# Efficacy and safety of moxidectin and albendazole compared to ivermectin and albendazole co-administration in adolescents infected with *Trichuris trichiura*: a randomized controlled trial

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| **Protocol Number** | 1 | | |
| **Version Number** | 1.1 | **Document Date** | 07 October 2020 |
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| **Funding Agency** | Bill and Melinda Gates Foundation | | |

# 1. General information

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| MSc | Sophie Welsche | Swiss TPH | Doctoral student | Co-PI |
| MSc | Emmanuel Mrimi | Swiss TPH | Doctoral student | Co-PI |
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| BSc | Ghanil Mohamed Khatib | Public Health Laboratory – Ivo de Carneri (PHL-IdC) Chake-Chake, Pemba (Tanzania) | Laboratory Technician | Co-PI |
| Dr. | Jan Hattendorf | Swiss TPH | Group leader | Statistician |

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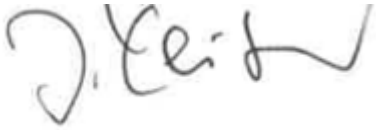
| Signature |  | Date of Signature<br>17.02.2020 |
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I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the trial as outlined herein and will complete the trial within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this trial. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the trial.

I will use only the informed consent forms approved by the Sponsor or its representative and will fulfill all responsibilities for submitting pertinent information to the Independent Ethics Committees responsible for this trial.

I agree that the Sponsor or its representatives shall have access to any source documents from which Case Report Form information may have been generated.

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## III. Abbreviations

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|---|---|
| AE | Adverse event |
| BMGF | Bill & Melinda Gates Foundation |
| CI | Confidence interval |
| CR | Cure rate |
| CRF | Case report form |
| EKNZ | Ethikkomission Nordwest- und Zentralschweiz |
| EML | Essential medicine list |
| EPG | Eggs per gram |
| ERR | Egg reduction rate |
| FDA | Food and Drug Administration |
| GCP | Good clinical practice |
| Hb | Hemoglobin |
| ICF | Informed consent form |
| ICH | International council for harmonization of technical requirements for pharmaceuticals for human use |
| IEC | Independent ethics committee |
| LC-MS/MS | Liquid chromatography tandem mass spectrometry |
| MDA | Mass drug administration |
| NLME | Nonlinear mixed-effects |
| PCR | Polymerase chain reaction |
| PI | Principal investigator |
| PD | Pharmacodynamics |
| PK | Pharmacokinetics |
| SAE | Serious adverse event |
| STH | Soil-transmitted helminth |
| WHO | World Health Organization |
| ZAHREC | Zanzibar Health Research Ethics Review Committee |

## IV. Synopsis

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|---|---|
| **Sponsor/Sponsor-Investigator** | Prof. Dr. Jennifer Keiser |
| **Study Title** | Efficacy and safety of moxidectin and albendazole compared to ivermectin and albendazole co-administration in adolescents infected with *Trichuris trichiura*: a randomized controlled trial |
| **Short title** | Efficacy and safety of MOX/ALB vs. IVM/ALB co-administration |
| **Protocol Number, Date and Version** | 1, 07.10.2020, v1.1 |
| **Trial registration** | Will be registered on https://www.clinicaltrials.gov/ |
| **Clinical phase** | Phase 2 trial |
| **Sample size** | 540 |
| **Indication** | *Trichuris trichiura* infection (eggs in stool) |
| **Investigational Product and Reference Treatment** | Moxidectin/ albendazole combination<br>Reference: ivermectin/ albendazole combination |
| **Study Rationale** | To provide evidence on the efficacy and safety of co-administered moxidectin and albendazole compared to co-administered ivermectin and albendazole, and to assess the efficacy of the drug combinations compared to monotherapies in adolescents aged 12-19 years against infection with *T. trichiura*. |

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| **Study Objectives** | Our **primary objective** is to demonstrate non-inferiority of<br>• Arm A: moxidectin (8 mg) / albendazole (400 mg) combination, compared to<br>• Arm B: ivermectin (200 µg/kg) / albendazole (400 mg)<br>in terms of egg reduction rates (ERRs) against *T. trichiura* infections in adolescents aged 12-19 years assessed at 14-21 days post-treatment by Kato-Katz microscopy.<br>The **secondary objectives** of the trial are:<br>a.) to assess superiority in terms of CRs of the drug combinations compared to their corresponding monotherapies: Arm C: Albendazole (400 mg) Arm D: Ivermectin (200 µg/kg) and Arm E: Moxidectin (8 mg)<br>b) to determine the cure rates (CRs) of combination therapies against *T. trichiura*<br>c) to evaluate the safety and tolerability of the treatment<br>d) to determine the CRs and ERRs of the treatment schemes study participants co-infected with hookworm and *A. lumbricoides*<br>e) to investigate potential extended effects on follow-up helminth prevalence (5-6 weeks and 3 months post-treatment) of the treatment regimens<br>f) to assess diagnostic performance and compare CRs based on egg counts retrieved from novel diagnostic tools (FECPAK-G2 and/or PCR) compared to standard Kato-Katz microscopy<br>g) to characterize pharmacokinetics (PK) or drug-drug interactions of study drugs following monotherapy or co-administration in *T. trichiura* infected adolescents. If a dose-response is observed, a Pharmacokinetic/-dynamic (PK/PD) analysis of the study drugs will be performed. |
| **Study design** | Randomized controlled trial, open-label with masked outcome assessor |
| **Study product / intervention** | Administration of a single oral dose of moxidectin (8mg) / albendazole (400mg) |

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| **Comparator(s)** | Primary: ivermectin (200 µg/kg) / albendazole (400mg)<br>Secondary: albendazole (400 mg), moxidectin (8 mg) and ivermectin (200 µg/kg) monotherapy |
| **Key inclusion / Exclusion criteria** | **Inclusion:** Adolescents aged 12-19 years infected with *T. trichiura* with a minimum count of 48 eggs per gram (EPG) of stool and at least two out of four Kato-Katz slides positive<br>• having given written informed consent signed by either the participant him/herself (if majority reached; ≥18 years) or by caregivers for minors; and written assent by minors (12-17 years)<br>• able and willing to be examined by a study physician before treatment<br>• able and willing to provide two stool samples at baseline and on the three follow-up assessments (14-21 days, 5-6 weeks and 3 months post-treatment),<br>**Exclusion:**<br>• No written informed consent by individual or caregiver and/or no written assent by minors<br>• Any trial or safety relevant abnormal medical conditions (including severe anemia, body temperature ≥ 38°C and or history of acute or severe chronic disease<br>• recent use of anthelminthic drugs (past 4 weeks)<br>• attending other clinical trials during study<br>• known allergy to study medication<br>• pregnancy, lactating or planning to become pregnant within the study period. |
| **Primary Endpoints** | ERR against *T. trichiura* at 14-21 days after treatment derived from EPG assessed by Kato-Katz |

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| **Secondary Endpoints** | • CRs against *T. trichiura* assessed at 14-21 days post-treatment<br>• CRs and ERRs against *A. lumbricoides* and hookworm assessed at 14-21 days post-treatment<br>• Adverse events<br>• Infection status and intensity assessed at baseline and 14-21 days post-treatment by novel diagnostic tools (FECPAK-G2 and/or PCR)<br>• Infection status and intensity assessed by Kato-Katz at 5-6 weeks and 3 months after treatment<br>• Characterization of population PK parameters and evaluation of possible drug-drug interactions between moxidectin and albendazole as well as ivermectin and albendazole and possibly PK/PD parameters of all drugs and combinations in *T. trichiura* infected adolescents |
| **Exploratory Endpoints** | None |
| **Interim Analyses** | None |
| **Study Duration** | 6 months total; up to 6 months per participant |
| **Schedule** | 05/2021 of first-participant in (planned)<br>11/2021 of last-participant out (planned) |
| **Study centres** | Pemba Island (Tanzania) |
| **Measurements & procedures** | Two stool samples will be collected if possible on two consecutive days or otherwise within a maximum of 7 days. The medical history of the participants will be assessed with a standardized questionnaire, in addition to a clinical examination carried out by the study physician before treatment.<br>All participants will be interviewed before treatment and after 3 and 24 hours (active surveillance) and retrospectively again at 14 - 21 days as well as 5-6 weeks and 3 months after treatment about the occurrence of adverse events (AEs). Any potential AEs happening between 24 hours and the respective follow-up time points will be monitored passively and medical intervention as determined suitable by a study physician provided if necessary. |

