# ISGlobal - Barcelona Institute for Global Health

*"An Adaptive phase II/III Single-Blinded, Randomized, Multi-Centre, Parallel-Group, Active-Controlled, Superiority Study to Evaluate the Safety and Efficacy of a Single Day or 3-day Single Dose of an ALBENDAZOLE-IVERMECTIN Co-formulation vs ALBENDAZOLE for the Treatment of Soil-Transmitted Helminth Infections (Trichuris trichiura, hookworm, Strongyloides stercoralis) in Paediatric and Young Adult Population (ALIVE Study)"*

## STATISTICAL ANALYSIS PLAN

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## Approval Page

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**ABBREVIATIONS**

| Abbreviations | Explanation |
|---|---|
| ADR | Adverse Drug Reaction |
| AE | Adverse Event |
| CDMS | Clinical Data Management System |
| CSV | Comma-Separated Values |
| DBMS | Database Management System |
| DMP | Data Management Plan |
| DSMB | Data Safety Monitoring Board |
| DTA | Data Transfer Agreement |
| CRF | Case Report Form |
| EDC | Electronic Data Capturing |
| EDTF | Electronic Data Transfer Form |
| FDC | Fixed Dose Co-formulation |
| GCP | Good Clinical Practice |
| ICH | International Conference on Harmonisation |
| IP | Investigational Product |
| ITT | Intention to treat |
| MedDRA | Medical Dictionary for Regulatory Activities |
| PK | Pharmacokinetics |
| PP | Per Protocol |
| PT | Preferred Term |
| SaaS | Software as a Service |
| SAS | Statistical Analysis System |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| SQL | Structured Query Language |
| TEAE | Treatment-Emergent Adverse Event |
| UBIOES-DM | Biostatistics and Data Management Unit |
| WHO | World Health Organization |

# 1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies to address the study's objectives in Adaptive phase II/III Single-Blinded, Randomized, Multi-Centre, Parallel-Group, Active-Controlled, Superiority Study to Evaluate the Safety and Efficacy of a Single Day or 3-day Single Dose of an ALBENDAZOLE-IVERMECTIN Co-formulation vs ALBENDAZOLE for the Treatment of Soil-Transmitted Helminth Infections (*Trichuris trichiura*, hookworm, *Strongyloides stercoralis*) in Paediatric and Young Adult Population (ALIVE Study).

# 2. STUDY OBJECTIVES AND OUTCOMES

## 2.1 Objectives

### 2.1.1 Phase II component

#### 2.1.1.1 Primary objective

To evaluate the **safety** of the FDC as a single dose or three-dose regimen for the treatment of *T. trichiura* in paediatric and young adult population.

#### 2.1.1.2 Secondary objectives

1. To evaluate the efficacy of FDC against *T. trichiura* in paediatric population.
2. To evaluate the efficacy of FDC against hookworms and *S. stercoralis* in those co-infected with species concomitantly to their infections with *T. trichiura.*
3. To describe the extent of albendazole and ivermectin exposure in different weight strata.
4. To evaluate the acceptability/palatability of the FDC 400mg-18mg and 400mg-9mg.

### 2.1.2 Phase III component

#### 2.1.2.1 Primary objective

To evaluate the efficacy of the FDC as a single dose or 3-day single dose regimen compared to the standard single dose regimen of ALB (400 mg) for the treatment of *T. trichiura* in paediatric and young adult population.

#### 2.1.2.2 Secondary objectives

1. To evaluate the **efficacy** of the FDC as a single dose or 3-day single dose regimen for the treatment of hookworm and *S. stercoralis*.
2. To evaluate the safety of the FDC as a single dose or 3-day dose regimen for the treatment of *T. trichiura*, hookworm and *S. stercoralis*.
3. To evaluate the performance of PCR in calculating the primary outcome measurement (efficacy) compared to an egg counting method (Kato-Katz).
4. To evaluate the frequency of known ALB resistant alleles in hookworm and *T. trichiura* in the three treatment arms before and after treatment.

#### 2.1.2.3 Exploratory objectives

1. To assess the efficacy of the FDC against *A. lumbricoides* in co-infected participants compared to single dose ALB in participants co-infected with this STH.

2. To describe the efficacy of the FDC in the prevalence of scabies compared to single dose ALB 400 mg.
3. To evaluate the efficacy of the FDC in co-infected participants compared to single dose ALB in participants co-infected with STH.

