as a categorical covariate in the model. Possible statistically significant interaction terms between treatment arm, infection intensity and visit will be examined (Example Table 7).

Schistosomiasis-related morbidity

All comparison of proportions will be done using the Chi-square or Fisher's exact test and comparison of continuous characteristics will be done using the Wilcoxon rank sum test. Comparisons will be done by *Schistosoma* species for each schistosomiasis-related endpoint as they are defined in sections 2.4 and 2.5. Results will be presented as in Example Tables 8.

Diagnostic tests

The CR and IRR per species will be estimated based on the results of each of the novel diagnostic tests together with a 95% confidence interval. (Example Table 9).

The objective of diagnostic accuracy of the various novel tests, based on the construction of composite reference standards and latency class analysis, as mentioned in the protocol, will not be a part of the current SAP. The methodology of these analyses will be described in a different document and the results will not be included in the study Statistical Analysis Report (SAR).

Malaria related endpoints

The estimate of prevalence of malaria at baseline and at weeks 24 and 48 will be calculated using standard formulas together with 95% CIs pooled and by treatment arm. Similarly, incidence of clinical malaria with 95% CI will be calculated at specific time points dividing the new cases of malaria over the person-time under study. Frequency and severity of anemia, as assessed by determination of hemoglobin levels, will be estimated as proportions with 95% CIs at baseline and at week 24 and 48 after initial treatment. No formal statistical comparison will be done for these endpoints (Example Table 10).

Molecular markers for antimalarial resistance

The prevalence of mutations related to mefloquine and artemisinin resistance will be estimated as a proportion over all available blood samples at each time point (baseline, week 24 and 48) with a 95%

CI. Patterns of resistant mutations will be reported similarly. Comparison between the two treatment groups will be done using Chi-square or Fisher's exact test at each time point (Example Table 11).

5.3. Safety objectives

General aspects

Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MEDDRA) and will be reported based on MEDDRA preferred terms and body systems (Example Table 12). All AEs will be analyzed based on counts of subjects with a specific category and not on counts of individual adverse events. The relationship between AEs and treatment is determined by the investigator and categorized as "drug-related" if possibly, probably or definitely related to treatment.

Malaria will be reported separate as an AE, even though it is also a secondary endpoint. Cases of malaria which meet the criteria of serious adverse event will be reported together with the other serious adverse events. Subjects who took the treatment of both treatment arms within or outside the study will be excluded from the safety analyses and their safety results will be described separately.

Primary analysis

The primary safety analysis on the frequency and pattern of (S)AEs between the two groups on week 4 will be assessed using patient counts and percentages with 95% CIs and will be compared using Fisher's exact test. The following categories of safety endpoints will be evaluated in the primary analysis (Example Table 13):

a. description of any deaths occurring during data collection
b. the total number of subjects with any serious AE
c. the total number of subjects with any drug-related SAE
d. the total number of subjects with AE
e. the total number of subjects with any drug-related AE
f. the total number of subjects with confirmed malaria infections

Secondary analyses

The primary objective at week 4 will additionally be evaluated using logistic regression with a binary (0/1) variable as an outcome for the absence/presence of AEs and the treatment group as the only covariate (Example Table 14). The secondary objective for the difference between repeated courses of AM (at 6 weeks intervals) and a single dose of PZQ will be evaluated using Poisson regression models, with a binary variable for each person in the study who has received at least one dose of treatment medication, the number of doses taken (1-9 doses) as an offset and the treatment arm (AM or PZQ) as the only covariate. This model will compare the safety outcomes between treatments adjusting per dose of treatment. In total 2 models will be created, one for each additional course of AM (Example Table 15).

The secondary objective for comparing the safety of two-additional courses of AM (at 6-week intervals) compared to a single course of AM will be assessed using Poisson mixed-effects regression models with a binary variable for each subject in the study who has received at least one dose of treatment medication indicating the presence or absence of AEs, the number of treatment rounds they received as the only covariate and the subjects as random intercepts. The effect of the number of the doses taken (1-9) as an offset term, will be examined. Only the subjects that received the AM arm will be included in this model (Example Table 16).