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| | The efficacy of the treatment and potential extended effects on follow-up prevalence will be determined around 14 - 21 days, 5-6 weeks and 3 months post-treatment by collecting another two stool samples.<br>All stool samples will be examined with duplicate Kato-Katz thick smears for *T. trichiura*, *A. lumbricoides,* and hookworm. From the remaining stool specimen, 3g will be analyzed with the FECPAK-G2 platform and 1.5-2g will be preserved in 70% ethanol and shipped to Swiss TPH, Basel or another reference laboratory for subsequent PCR-analysis.<br>Each participant will be asked to provide a finger-prick blood sample for hemoglobin measurement at baseline. At the same time, anthropometric measurements (i.e. height, and weight) will be taken for all participants.<br>To determine PK/PD parameters of the study drugs, a subsample of 60 willing study participants of all treatment arms will be asked to provide a maximum of 4 micro blood samples using finger pricks at defined time points between day 0 and day 7. |
| **Statistical Analyses** | An available case analysis according to the intention to treat principles will be performed, including all subjects with primary endpoint data. Additionally, a per-protocol analysis will be conducted. Geometric and arithmetic mean egg counts will be calculated for the different treatment arms before and after treatment to assess the corresponding ERRs. Bootstrap resampling method with 5,000 replicates will be used to calculate 95% confidence intervals (CIs) for ERRs and differences between ERRs.<br>CRs will be calculated as the percentage of egg-positive subjects at baseline who become egg-negative after treatment, assessed at 14-21 days with quadruple Kato Katz assays. Differences among CRs (between treatment arms and between diagnostic approaches) will be analyzed by using crude and adjusted logistic regression modelling (adjustment for infection intensity, age, sex and weight). Adverse events will be compiled into frequency tables and compared between treatment groups using descriptive summaries.<br>To determine PK/PD parameters, nonlinear mixed-effects (NLME) modeling will be used. To assess differences in diagnostic performance of the different tools used as compared to the standard Kato-Katz |

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| | microscopy (FECPAK-G2 and/or PCR) a readily available hierarchical Bayesian egg-count model will be applied to the individual level data. |
| **GCP statement** | This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, ICH-GCP E6 (R2) as well as all national legal and regulatory requirements. |
| **Key explanation for the inclusion of children** | This study will involve adolescents of at least 12 years of age, since an infection with *T. trichiura* occurs most often in children who are therefore the main target group of deworming campaigns. Younger children will not be included as moxidectin is registered only for children ≥ 12 years. |
| **Recruitment procedure** | The trial will be conducted in Pemba (Zanzibar, Tanzania).<br>It will take place in areas with moderate to high *T. trichiura* endemicity (communities/schools with a prevalence ≥ 25%) identified from earlier studies and/or based on experience of the local collaborating team. It will be implemented as a school-based study. |
| **Coverage of damages** | Winterthur Police Nr. 4746321, National Insurance Corporation of Tanzania (to be issued) |
| **Storage of data and samples for future research aims** | After the study has been completed, all samples will be destroyed. Case report forms and electronic source data will be kept for a minimum of 15 years. |
| **Conflict of interest in relation to the investigated drugs** | We declare no conflict of interest in relation to the investigated drugs. |

# 2. Background information

Albendazole and mebendazole are the most widely used drugs for preventive chemotherapy campaigns against soil-transmitted helminth (STH) infections. Albendazole is characterized by high cure rates (CRs) against infections with *Ascaris lumbricoides* (96%) and hookworm infections (80%). Mass drug administration (MDA) with single dose albendazole has already been considered insufficient to achieve World Health Organization (WHO) goals of morbidity reduction, in the case of *Trichuris trichiura* infections due to the low efficacy of this drug (CR 31%) [1, 2].

Therapies combining two or more drugs are widely advocated in different therapeutic areas such as tuberculosis, malaria, HIV/AIDS or cancer. The underlying rationale for multifactorial pharmacological treatment varies with the disease and includes the protection against the selection of drug-resistance, and hence, a prolongation of the life-span of effective and available drugs, and to increase and broaden the efficacy over drugs being administered in monotherapy [3].

To help prioritize candidate STH combinations, the Bill & Melinda Gates Foundation (BMGF) defined different levels of investment risk. In more detail, the prioritization includes four tiers and takes into account (i) the current efficacy and safety data of existing anthelmintic drugs; (ii) the financial and time investment required to generate the necessary evidence to change WHO treatment guidelines; and (iii) the potential for transformational change. The ivermectin/ albendazole combination was identified as a first-tier priority. Moxidectin/ albendazole was classified as second tier priority (since at this stage the drug was not yet approved).

In 2017, ivermectin in combination with albendazole for treatment of STH was added on the Essential Medicines List (EML) paving the way to further evaluate efficacy of this combination among school-aged children and communities in a range of epidemiological settings [4]. While for ivermectin/ albendazole evidence of superiority compared to single standard treatments in different settings and over varying time points is growing [5, 6], we still have very few studies providing similar results for the co-administration of moxidectin and albendazole in STH infections [7].

Moxidectin was recently approved by the Food and Drug Administration (FDA) for the treatment of onchocerciasis at a dose of 8 mg. Our own studies have shown that the combination of moxidectin/ albendazole might reveal high potential in the treatment of STH infections [7, 8]. Moreover, our recent dose-finding study against *T. trichiura* infections revealed that the approved 8 mg dose (regardless whether combined with albendazole or monotherapy) performs as good as higher doses against this STH [9]. However, there is a need to explore whether the longer half-life of moxidectin (compared to ivermectin) could reveal a benefit in the treatment of STH infections [10]. Finally, there is a need to thoroughly evaluate the efficacy of the marketed tablet moxidectin formulation (our previous studies used a different formulation).

In this trial, we test for non-inferiority of both currently approved and available drug combinations (i.e. moxidectin/ albendazole against ivermectin/ albendazole) and for superiority of the combination therapy compared to the respective monotherapies. Furthermore, potential extended effects through a prolonged efficacy assessment scheme in adolescents in Pemba (Tanzania) are examined. Follow-up will be conducted at approx. 14-21 days, 5-6 weeks and 3 months post-treatment to assess co-benefits on worm burden reduction from the relatively long half-life of moxidectin ($T_{1/2}$: 491-832 hours) [11, 12] compared to ivermectin ($T_{1/2}$:16-32 hours; increasing with age) [13]. Given the comparatively long half-live of moxidectin, the standard follow-up period of 14-21 days recommended by WHO might not be sufficient to reveal the full potential of the drug. Therefore, two more follow-up time points will give the opportunity to investigate potential extended effects on infection status and intensity. Results from this trial will inform decisions on how anthelminthic combination therapy could be introduced into existing MDA programs and, therefore, provide a valuable adjunct tool for interrupting STH transmission.

The pharmacokinetic/-dynamic (PK/PD) characterization of a drug is essential to understand the response of the human body to a drug and vice versa, especially in populations that physiologically differ from healthy adults. Physiological characteristic like mal- or undernutrition or intestinal worms, such as *T. trichiura*, can potentially affect the PK of a drug.[14, 15]. For moxidectin, clinical pharmacokinetics have so far exclusively been assessed in a limited number of healthy adults [16, 17].

A trial currently being conducted by our group will characterize PK and evaluate PK/PD parameters of moxidectin in adults infected with *Strongyloides stercoralis*. Our study will be the first one to provide information on the PK, PK/PD on all three drugs in this population and thus guide optimal drug dosing in *T. trichuris*-infected adolescence in Pemba. Additionally, we aim to provide novel information on the possible interplay of moxidectin and ivermectin with albendazole when co-administered. For this it is necessary to take not only samples in the combination arm but also in the monotherapy arms.

Micro blood sampling by collecting blood from a finger prick has become the preferred method not only for molecular epidemiological studies but also for PK studies due to its minimal invasiveness, and simple and fast handling [18]. Furthermore, the results gained by this method have been repeatedly shown to correlate well with the standard venous blood sampling, which also holds true for ivermectin and albendazole and is currently under investigation for moxidectin by our group [19, 20]. A sparse sampling approach will be used in 60 participants, which allows to use only 4 samples per individual.

# 3. Trial objective and purpose

We designed a non-inferiority trial to show that co-administered moxidectin and albendazole is non-inferior compared to co-administered ivermectin and albendazole in adolescents aged 12-19 years on Pemba Island (Tanzania). From previous studies conducted by our group, we expect similar efficacies from the combination moxidectin/ albendazole compared to ivermectin/ albendazole [7, 8]. However, moxidectin might be advantageous in terms of the drug's longer half-life and in areas with possible emerging ivermectin resistancy [17, 21]. This study will allow comparing the efficacy of the two available co-administrations and will provide further insights on the potential value of moxidectin/ albendazole. Our data will pave the way for possible large scale, multi country follow-up studies. As recommended for new combination therapies, we simultaneously assess superiority of the drug combinations compared to monotherapies.

The **primary objective** is to demonstrate that co-administered moxidectin (8 mg) / albendazole (400 mg) is non-inferior to ivermectin (200 µg/kg) / albendazole (400 mg) in terms of egg reduction rates (ERRs) against *T. trichiura* infections assessed by Kato-Katz at 14-21 days post-treatment in adolescents aged 12-19 years using a non-inferiority margin of 2 percentage-points.

The **secondary objectives** of the trial are:

a) Efficacy assessments of combination therapies require demonstration of superiority against the respective monotherapies. Therefore, the trial has five different treatment groups: moxidectin (8 mg) / albendazole (400 mg) combination, ivermectin (200 µg/kg) / albendazole (400 mg) combination, albendazole (400 mg) monotherapy, ivermectin (200 µg/kg) monotherapy and moxidectin (8 mg) monotherapy.

b) to determine the CRs of the drug regimens against *T. trichiura*

c) to evaluate the safety and tolerability of the treatment

d) to determine the CRs and ERRs of the treatment schemes in study participants infected with hookworm and *A. lumbricoides*

e) to investigate potential extended effects on follow-up helminth prevalences (5-6 weeks and 3 months post-treatment) of the treatment regimens

f) to assess diagnostic performance and compare CRs based on egg counts retrieved from novel diagnostic tools (FECPAK-G2 and/or PCR) compared to standard microscopy

g) to characterize population PK parameters, as well as drug-drug interactions of active study treatments following single and co-administration in *T. trichiura* infected adolescents. If a dose-response is observed, a PK/PD analysis will further be performed

# 4. Methodology

## 4.1 Primary and secondary endpoint

**Primary endpoint:** the geometric mean based ERR of *T. trichiura* egg counts assessed by Kato-Katz microscopy pre-treatment and 14-21 days post-treatment.

**Secondary endpoint:** CR of *T. trichiura* as well as CRs and ERRs for *A. lumbricoides* and hookworm assessed by Kato-Katz at 14-21 days post-treatment. In addition, tolerability of treatment (AEs), infection status and intensity assessed at baseline and 14-21 days post-treatment by novel diagnostic tools (FECPAK-G2 and/or PCR), infection status and intensity derived by Kato-Katz at 5-6 weeks and 3 months post-treatment and PK/PD parameters of the study drugs will be assessed.

## 4.2 Type of trial

Randomized controlled trial, open-label with masked outcome assessor.

## 4.3 Trial design

### 4.3.1 Baseline survey and screening

A randomized-controlled trial will be conducted with five treatment arms to be followed-up over a 3-month period. This trial will be conducted as a school-based study on Pemba Island (Zanzibar, Tanzania). Several secondary schools in areas with suspected high *T. trichiura* endemicity as identified from previous research and experience of our local collaborators will be visited. In each selected school, adolescents aged 12-19 years will be invited for study participation. The Zanzibar education system has adopted a 12-year compulsory basic education cycle with abolished school fees and parents' contributions since 2015 leading to high general enrolment rates for secondary schools (85% of eligible children finalize the last primary grade level) but also comparably high ordinary secondary level survival of more than half of all pupils (54%) [22]. Entering school over-age is a common occurrence in Zanzibar, thus ages of secondary level pupils may well range from 12 to 19 years of age. We are thus confident that recruitment of adolescents aged 12-19 years through a school-based approach is not only more efficient than working in communities but also still representative for the total population of this age strata. Adolescents are within the main target group of helminth control programs and are listed among potential receivers of moxidectin that is, so far, only approved from 12 years of age onwards [23].

The study includes one baseline and three follow-up assessments at 14 – 21 days, 5-6 weeks and 3 months. The study is designed as a five-arm trial including two arms with combined treatment through co-administration of separate tablets (arm A; moxidectin/ albendazole, arm B; ivermectin/ albendazole) and three arms with single drug administration (arm C; albendazole, arm D; ivermectin, arm E; moxidectin).