Exploratory analyses

In all regression models described in the secondary objectives above, weight, age, gender and anemia will be added in the models in order to examine how adjusting for those characteristics affect the treatment effect in safety outcomes. Potential statistically significant interaction terms between treatment and the predictors will be examined.

5.4. Other aspects of Statistical Methods

5.4.1. Subgroup analyses

No differences in the endpoints are expected due to the different villages in the study or the fieldwork teams who did the data collection. Thus, no subgroup analyses have been planned, except for the two *Schistosoma* species.

5.4.2. Other aspects

a. Analysis strategy
For the main efficacy and safety analyses the data from all sites will be pooled and analyzed together.

b. Multiplicity adjustment

Non-inferiority will be established using 95% two-sided confidence intervals. No adjustment for multiplicity is performed as the focus of the study is on individual treatment comparisons.

c. Missing data and sensitivity analysis

The number of missing values will be reported together with the descriptive statistics of the study. Sensitivity analyses will be performed in case of missing data in microscopy samples as following:

For CR, a best-case scenario will be used, treating all missing samples as schistosomiasis-negative (Example Table 4).

For CR, a worst-case scenario will be used, treating all missing cases as schistosomiasis positive (Example Table 4).

For all sensitivity analyses, the results will be compared to the ITT estimations from the CR and ERR. For all other data points, we will use an available-case approach. No statistical methods will be used to retrieve the missing values, like multiple imputation.

d. Exploratory analysis
A potential effect among the different lots/brands of medication will be examined in case different lots/brands of medication are used during the study. The lot/brand will be included in the efficacy analysis as an extra covariate in the model.

6. Example Tables and Figures

Example Table 1a: Patient accounting by site

	Total n (%)	Colona n (%)	Gnith n (%)	Nder n (%)	Pakh n (%)	Ronkh n (%)	Yetti-Yone n (%)
Screened	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Screening failures	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Enrolled in the study	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Did not complete visit at week 4	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Lost to Follow Up	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Withdrawal of Consent	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Subject Withdrawn by investigator or due to Adverse Events, development of illness, or chronic medication	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Vomiting of treatment	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Other	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Completed visit at week 4	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Did not complete visit at week 10	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Lost to Follow Up	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Withdrawal of Consent	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Subject Withdrawn by investigator or due to Adverse Events, development of illness, or chronic medication	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Vomiting of treatment	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Other	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Completed visit at week 10	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Did not complete visit at week 16	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Lost to Follow Up	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Withdrawal of Consent	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Subject Withdrawn by investigator or due to Adverse Events, development of illness, or chronic medication	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Vomiting of treatment	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
- Other (Removed from study due to negative or missing sample at baseline)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)
Completed visit at week 16	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)	xx (xx)

Example Table 1b: Patient accounting by treatment

	Total n (%)	AM n (%)	PZQ n (%)
Screened	xx (xx)	xx (xx)	xx (xx)
Screening failures	xx (xx)	xx (xx)	xx (xx)
Enrolled in the study	xx (xx)	xx (xx)	xx (xx)
Did not complete visit at week 4	xx (xx)	xx (xx)	xx (xx)
- Lost to Follow Up	xx (xx)	xx (xx)	xx (xx)
- Withdrawal of Consent	xx (xx)	xx (xx)	xx (xx)
- Subject Withdrawn by investigator or due to Adverse Events, development of illness, or chronic medication	xx (xx)	xx (xx)	xx (xx)
- Vomiting of treatment	xx (xx)	xx (xx)	xx (xx)
- Other	xx (xx)	xx (xx)	xx (xx)
Completed visit at week 4	xx (xx)	xx (xx)	xx (xx)
Did not complete visit at week 10	xx (xx)	xx (xx)	xx (xx)
- Lost to Follow Up	xx (xx)	xx (xx)	xx (xx)
- Withdrawal of Consent	xx (xx)	xx (xx)	xx (xx)
- Subject Withdrawn by investigator or due to Adverse Events, development of illness, or chronic medication	xx (xx)	xx (xx)	xx (xx)
- Vomiting of treatment	xx (xx)	xx (xx)	xx (xx)
- Other	xx (xx)	xx (xx)	xx (xx)
Completed visit at week 10	xx (xx)	xx (xx)	xx (xx)
Did not complete visit at week 16	xx (xx)	xx (xx)	xx (xx)
- Lost to Follow Up	xx (xx)	xx (xx)	xx (xx)
- Withdrawal of Consent	xx (xx)	xx (xx)	xx (xx)
- Subject Withdrawn by investigator or due to Adverse Events, development of illness, or chronic medication	xx (xx)	xx (xx)	xx (xx)
- Vomiting of treatment	xx (xx)	xx (xx)	xx (xx)
- Other (Removed from study due to negative or missing sample at baseline)	xx (xx)	xx (xx)	xx (xx)
Completed visit at week 16	xx (xx)	xx (xx)	xx (xx)