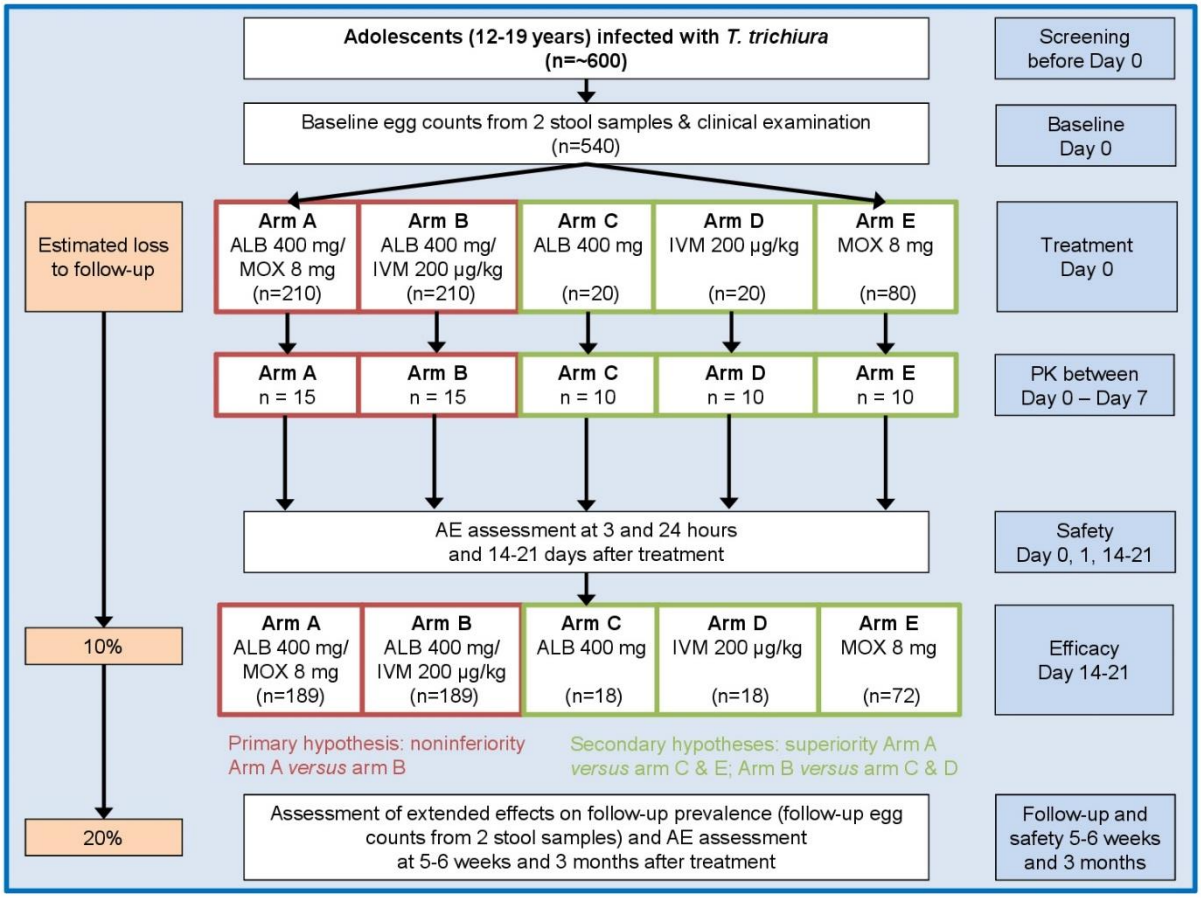


**Figure 1:** Design and timeline of the randomized-controlled trial to be implemented on Pemba Island (Tanzania).

### 4.3.2 Diagnosis

At baseline, all participants will be asked to provide two stool samples (within a maximum of 7 days). From each stool specimen, duplicate Kato-Katz thick smears (41.7 mg each) [24] will be prepared and read under a microscope for eggs of *T. trichiura*, *A. lumbricoides* and hookworm by experienced technicians. To ensure quality of hookworm diagnosis, 10% of the samples will be divided into two sub-samples; one of the containers will keep its original participant ID, whereas the second container will be labeled with a new ID (assigned by the co-PI). Duplicate Kato-Katz will be prepared from both containers and the findings compared. For hookworm, results are considered correct if no difference in presence/absence is found. For quality control of *A. lumbricoides* and *T. trichiura* egg counts, 10% of slides will be re-read by another laboratory technician. Results are considered correct if the following tolerance margin is not exceeded: (i) No difference in presence/absence of *A. lumbricoides* and *T. trichiura* (ii) egg counts are +/-10 eggs for counts ≤100 eggs or +/-20% for counts >100 eggs (for each species separately). In case discrepancies above the tolerance margin are noted in one or more slides, the respective slides are re-read by the local technicians. The new results are discussed, so that in case of discordant results, slides can be re-evaluated to reach consensus. The same slides might further be re-read by an automatized reading system (Kato-Katz 2.0) if available at study start.

All microscopically analyzed quadruplicate Kato-Katz thick smears will be destroyed after passing the quality control. Additionally, a portion of 1.5-2 g of stool from each specimen will be preserved in 70% ethanol and shipped to a reference laboratory for polymerase chain reaction (PCR) analysis (to be determined) [25]. The same sampling procedure and diagnostic approach will be applied at days 14-21, 5-6 weeks and 3 months post-treatment. At baseline and 14-21 days post-treatment, 3g of the remaining stool specimen will be used for analysis with a further developed version of the FECPAK-G2 device using a similar laboratory protocol as applied in previous studies [26, 27].

#### 4.3.3 Clinical examination

A clinical examination of the study participants assessing general health, anthropometric parameters including height and weight as well as tympanic temperature using an ear thermometer will precede the treatment. Each participant will be asked to provide a finger-prick blood sample for hemoglobin (Hb) levels, which will be detected using a HemoCue analyzer (Hb 301 system, Angelholm, Sweden). To avoid accidental treatment of pregnant girls/women all female participants will be asked to provide a urine sample to be subjected to a pregnancy RDT at baseline and at the end of the study 3 months after treatment. Girls/women will be individually counselled that they should not become pregnant during the entire study period. All trial participants will further be asked about existing clinical symptoms before drug administration.

#### 4.3.4 Adverse events assessment

Participants will be kept for 3 hours after treatment administration to observe any possible acute AEs and reassessment will be done at 24h post-treatment. Additionally, interviews will be conducted to determine the emergence of clinical symptoms such as headache, abdominal pain, itching, nausea, vomiting and diarrhea directly before treatment within the scope of baseline assessment. At 3 and 24 hours after treatment and retrospectively at days 14 – 21 as well as 5-6 weeks and 3 months post-treatment, participants will again be interviewed for the assessment of adverse events (AEs). Symptoms arising within the timespan of 24 hours after treatment and the respective follow-up time points will be monitored passively by teachers or local health workers who will report incidences to the study team. Any symptoms are recorded in the CRF and immediate action will be undertaken according to the judgement of a study physician if indicated.

#### 4.3.5 Assessment of efficacy after treatment

The efficacy of the treatment will be determined 14-21 days post-treatment by collecting another two stool samples, which will be microscopically examined for *T. trichiura* using duplicate Kato-Katz thick smears. Participants will be considered cured if no *T. trichiura* eggs are found in the follow-up stool samples. Eggs per gram will be assessed by adding up the egg counts from the quadruplicate Kato-Katz thick smears and multiplying this number by a factor of six. Geometric and arithmetic mean egg counts

will be calculated for the different treatment arms before and after treatment to assess the corresponding ERRs. At the end of the study all participants remaining positive for any STH infection will be treated with the currently best recommended treatment, i. e. ivermectin/ albendazole against *T. trichiura* and hookworm and albendazole against *A. lumbricoides*.

### 4.3.6 Pharmacokinetic studies

The exposure-response correlation study will be performed in a maximum of 15 participants in the combination chemotherapy treatment arms and 10 participants in the monotherapy treatment arms, amounting to a subsample of 60 participants overall. This number of participants was determined to be representative of the study community taking into account the relatively low variability among PK values within a population. The absorption of all study drugs is known to be better after consumption of a high-fat meal, therefore study participants will receive a local high-fat breakfast before treatment [16]. Since PK population parameters of all study drugs are available [31], a sparse sampling approach can be applied to describe the PK profiles of the individual drugs upon co-administration as well as a potential interference between moxidectin or ivermectin and albendazole. Within a sparse sampling approach, instead of describing the PK profile for each participant separately, samples are allocated to different time points and individuals within the same treatment. Statistical inferences are performed to characterize the population-based PK profile of a specific treatment arm. This approach allows for a reduced number of samplings per participant and renders a PK characterization well tolerable. To this end, a small drop of blood from the fingertip will be taken. The sampling will be performed according to the following scheme:

- A maximum of 4 finger pricks will be done per participant throughout the whole PK study
- Of these 4 finger pricks per participant, a maximum of 3 pricks will be done on the same day
- The intervals between the pricks done on the same day will be at least 1h
- The whole PK study will be completed between day 0 and day 7

The sparse sampling scheme will allow for a sufficient number of data points at a subsample of 60 participants to model the PK profiles of the drugs. The exact time points will have to be adapted according to PK data gathered during a current trial conducted by our group for the evaluation of the efficacy of moxidectin against *Strongyloides stercoralis*. Capillary blood (≤ 30µL, i.e. 10µL for ivermectin and albendazole and 30µL for moxidecin) will be collected by puncture with a finger prick. Two microsamples (duplicates) will be taken with one finger prick. Each time, the drop of blood (10 – 30 µL) will be directly transferred on filter paper or Mitra® sticks which should dry for approximately one hour. The dried sticks and filter paper will be transported to Swiss TPH, Basel, and stored at -20°C until analysis. The quantification of the study drugs will be performed using the validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method as described elsewhere [20, 31]. Drug concentrations will be calculated by interpolation from a calibration curve with a lower limit of quantification of 1-5ng/ml. 7% of the sample duplicates will be analyzed for quality control, and the

measured concentrations will be used to determine between-run and overall precision and accuracy of the analysis.

## 4.4 Measure to minimize bias

Study participants eligible for treatment will be randomly assigned to one of the five treatment arms using a computer-generated stratified randomization code. The random allocation sequence will be generated by using an algorithm which minimizes deviations from the anticipated arm sizes stratified by 2 levels of baseline infection intensity (light: 1-999 EPG, and moderate plus heavy: ≥ 1000 EPG *T. trichiura* infections) which will be provided by the trial statistician not involved in enrolment, treatment and data collection. This way, all treatment arms will have a similar proportion of participants with light infection intensity. The number of light *versus* moderate/heavy infections however are not expected to be equal in each arm, depending on the distribution of infection intensity in the recruited cohort. Allocation concealment will be warranted by masking the randomization sequence from the team member conducting the recruitment. Due to the complexity of the treatment scheme, blinding will not be feasible in order to avoid an unnecessary administration of a large amount of placebo pills. Masking of the outcome assessor is warranted since the laboratory technicians determining the egg counts for the efficacy assessment will have no knowledge of the participants' assignment to treatment arms.

## 4.5 Study duration and duration of subject participation

The trial will last 6 months, and screening for the baseline is scheduled to start 3 months prior to treatment. Follow-up screenings will take place between 14-21 days, 5-6 weeks and 3 months post-treatment and will last approximately three weeks respectively. Thus, the maximum time for subject participation will be 6 months. Schedules of visits are summarized below.

### 4.6 Schedule of visits

**Table 1.** Schedule of visits of during study.

| | Screening | Baseline/Treatment/Safety | | | | | Follow up | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before day 0 | 0h | Randomization and treatment | 3h | 24h | Until day 7 | 14-21 days | 5-6 weeks | 3 months |
| **Informed consent** | X | | | | | | | | |
| **Diagnosis (stool examination)** | X | | | | | | X | X | X |
| **Medical history** | | X | | | | | | | |
| **Clinical examination** | | X | | | | | | | |
| **Pregnancy testing** | | X | | | | | | | X |
| **Hemoglobin measurement** | | X | | | | | | | |
| **PK (micrsosampling)** | | X | | X | X | X | | | |
| **Capturing AEs** | | | | X | X | | X | X | X |
| **Capturing SAE** | | | | X | X | | X | X | X |

## 5. Selection of the trial subjects

### 5.1 Recruitment

The study will be carried out in adolescents aged 12-19 years attending secondary schools on Pemba Island, Tanzania. Schools in areas with moderate to high *T. trichiura* infection intensities will be selected and identified based on experience from earlier studies and/or knowledge of the local collaborating teams. The trial will be implemented as a school-based study in order to simplify recruitment of adolescents. Caregivers of potential participants and adolescents aged ≥18 years will be invited to participate in an information session. The research team will explain the purpose and procedures of the study, as well as potential benefits and risks of participation. Attendees will be encouraged to ask questions which will be discussed in an open setting.

Caregivers interested in having their child/children of 12-17 years of age participate in the study or adolescents aged 18-19 years willing to participate will be invited to complete the process of informed consent by signing the informed consent form (ICF). In addition, written assent will be obtained from underage participants. Participants having a signed ICF will be assessed for eligibility during screening procedures.

## 5.2 Inclusion criteria

1. Aged between 12 and 19 years.
2. Written informed consent signed by either parents/caregivers for underage adolescents (aged 12-17 years) or by the participant him/herself (18-19 years of age); and written assent by underage participant.
3. Agree to comply with study procedures, including provision of two stool samples at the beginning (baseline) and on three follow-up assessments (14-21 days, 5-6 weeks and 3 months after treatment).
4. Willing to be examined by a study physician prior to treatment.
5. At least two slides of the quadruple Kato-Katz thick smears positive for *T. trichiura* and infection intensities of at least 48 EPG.

## 5.3 Exclusion criteria

1. No written informed consent by individual or caregiver and/or no written assent by minors
2. Presence or signs of major systemic illnesses, e.g. body temperature ≥ 38°C, severe anemia (below 80g/l Hb according to WHO [32]) upon initial clinical assessment.
3. History of acute or severe chronic disease.
4. Recent use of anthelmintic drug (within past 4 weeks).
5. Attending other clinical trials during the study.
6. Pregnancy, lactating, and/or planning to become pregnant within the next 6 months.
7. Known allergy to study medications (*i.e.* albendazole, ivermectin or moxidectin).
8. Taking medication with known interaction on study drugs.

## 5.4 Criteria for discontinuation of trial

A subject can be discontinued from the study for the following reasons:

1. Withdraws from the study (this can happen anytime as participation is voluntary and there are no further obligations once a participant withdraws).
2. At the discretion of the Principal Investigator (PI) or co-PI, if the participant is not compliant to the requirements of the protocol.

Discontinued subjects will not be replaced. If, for any reason, a subject is discontinued from the study before the end of treatment evaluations, the AE assessment will still be conducted. Data obtained prior to the withdrawal will be included in the analysis to ensure the validity of the trial. Data of withdrawn participants are fully anonymized once analysis is complete.

## 5.5 Treatment of subjects

After randomization, all eligible adolescents will be treated with the respective single or combination treatment regimen according to their assigned treatment arm at day 0. Albendazole will be the product of Glaxo Smith Kline (Zentel®) and a single tablet of 400 mg will be administered. Moxidectin tablets will be obtained from Medicines Development for Global Health (MDGH), Australia, and four tablets of 2 mg will be administered. Ivermectin tablets (3 mg) will be obtained from Merck (Stromectol®), and to administer a dose of 200 µg/kg, the body weight of each participant will be recorded to calculate and administer the correct dose of ivermectin.

Since the study drugs are known to have an increased absorption in humans after a high-fat meal was consumed, participants will receive a local high-fat breakfast prior to treatment [16, 33, 34]. The tablets will be handed out from the drug container according to the randomization list. Each person will receive either:

(i) A single tablet of albendazole plus four tablets of moxidectin

(ii) A single tablet of albendazole plus the appropriate number of ivermectin tablets with regard to their body weight category

(iii) A single tablet of albendazole

(iv) The appropriate number of ivermectin tablets with regard to their weight category

(v) Four tablets of moxidectin

All drugs will be administered in the presence of the PI and/ co-PI, and ingestion confirmed. This will be recorded with the time and date of dosing. Subjects will be asked not to take any drugs other than those prescribed by the study medical team. After ingestion of the medication, the subjects will be observed for 3 hours to ensure retention of the drug. Vomiting within 1-hour post-dosing will require re-dosing. The subjects will not be allowed more than one repeated dose. No re-administration will be needed for subjects vomiting after one hour. The PI or the co-PIs are responsible for drug accountability at the study site. Maintaining drug accountability includes careful and systematic study drug storage, handling, dispensing, and documentation of administration. Prior to administration, drugs will be stored at room temperature and protected from light and exposure to moisture in a secure area with limited access at the study site. The tablets must not be frozen or stored at temperatures above 30°C. Temperature monitors must be used for shipment and storage.

Treatment and capillary blood sampling (finger pricking) for the population PK and PK/PD assessment for a maximum of 60 study participants (maximum of 15 participants in the combination treatment arms and 10 participants in the monotherapy treatment arms) will be done in a quiet location at the school. Infrastructure as required for the study needs will be installed as necessary at the specific treatment days.

To avoid interference of potential on-going control programs against helminthiases with the infection status of the trial participants, communication with local stakeholders will be established to ascertain that trial participants will not undergo MDA treatment. Missed-out rounds of planned MDA against STH in the participating schools will be substituted with a free single-dose treatment (i.e., ivermectin 200 µg/kg/ albendazole 400 mg ) against STH infection at the study endpoint (after the day 90 follow-up assessment) offered by the study team. At each follow-up time point, participants will be asked whether they had taken anthelmintic treatment.

### 5.6 Concomitant therapy

All medications taken one month before and during the study period until the last stool examination at the day 90 follow-up assessment must be recorded with indication, dose regimen, date and time of administration.

Medication(s)/treatment(s) permitted during the trial:
- Analgesics and antipyretics are allowed to be given to the subjects in case of fever, antiemetics to prevent nausea and vomiting and/or antibiotics to prevent or treat bacterial superinfection.

Medication(s)/treatment(s) NOT permitted during the trial:
- No other active drugs against helminths are permitted during the trial. Participants receiving active anthelminthic concomitant medication during the trial will not be discontinued, however a case-specific assessment will be done at the point of data-analysis.