Example Table 2: Baseline characteristics

	Total n (%)/ Median (IQR)	AM n (%)/ Median (IQR)	PZQ n (%)/ Median (IQR)
Total randomized	xx (xx)	xx (xx)	xx (xx)
Gender	xx (xx)	xx (xx)	xx (xx)
- Female	xx (xx)	xx (xx)	xx (xx)
- Male	xx (xx)	xx (xx)	xx (xx)
Age (years)	xx (xx)	xx (xx)	xx (xx)
Height (cm)	xx (xx)	xx (xx)	xx (xx)
Weight (kg)	xx (xx)	xx (xx)	xx (xx)
Schistosoma species	xx (xx)	xx (xx)	xx (xx)
- Negative	xx (xx)	xx (xx)	xx (xx)
- Single *S. mansoni* infection	xx (xx)	xx (xx)	xx (xx)
- Single *S. haematobium* infection	xx (xx)	xx (xx)	xx (xx)
- Mixed infection	xx (xx)	xx (xx)	xx (xx)

Example Table 3: Analysis population

	Total n (%)	AM n (%)	PZQ n (%)
Screened	xx (xx)	xx (xx)	xx (xx)
Screening failures	xx (xx)	xx (xx)	xx (xx)
Excluded from ITT analysis – Week 4	xx (xx)	xx (xx)	xx (xx)
Included in the ITT analysis – Week 4	xx (xx)	xx (xx)	xx (xx)
Excluded from PP analysis – Week 4	xx (xx)	xx (xx)	xx (xx)
- Deviation of inclusion/exclusion criteria	xx (xx)	xx (xx)	xx (xx)
- Missed treatment	xx (xx)	xx (xx)	xx (xx)
- Not taken the randomized treatment	xx (xx)	xx (xx)	xx (xx)
- Intake of PZQ and AM	xx (xx)	xx (xx)	xx (xx)
- Repeated vomiting	xx (xx)	xx (xx)	xx (xx)
- Discontinued treatment	xx (xx)	xx (xx)	xx (xx)
- Treatment administration outside time window	xx (xx)	xx (xx)	xx (xx)
- Sample analysis outside time window	xx (xx)	xx (xx)	xx (xx)
- Dosage miscalculation	xx (xx)	xx (xx)	xx (xx)
- Missing stool/urine samples	xx (xx)	xx (xx)	xx (xx)
Included in PP analysis – Week 4	xx (xx)	xx (xx)	xx (xx)
Excluded from ITT analysis – Week 10	xx (xx)	xx (xx)	xx (xx)
Included in the ITT analysis – Week 10	xx (xx)	xx (xx)	xx (xx)
Excluded from PP analysis – Week 10	xx (xx)	xx (xx)	xx (xx)
- Deviation of inclusion/exclusion criteria	xx (xx)	xx (xx)	xx (xx)
- Missed treatment	xx (xx)	xx (xx)	xx (xx)
- Not taken the randomized treatment	xx (xx)	xx (xx)	xx (xx)
- Intake of PZQ and AM	xx (xx)	xx (xx)	xx (xx)
- Repeated vomiting	xx (xx)	xx (xx)	xx (xx)
- Discontinued treatment	xx (xx)	xx (xx)	xx (xx)
- Treatment administration outside time window	xx (xx)	xx (xx)	xx (xx)
- Sample analysis outside time window	xx (xx)	xx (xx)	xx (xx)
- Dosage miscalculation	xx (xx)	xx (xx)	xx (xx)
- Missing stool/urine samples	xx (xx)	xx (xx)	xx (xx)
Included in PP analysis – Week 10	xx (xx)	xx (xx)	xx (xx)
Excluded from ITT analysis – Week 16	xx (xx)	xx (xx)	xx (xx)
Included in the ITT analysis – Week 16	xx (xx)	xx (xx)	xx (xx)
Excluded from PP analysis – Week 16	xx (xx)	xx (xx)	xx (xx)
- Deviation of inclusion/exclusion criteria	xx (xx)	xx (xx)	xx (xx)
- Missed treatment	xx (xx)	xx (xx)	xx (xx)
- Not taken the randomized treatment	xx (xx)	xx (xx)	xx (xx)
- Intake of PZQ and AM	xx (xx)	xx (xx)	xx (xx)
- Repeated vomiting	xx (xx)	xx (xx)	xx (xx)
- Discontinued treatment	xx (xx)	xx (xx)	xx (xx)
- Treatment administration outside time window	xx (xx)	xx (xx)	xx (xx)
- Sample analysis outside time window	xx (xx)	xx (xx)	xx (xx)
- Dosage miscalculation	xx (xx)	xx (xx)	xx (xx)
- Missing stool/urine samples	xx (xx)	xx (xx)	xx (xx)
Included in PP analysis – Week 16	xx (xx)	xx (xx)	xx (xx)