## 6. Safety assessments

### 6.1 Adverse event definitions

The term “adverse event” is defined as follows:
*Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment*

An AE could therefore include any of the following events which develop or increase in severity during the course of the study after administration of the study product at treatment on day 0:

a. Any unfavorable and unintended signs, symptoms or disease temporally associated with the use of a medicinal product, whether or not considered related to the condition under study and the study product
b. Any abnormality detected during physical examination.

The medical conditions present at the initial trial visit that do not worsen in severity or frequency during the trial will not be defined as adverse events but considered as baseline medical conditions. For the purpose of this trial, disease progression and relapse will be considered as treatment failure, not as an Adverse Event.

The observation time for adverse events starts when the treatment is initiated until the end of the study. These data will be recorded on the appropriate CRF sections, regardless of whether they are thought to be associated with the study or the drug under investigation. Associated with the use of the drug means that there is a reasonable possibility that the event may have been caused by the drug (see also relatedness definitions below).

### 6.1.1 Severity grading

Adverse signs or symptoms will be graded by the physician or nurse of the trial as mild, moderate, severe or life threatening according to the following definitions:

| Grade | Definition |
|---|---|
| 1 | Mild: the subject is aware of the event or symptom, but the event or symptom is easily tolerated. |
| 2 | Moderate: the subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity. |
| 3 | Severe: significant impairment of functioning: the subject is unable to carry out his or her usual activities. |
| 4 | Life threatening or disabling |
| 5 | Death related to adverse events |

### 6.1.2 Relatedness

Relatedness will be assessed as defined below based on the temporal relationship between the adverse event and the treatment, known side effects of treatment, medical history, concomitant medication, course of the underlying disease and trial procedures.

Possibly related: an adverse event which can medically (pharmacologically/clinically) be attributed to the study treatment.

Unrelated: an adverse even which is not reasonably related to the study treatment. A reasonable alternative explanation must be available.

An adverse event that is determined to be related to the administration of a study product is referred to as an “adverse drug reaction.”

### 6.1.3 Expectedness

Expected adverse drug reaction: Any adverse event possibly related to the co-administration of ivermectin/ albendazole or moxidectin/ albendazole reported in the literature or on the drug package leaflets and listed in the consent form.

Unexpected adverse drug reaction: Any adverse event possibly related to the study product administration, the nature, frequency, specificity or severity of which is unanticipated and not consistent with the available risk information described for these drugs.

### 6.1.4 Serious adverse events

According to the ICH "Clinical Safety Data Management: Definitions and standards for expedited Reporting E2A" [35], a serious adverse event includes any event (experience) or reaction in any untoward medical occurrence that at any dose:

1. results in death;
2. is life threatening, meaning, the subject was, in the view of the Investigator, at immediate risk of death from the reaction as it occurred, *i.e.* it does not include a reaction that, had it occurred in a more serious form, might have caused death;
3. results in persistent or significant disability/incapacity, *i.e.* the event causes a substantial disruption of a person's ability to conduct normal life functions;
4. requires in patient hospitalization or prolongation of existing hospitalization;
5. creates a congenital anomaly or birth defect (not relevant for this study);
6. is an important medical event, based upon appropriate medical judgment, that may jeopardize the patient or subject or may require medical or surgical intervention to prevent one of the other outcomes defining serious.

A "severe" adverse event does not necessarily meet the criteria for a "serious" adverse event. Serious adverse events are reported from treatment until the end of the study.

Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome.

The causality of any serious adverse event that occurs after the study period and its possible relatedness to the study treatment or study participation will also be assessed by investigators as described in section 6.1.2.

### 6.1.5 Suspected unexpected serious adverse reactions

A suspected unexpected serious adverse reaction (SUSAR) is an unexpected adverse drug reaction which also meets the definition of serious adverse events.

## 6.2 Methods of recording and assessing adverse events

Few adverse events have been reported following albendazole, ivermectin or moxidectin administration in STH-infected individuals. The most common adverse events were abdominal cramps, headache, fatigue, nausea, diarrhea, fever and vertigo [7, 8, 17, 36-38].

The observation time for AE starts when the treatment is initiated. Subjects will be kept for observation for at least 3 hours following treatment for any acute AE and. If there is any abnormal finding, the local study physician will perform a full clinical examination and findings will be recorded. An emergency kit will be available on site to treat any medical conditions that warrant urgent medical intervention. Participants will also be interviewed at 3h and 24h as well as retrospectively 14 -21 days, 5-6 weeks and 3 months after treatment about the occurrence of AEs.

Information on all AE (incidence, intensity, seriousness and causality) will be entered immediately in the source document, and also in the appropriate AE module of the case report form. For all AEs, sufficient information will be pursued and/or obtained so as to permit i) an adequate determination of the outcome of the event (i.e. whether the event should be classified as a SAE); and; ii) an assessment of the casual relationship between the AE and the study treatments. Intensity of AE will be judged by the study physician, following guidelines by the European Medicine Agency (Note for Guidance on Clinical Safety Data Management).

All SAE, unexpected adverse drug reactions or SUSARs must be reported as described in Section 6.3.

## 6.3 Reporting of serious adverse events

Any study-related unanticipated problem posing risk of harm to subjects or others (including all unexpected adverse drug reactions), and any type of serious adverse event (SAE) will be immediately (within a maximum of 24 hours after becoming aware of the event) notified to the study sponsor-investigator and co-PIs:

**Prof. Dr. Jennifer Keiser (Sponsor-investigator)**
Swiss Tropical and Public Health Institute
Socinstrasse 57, 4051 Basel, Switzerland
Tel.: +41 61 284-8218
Fax: +41 61 284-8105
E-mail: jennifer.keiser@swisstph.ch

**Mr. Said Ali (Co-PI)**
Public Health Laboratory Ivo de Carneri
P.O. Box 122 Wawi, Chake Chake
Pemba, Zanzibar (Tanzania)
Tel.: +255 24 245-23
Fax: +255 24 245-2003
Mobile: +255 77 741-6867
Email: saidmali2003@yahoo.com

Within the following 48 hours, the local co-investigator must provide to study sponsor-investigator further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of a completed SAE form, and any other diagnostic information that will assist the understanding of the event. In exceptional circumstances, a serious adverse event may be reported by telephone. In these cases, a written report must be sent immediately thereafter by fax or e-mail. Names, addresses and telephone for serious adverse event reporting will be included in the trial-specific SAE form. Relevant pages from the CRF may be provided in parallel (e.g., medical history, concomitant medications).

All pregnancies must be reported to the Sponsor-Investigator promptly after becoming aware of the pregnancy. Treatment will not be administered at follow-up time points in the event of pregnancy. A study physician recruited from a local health facility/ hospital will serve as medical contact. The treating physician will follow up on the study participant until the pregnancy is resolved. The outcome of the pregnancy must be reported to the Sponsor-Investigator.

## 6.4 Safety reporting to Health Authorities and Ethics Committees

The sponsor-investigator will send appropriate safety notifications to Health Authorities in accordance with applicable laws and regulations. Additionally, this information will be provided to 'Ethikkomission Nordwest- und Zentralschweiz' (EKNZ, Switzerland), and the 'Zanzibar Health Research Ethics Review Committee' (ZAHREC, Tanzania) in Zanzibar according to national rules. Fatal or life-threatening serious adverse events or SUSARs will be reported within 24 hours followed by a complete report within 7 additional calendar days. Other SAEs and SUSARs that are not fatal or life-threatening will be filed as soon as possible but no later than 14 days after first knowledge by the sponsor.

# 7. Data management and data quality control

The investigators are responsible for an adequate data quality. Prior to the initiation of the study, a short investigator's meeting will be held between investigators of Swiss TPH and PHL-IdC. This meeting will include a detailed discussion of the protocol, performance of study procedures (SOPs from previous studies available on site), CRF completion, specimen collection and diagnostic methods.

Information about study subjects will be kept confidential and managed accordingly. Screened participants will be listed in a confidential "subject screening log" and attributed a unique study number. Enrolled participants will be listed in a confidential "subject enrolment log"; this document will constitute the only source to decode the pseudonymized data and will only be accessible to the investigators. CRF data will be double-entered and compared using Beyond Compare 4 (Scooter Software Inc., Madison, Wisconsin). Any discrepancies will be reviewed against the hard copies of the CRF and corrected accordingly. Electronic data files will be stored on secured network drives with restricted access for study personnel only. Data analysis will be conducted with pseudonymized data and reporting of findings will be fully anonymized.
Essential infrastructure such as a locked room for safe storage of hardcopy data will be made available.

## 7.1 Source data

Source data are comprised of clinical findings and observations as well as laboratory data maintained and compiled at the study site. Source data are contained in source documents and are allowed to be accessed by local authorities. Source data will be directly entered in the following documents:

1. CRF: Primary data collection instrument for the study. It holds records of all clinical and physical examination data, treatment information, AEs, and infection status at the follow-up time points. For every subject enrolled in the clinical trial, a corresponding CRF exists. All data requested on the CRF must be recorded, and investigators will review and approve each CRF for completion.
2. Laboratory parasitology sheets: Record of the STH egg counts at all sample collection time points
3. PK: Time records of PK samplings for 60 willing participants

## 7.2 Data collection and documentation

Data collected and produced within this trial will fall into one of the following categories:

a) Egg counts of *T. trichiura*, Hookworm (*Necator americanus* and *Ancylostoma duodenale*, no differentiation between the two species will be made) and *A. lumbricoides* derived from standard Kato-Katz microscopy performed at baseline as well as at 14-21 day, 5-6 weeks and 3 months post-treatment.
b) Egg counts of *T. trichiura*, Hookworm and *A. lumbricoides* derived from FECPAK-G2 platform analysis at baseline and 14-21 days post-treatment.
c) Anthropometric and clinical characteristics of the trial participants collected using the study's CRF such as weight, height, blood pressure, temperature, pregnancy status (for female subjects), overall health status and any abnormal medical condition or chronic disease.
d) PK time recording of each sample per person
e) Measured concentrations analyzed from micro blood samples and subsequently derived PK/PD parameters
f) Infection status of *T. trichiura*, Hookworm and *A. lumbricoides* derived by PCR assessment of ethanol-fixated stool sample aliquots

Data of categories c) and d) will be recorded both paper-based and directly into tablets using CommCare (Dimagi, Inc., Cambridge, MA) or a comparable data-entry software, whereas data in categories a), b) and e) will be captured by software only. Data compiled using the software will be directly saved on the personal, password-protected laptop of one of the Co-PIs and uploaded to a server hosted at Swiss TPH, Basel. In paper-based data collection, all missing data must be explained. If an item on the CRF is left blank because the procedure was not done or the question was not asked "N/D" will be entered. If the item is not applicable to the individual case "N/A" will be written. All entries will be printed in black ink. All corrections must be noted with the initials of the respective team member and dated. Data in categories a) and c) will be merged into a masterfile and saved in .xlsx, .mdb and/or .csv. Data in categories b) and d) - f) will be saved as .mdb, .csv, .xlsx, .txt and/or .pdf files. For categories a) – d), paper-based data will serve as physical backup. Hard copies of the data such as parasitological sheets and CRFs will remain at PHL-IdC. Digital copies along with single databases and compiled masterfiles will be transferred to the Swiss TPH, Basel. Data will then be analyzed as described in section 8.

### 7.3 Ethical, legal and security issues

Screened participants will be listed in a confidential "subject screening log" and attributed a unique study ID. In case of enrolment, participants will be listed in a confidential "subject enrolment log" utilizing the same study IDs. The codes will be linked with the participant's identity on a separate file (subject identification list), filed in a secured place at PHL-IdC and will only be accessible to investigators. Personal data will be coded for data analysis. No names will be published at any time, and published reports will not allow for identification of single subjects. Confidentiality will be ensured throughout the entire research project. All databases will be password secured. None of the investigators declare to have any conflicts of interest.

### 7.4 Data storage and preservation

All samples will be destroyed after completion of the study. Paper-based and electronic source data and related material will be preserved for a minimum of 15 years to enable understanding of the study procedures, which allows the work to be assessed retrospectively and repeated if necessary. The study site will retain a copy of the documents to ensure that local collaborators can provide access to the source documents to a monitor, auditor, or regulatory agency. Electronic source documents will be stored on a flash drive and kept at the study site (IdC PHL, Pemba, Tanzania). The primary data storage and backup will be in the Swiss TPH shared server and secondary data storage will be on personal, password-protected laptops of Jennifer Keiser, Sophie Welsche and Said Ali, and on SWICTHdrive (a cloud storage supported by University of Basel). Electronic data files and archiving conditions will be made strictly confidential by password protection.

### 7.5 Study documents: translations – reference language

- The protocol master document will be in English, all further language versions are translated thereof
- The ICF master document will be in English, all language versions are translated thereof.

## 8. Statistics

### 8.1 Definition of primary endpoint

Egg reduction rate calculated from the geometric means of co-administered moxidectin/ albendazole and ivermectin/ albendazole against *T. trichiura* assessed at 14-21 days post-treatment is the primary endpoint in our study.

## 8.2 Justification of number of trial subjects

For the primary analysis the trial is designed as 2 arm parallel group randomized-controlled trial. We test the primary hypothesis that the treatment combination moxidectin and albendazole is not inferior compared to ivermectin and albendazole. To determine the required sample size, we run a series of simulation using artificial data which behaved roughly in the same way as found by [7]. Assuming true ERR of 98% in both arms and 98.5% in both arms, we estimate that 160 participants are required in each group to be at least 90% sure that the limits of a two-sided 95% confidence interval will exclude a difference in favor of the standard group of more than 2 percentage points. To account for a potential loss to follow-up of 10% and including a safety margin of 20% to account for uncertainty in our assumptions underlying the simulations, we anticipate enrolling 210 participants in each combination treatment arm (arm A and B). Secondary hypothesis will compare monotherapies against the combinations. Assuming cure rates below 25% for albendazole and ivermectin and 40% for moxidectin monotherapy respectively, we need to enroll 20, 20 and 80 children, respectively, to identify a statistical significant difference with 85 to 90% power (arm C, D, E) [1, 5, 8, 39]. We thus aim to recruit 210 + 210 + 20 + 20 + 80 = 540 participants in total.

The suggested sample size of a maximum of 4 PK samples from 60 willing participants (10-15 per study arm) is sufficiently high to determine the population PK parameters and investigate drug-drug interactions with a sparse sampling scheme, considering that PK variability is moderate. A moderate PK variability is a reasonable assumption when dealing with adolescents.

## 8.3 Description of statistical methods

In non-inferiority trials, non-inferiority has to be demonstrated in the intention-to-treat and in the per protocol population. The primary analysis will be performed according to the intention-to-treat principles using the available case population which includes all participants with any primary end point data. Subsequently, a per-protocol analysis will be performed. EPG will be assessed by calculating the mean egg count from the quadruplicate Kato-Katz thick smears and multiplying this number by a factor of 24. The ERR will be calculated as:

$$ERR = 1 - \frac{\frac{1}{n} e^{\sum \log\left(EPG_{follow-up} + 1\right)} - 1}{\frac{1}{n} e^{\sum \log\left(EPG_{baseline} + 1\right)} - 1}$$

Geometric mean egg counts will be calculated for the different treatment arms before and at 14-21 days after treatment to assess the corresponding ERRs. Bootstrap resampling method with 5,000 replicates will be used to calculate 95% confidence intervals (CIs) for ERRs and the difference between the ERRs. CRs will be calculated as the percentage of egg-positive children at baseline who become egg-negative after treatment. Differences among CRs will be assessed by using unadjusted logistic regressions. In a subsequent analysis an adjusted logistic regression (adjustment for baseline infection intensity, age, sex, weight) will be performed.