Example Table 4: Efficacy analysis: Primary and secondary analysis of CR estimation and comparison to the control arm using microscopy results

		Treatment arm		
Visit	Species	AM Cured (CR; 95% CI)	PZQ Cured (CR; 95% CI)	Difference: AM - PZQ (95% CI)
Week 4	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 10	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 16	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 24	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 48	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)

Note: This table will be presented in total 5 times: for the PP analysis population, for the ITT analysis population with complete samples, for the ITT analysis population with all available samples and for the best- and worst-case scenarios. An additional version of the table using the cumulative CR will also be presented (PP and ITT populations).

Example Table 5: Efficacy analysis: Secondary analysis of CR estimation and comparison to a single dose of AM using microscopy results.

Visit	Species	AM Cured (CR; 95% CI)	AM week 4 (AMw4) Cured (CR; 95% CI)	Difference: AM – AMw4 (95% CI)
Week 10	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 16	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 24	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 48	Haematobium	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Mansoni	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
	Pooled	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)

Note: This table will be presented in total 5 times: for the PP analysis population, for the ITT analysis population with complete samples, for the ITT analysis population with all available samples and for the best- and worst-case scenarios. An additional version of the table using the cumulative CR will also be presented (PP and ITT populations).

Example Table 6: Efficacy analysis: Secondary analysis of ERR estimation using microscopy results.

Visit	Species	AM ERR (95% CI)	PZQ ERR (95% CI)
Arithmetic mean			
Week 4	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 10	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 16	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 24	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 48	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Geometric mean			
Week 4	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 10	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 16	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 24	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)
Week 48	Haematobium	xx (xx – xx)	xx (xx – xx)
	Mansoni	xx (xx – xx)	xx (xx – xx)

Note: This table will be presented in total 3 times: for the PP analysis population, for the ITT analysis population with complete samples and for the ITT analysis population with all available samples.

Example Table 7: Secondary analyses of treatment efficacy - regression models

Covariates	Levels	Estimate (95% CI)	p-value
CR			
Treatment	PZQ	1 (Reference)	
	AM	xx (xx – xx)	0.xxx
Visit	Week 4	1 (Reference)	
	Week 10	xx (xx – xx)	0.xxx
	Week 16	xx (xx – xx)	0.xxx
Other covariates			
…			
ERR			
Treatment	PZQ	1 (Reference)	
	AM	xx (xx – xx)	0.xxx
Visit	Week 4	1 (Reference)	
	Week 10	xx (xx – xx)	0.xxx
	Week 16	xx (xx – xx)	0.xxx

Other covariates			
…			

Note: This table will be presented in total 2 times, one for each *Schistosoma* species.