Questionnaires on physical functioning and treatment satisfaction will be evaluated by creating summary scores by summing up and transforming the single question scores according to the following formula: [(actual raw score-lowest possible raw score)/(possible raw score range)]*100 [40].

Adverse events will be summarized in frequency tables and compared between treatment groups using descriptive statistics.

To assess differences in diagnostic performance of the different tools used as compared to the standard Kato-Katz microscopy (FECPAK-G2 and/or PCR) a readily available hierarchical Bayesian egg-count model will be applied to the individual level data.

A nonlinear mixed-effects (NLME) modelling will be used to determine pharmacokinetic parameters. Concentrations are measured with a validated LC-MS/MS method [20, 31]. Using NLME, the key population PK parameters will be calculated based on which an effect on the drug-drug interaction will be determined:

- $C_{max}$ maximal plasma concentration
- $t_{max}$ time to reach $C_{max}$
- AUC area under the curve, from 0 to 24h and 0 to inf.
- $t_{1/2}$ elimination half-life

$C_{max}$ and $t_{max}$ will be observed values derived from the plasma concentration-time profile. Total drug exposure (AUC) and $t_{1/2}$ will be calculated with the NLME modeling software Monolix 2018R2 (Lixoft, Antony, France) using compartmental analysis. The elimination half-life will be estimated by the equation: $t_{1/2} = \ln 2/\lambda$, where $\lambda$ (the elimination rate constant) will be determined by performing a regression of the natural logarithm of the concentration values during the elimination period. Primary PK parameters including absorption rate ($k_a$), volume of distribution (V), and clearance (CL) will be estimated utilizing NLME modeling.

The exact design of the sparse sampling scheme will depend on the results of current PK trials of our group. Optimization in nonlinear regression will be based on the Fisher information matrix $M_F$. Number of groups, subjects per group, samples per subjects and sampling times in each group will be adjusted to maximize the determinant of the Fisher matrix and hence minimizing the standard errors (SE < 25%).

# 9. Duties of the investigator

## 9.1 Investigator's confirmation

This trial will be conducted in accordance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (R2) (ICH-GCP) and the current version of the Helsinki Declaration.

All protocol modifications must be documented in writing. A protocol amendment can be initiated by either the Sponsor/PI or any Investigator. The Investigator will provide the reasons for the proposed amendment in writing and will discuss with the Sponsor/PI and Co-PIs. Any protocol amendment must be approved and signed by the Sponsor/PI and must be submitted to the appropriate Independent Ethics Committee (IEC) for information and approval, in accordance with local requirements, and to regulatory agencies if required. Approval by IEC must be received before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial, e.g. change of telephone number(s).

## 9.2 Damage coverage

A general liability insurance of the Swiss TPH is in place (Winterthur Police Nr. 4746321) and participant liability insurances will be issued in Pemba (Tanzania).

## 9.3 Project management

The trial team will include the PI (Prof. Jennifer Keiser), five Co-PIs (Mr. Said Mohammed Ali, Mr. Ghanil Mohamed Khatib, Dr. Daniela Hofmann, Ms Sophie Welsche and Mr. Emmanuel Mrimi), a trial statistician (Dr. Jan Hattendorf), as well as two local physicians and several laboratory technicians. Prof. Jennifer Keiser, Mr. Said Mohammed Ali, Mr. Ghanil Mohamed Khatib, Ms. Sophie Welsche and Mr. Emmanuel Mrimi will be responsible for staff management, communication with the collaborative group, recruitment monitoring, data management, safety reporting, analysis, report writing and dissemination of the trial results. Mr. Said Mohammed Ali and Mr. Ghanil Mohamed Khatib are responsible for supervision of the lab- and field technicians, staff management, recruitment monitoring, supply of the material, contact to the local authorities and participating schools.

The investigator team is responsible for ensuring that the protocol is strictly followed. The investigator should not make any changes without the agreement of the Principal Investigator and the Co-Investigators, except when necessary to eliminate an apparent immediate hazard or danger to a study participant. The investigator will work according to the protocol and GCP. The investigator may take any steps judged necessary to protect the safety of the participants, whether specified in the protocol or not. Any such steps must be documented. During the treatment, the records are maintained by the responsible medical doctor. All entries have to be made clearly readable with a pen. The investigator must be thoroughly familiar with the properties, effects and safety of the investigational pharmaceutical product.

# 10. Ethical considerations

## 10.1 Independent Ethics Committee (IEC)

The study will be submitted for approval by the institutional research commission of the Swiss TPH and the ethical committees of Switzerland and Zanzibar. The study will be undertaken in accordance with the Declaration of Helsinki and good clinical practice (GCP).

## 10.2 Evaluation of the risk-benefit ratio

Albendazole, ivermectin, and moxidectin are well-known drugs and have little and mainly mild adverse events as described to date (headache, abdominal pain etc.). Albendazole and ivermectin are widely used drugs in mass treatment programs against filariasis and moxidectin is an FDA-approved drug against onchocerciasis. All community members enrolled in the study will benefit from a clinical examination and a treatment against STHs. All participating subjects remaining positive for *T. trichiura* will be treated with ivermectin (200 µg/kg)/ albendazole (400 mg), considering this combination showed higher efficacy compared to the existing standard treatment (albendazole alone) and the recent inclusion of ivermectin as recommended treatment scheme on the Essentials Medicines List [4].

## 10.3 Subject information and consent

All parents or caregivers of eligible adolescents and all participants <18 years will be asked to sign a written informed consent sheet. In case the person is illiterate, an impartial witness that can read and write has to sign the consent and the illiterate participant has to give a thumb print. Parents or caregivers and adult participants will have sufficient time for reflection of their child's or their own participation, respectively. Additionally, adolescent children (aged 12-17 years) will be briefed verbally, and written assent will be sought in form of their name written down or if illiterate by providing a thumb print.

Information sessions at the respective schools will be conducted to explain to caregivers and potential participants the purpose and procedures of the study. Parents or caregivers attending this meeting will receive a small provision to cover their costs for transportation (~US$ 2). One of the parents/caregiver of an eligible individual will be asked to sign a written informed consent form (translated into the local language, i.e. Kiswahili) after having had sufficient time for reflection of their child's participation. Adolescents will be asked orally for assent. Participation is voluntary and individuals have the right to withdraw from the study at any given point in time with no further obligations. Participation itself will not be awarded with compensation.

### 10.4 Subjects requiring particular protection

Our study will include school-based adolescents, since *T. trichiura* infection occurs often in children and adolescents; hence this age group is at high risk of infection. Pharmacokinetic/-dynamic and non-inferiority studies have not been conducted to date in this population between co-administration of ivermectin and albendazole against co-administration of moxidectin and albendazole. Our trial will produce more evidence to support the search for a safe and effective treatment of STH infections in adolescents and whole community.

## 11. Quality control and quality assurance

### 11.1 Monitoring and auditing

We will work with a locally based external monitor. He/she will conduct site visits to the investigational facilities for the purpose of monitoring the study. Details will be described in a separate monitoring plan. The investigator will permit them access to study documentation and the clinical supplies dispensing and storage area. Monitoring observations and findings will be documented and communicated to appropriate study personnel and management. A corrective and preventative action plan will be requested and documented in response to any significant deviation. No sponsor-initiated audits are foreseen, but audits and inspections may be conducted by the local regulatory authorities or ethics committees. The investigator agrees to allow inspectors from regulatory agencies to review records and is encouraged to assist the inspectors in their duties, if requested.

### 11.2 Data and safety monitoring board (WHO) / data monitoring committee (EU/FDA)

In our study, no data and safety monitoring board will be established, since we work with well-known drugs in a small sample size and using a single dose treatment. However, advisors will be informed regularly and the findings discussed.

## 12. Dissemination of results and publication

The final results of this study will be published in a scientific journal and presented at scientific conferences. BMGF will be acknowledged as study funder. All results from this investigation are considered confidential and shall not be made available to any third party by any member of the investigating team before publication. A summary of study conclusions will be shared with ZAHREC. After publication, study results will be made available to study participants.

# 13. References


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