Example Table 8: Morbidity markers

Covariates	AM n (%)	PZQ n (%)	p-value
Anemia	xx (xx)	xx (xx)	0.xxx
Wall irregularities	xx (xx)	xx (xx)	0.xxx
Wall thickness	xx (xx)	xx (xx)	0.xxx
…			

Note: This table will be presented 5 times in total for different comparison between groups: PZQ vs single treatment of AM, PZQ vs two treatment rounds of AM, PZQ vs three treatment rounds of AM, single treatment of AM vs 2 treatment rounds of AM and single treatment of AM vs three treatment rounds of AM. All comparisons will be done in the ITT analysis population.

Example Table 9: Diagnostic performance of novel tests
Similar tables as Example Tables 4,5 and 6 will be produced, one set for each diagnostic test. Only PP and ITT analyses populations will be used for these tables.

Example Table 10: Malaria incidence and *P. falciparum* infection prevalence

Covariates	Pooled n(%; 95% CI)	AM n(%; 95% CI)	PZQ n(%; 95% CI)
P. falciparum prevalence			
Baseline	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 24	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 48	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Clinical malaria incidence			
Baseline	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Baseline + 7 days	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 4	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 6	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 6 + 7 days	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 10	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 12	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 12 + 7 days	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 16	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 24	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 48	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Anemia			
Baseline	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 24	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)
Week 48	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)

Note: The part of the table about clinical malaria will be presented also for the worst-case scenario in case of missing test results, treating all missing results as malaria positive.

Example Table 11: Malaria resistance and patterns of mutation

Covariates	Pooled n(%; 95% CI)	AM n(%; 95% CI)	PZQ n(%; 95% CI)	p-value
Baseline				
Mefloquine resistance markers				
Polymorphism pfmdr1: N86Y	xx (xx; xx – xx)	xx (xx; xx – xx	xx (xx; xx – xx	0.xxx
Polymorphism pfmdr1: Y184F	xx (xx; xx – xx)	xx (xx; xx – xx	xx (xx; xx – xx	0.xxx
Polymorphism pfmdr1: D1246Y	xx (xx; xx – xx)	xx (xx; xx – xx	xx (xx; xx – xx	0.xxx
Haplotype (in bold the mutants)				
NYD	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
N**F**D	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
N**FY**	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
NY**Y**	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
YYD	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
YFD	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
YYY	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
YFY	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
Artesunate resistance markers				
Polymorphism pfk13	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
Week 24				
Mefloquine resistance				
…	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
Artesunate resistance				
…	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
Week 48				
Mefloquine resistance				
…	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
Artesunate resistance				
…	xx (xx; xx – xx)	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx

Example Table 12: Counts of patients (%) with Adverse Events by Body System and Preferred Term (Meddra) for the whole study period.

	AM (N = xx)	PZQ (N = xx)
Blood and Lymphatic System	xx (xx)	xx (xx)
Anemia	xx (xx)	xx (xx)
Gastrointestinal Disorders	xx (xx)	xx (xx)
Diarrhea	xx (xx)	xx (xx)
...	xx (xx)	xx (xx)

Note: Similar tables will be presented for drug-related AEs, serious AEs, and serious drug-related AEs.

Example Table 13: Summary of safety analyses at Week 4/for the complete study period.

Number of patients (%; 95% CI) with:	AM (N = xx)	PZQ (N = xx)	Fisher's exact test
- Death	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
- Serious adverse events	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
- Drug-related serious adverse events	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
- any adverse events	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
- any drug-related adverse events	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx
- Confirmed malaria cases	xx (xx; xx – xx)	xx (xx; xx – xx)	0.xxx

Note: This table will be presented for the safety results of week 4 and for the whole study period separately.

Example Table 14: Summary of safety analyses at week 4.

	Odds Ratio compared to PZQ (95 % CI)	p-value
Death	xx (xx – xx)	0.xxx
Serious adverse events	xx (xx – xx)	0.xxx
Drug-related serious adverse events	xx (xx – xx)	0.xxx
Any adverse events	xx (xx – xx)	0.xxx

Example Table 15: Summary of safety analyses adjusting for administered doses of treatment.

	Incidence rate compared to PZQ (95 % CI)	p-value
Death	xx (xx – xx)	0.xxx
Serious adverse events	xx (xx – xx)	0.xxx
Drug-related serious adverse events	xx (xx – xx)	0.xxx
Any adverse events	xx (xx – xx)	0.xxx
Any drug-related adverse events	xx (xx – xx)	0.xxx
Any drug-related adverse events	xx (xx – xx)	0.xxx

Note: This table will be produced two times, one for each treatment round separately.

Example Table 16: Summary of safety analyses adjusting for administered doses of treatment.

	Incidence rate compared to a single AM treatment (95 % CI)	p-value
Death	xx (xx – xx)	0.xxx
Serious adverse events	xx (xx – xx)	0.xxx
Drug-related serious adverse events	xx (xx – xx)	0.xxx

Any adverse events	xx (xx – xx)	0.xxx
Any drug-related adverse events	xx (xx – xx)	0.xxx
Any drug-related adverse events	xx (xx – xx)	0.xxx

Note: Only the subjects that received the AM arm will be included in this model.

7. References

1. WHO. *Assessing the Efficacy of Anthelminthic Drugs against Schistosomiasis and Soil-Transmitted Helminthiases*.; 2013.
2. Casacuberta-Partal M, Hoekstra PT, Kornelis D, van Lieshout L, van Dam GJ. An innovative and user-friendly scoring system for standardised quantitative interpretation of the urine-based point-of-care strip test (POC-CCA) for the diagnosis of intestinal schistosomiasis: a proof-of-concept study. *Acta Trop*. 2019;199(June):4-7. doi:10.1016/j.actatropica.2019.105150
3. Pillay P, Taylor M, Zulu SG, et al. Real-time polymerase chain reaction for detection of Schistosoma DNA in small-volume urine samples reflects focal distribution of urogenital schistosomiasis in primary school girls in KwaZulu Natal, South Africa. *Am J Trop Med Hyg*. 2014;90(3):546-552. doi:10.4269/ajtmh.13-0406
4. Corstjens PLAM, Nyakundi RK, De Dood CJ, et al. Improved sensitivity of the urine CAA lateral-flow assay for diagnosing active Schistosoma infections by using larger sample volumes. *Parasites and Vectors*. 2015;8(1):1-9. doi:10.1186/s13071-015-0857-7
5. Olliaro PL, Vaillant M, Diawara A, Coulibaly JT. Toward Measuring Schistosoma Response to Praziquantel Treatment with Appropriate Descriptors of Egg Excretion. 2015:1-20. doi:10.1371/journal.pntd.0003821
6. Moser W, Keiser J, Id BS, Sayasone S, Knopp S, Id JH. One mean to rule them all ? The arithmetic mean based egg reduction rate can be misleading when estimating anthelminthic drug efficacy in clinical trials. 2020:1-14. doi:10.1371/journal.pntd.0008185
7. Dobson RJ, Sangster NC, Besier RB, Woodgate RG. Veterinary Parasitology Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test (FECRT). 2009;161:162-167. doi:10.1016/j.vetpar.2008.12.007
8. Atalabi TE, Adoh SD, Eze KM. The current epidemiological status of urogenital schistosomiasis among primary school pupils in Katsina State , Nigeria : An imperative for a scale up of water and sanitation initiative and mass administration of medicines with Praziquantel. 2018:1-19.
9. Vercruysse J, Behnke JM, Albonico M, et al. Assessment of the Anthelmintic Efficacy of Albendazole in School Children in Seven Countries Where Soil-Transmitted Helminths Are Endemic. 2011;5(3). doi:10.1371/journal.pntd.0000948
10. Walker M, Mabud TS, Olliaro PL, et al. New approaches to measuring anthelminthic drug efficacy : parasitological responses of childhood schistosome infections to treatment with praziquantel. *Parasit Vectors*. 2016:1-15. doi:10.1186/s13071-016-1312-0
11. Richter J, Hatz C, Campagne G, Bergquist N, Jenkins J. *Ultrasound in Schistosomiasis: A Practical Guide to the Standard Use of Ultrasonography for Assessment of Schistosomiasis-Related Morbidity*. Geneva; 2000.
12. Meurs L, Mbow M, Vereecken K, Menten J, Mboup S, Polman K. Bladder Morbidity and Hepatic Fibrosis in Mixed Schistosoma haematobium and S . mansoni Infections : A Population-Wide Study in Northern Senegal. 2012;6(9):1-8. doi:10.1371/journal.pntd.0001829