## 2.2 Outcomes

### 2.2.1 Primary outcome

**Safety evaluations:** Safety evaluations and measurements, including adverse events (AEs), vital signs, physical examination, weight, height and BMI will be assessed from the time of signing the informed consent through to post-treatment follow-up visit. To evaluate safety, participants will be evaluated as detailed in the schedule of visits (Table 1 and Table 2). All AEs during all study visits, will be noted in the CRF for each participant. A close surveillance, 3 hours post-treatment, will be conducted each day a participant receives treatment.
Any clinically significant abnormalities persisting at the end of the study will be followed up by the study physician until resolution or until a clinically stable endpoint is reached.
A Data Safety Monitoring Board (DSMB) will monitor the ongoing safety of the participants during the study.

**Efficacy evaluation**: Anti-helminthic primary efficacy, measured by cure rate will be determined by analysing a stool sample taken 21(+/-7) days after completing treatment with Kato Katz, Baermann. [1,2]
Cure is defined as absence of the species of STH in participants who had a positive egg count and/or larva for that STH species at baseline. Cure rate (CR) is defined as the proportion of individuals cured (absence of any egg and/or larva) to the total of those infected at baseline with each particular species of STH.

**Table 1. Schedule of Activities Phase II Component (KENYA only)**

| Procedures | Study Visit 0 Screening Day -7 to -1 | Study Visit 1 Enrolment/ Baseline Day 0 | Study Visit 2 Day 1 | Study Visit 3 Day 2 | Study Visit 4 Day 3 | Study Visit 5 Day 7 | Study Visit 6 Day 21+/-7 day post treatment | Unscheduled | Withdrawal |
|---|---|---|---|---|---|---|---|---|---|
| Informed consent[1] | X | | | | | | | | |
| Informed assent[2] | X | | | | | | | | |
| Stool collection kit delivered | X | | | | | | X | | |
| Stool collection[3] | X | | | | | | X | | X |
| Stool analysis (Baermann and Kato-Katz)[4] | X | | | | | | X | | X |
| Inclusion and Exclusion criteria | X | X | | | | | | | |
| Demographics[5] | X | | | | | | | | |
| Physical exam[6] | X | X | | | | | X | X | X |
| Vital signs[7] | X | X | X | X | X | X | X | X | X |
| Height | X | X | | | | | | | |
| Weight | X | X | | | | | | | |
| Urine Pregnancy test[8] | X | | | | | | | | |
| Haematuria strip test as proxy *for Schistosoma* [h] | X | | | | | | | | |
| Randomization and treatment assignation | | X | | | | | | | |
| Study drug administration | | X[9] | X[10] | [11]X | | | | | |
| Acceptability questionnaire Arm 1 (ALB) | | | | | | | | | |
| Acceptability questionnaire Arm 2 (FDCx1) | | X | | | | | | | |
| Acceptability questionnaire Arm (FDCx3) | | | | X | | | | | |
| PK blood sampling | | X[12] | X[l] | X[l] | | | | | |
| Study drug accountability | | X | X | X | | | X | | X |
| Concomitant medication & disease review | X | X | X | X | X | X | X | X | X |
| Adverse event monitoring[13] | X | X | X | X | X | X | X | X | X |
| Complete Case Report Form (CRF) | X | X | X | X | X | X | X | X | X |

[1] Informed consent will be obtained from parents or guardians of all participants <18years

[2] Assent to participate in the trial will be obtained from participants 12-17 years old.

[3] A stool collection kit will be provided to participants for the collection of stool samples after providing assent and consent. The stool should be collected <24 h before examination for eligibility check. An aliquot of the collected stool sample will be stored for QC by PCR to confirm microscopic results from Kato-Katz and Baermann test if microscopy positive at screening and for all follow-up stool samples. All samples will be tested at site. Should technical issues arise or in case of discrepancy in results, an aliquot of the stool sample will be tested abroad for QC purposes.

[4] Lab tests will be performed on a fresh stool sample collected <24 hours before examination.

[5] Age, Date of Birth, Sex, Participant ID, Guardian relationship, age and contact details

[6] Physical exam should include clinical diagnosis of scabies (rush/lesions on skin) before and after treatment. During unscheduled visits Physical exam will be performed only if necessary.

[7] Vital signs should Include heart rate, respiration rate, body temperature and blood pressure.

[8] A urine test kit will be provided in order to collect a urine sample from which will be examined by a haematuria strip test as a proxy for *Schistosoma* eggs and to confirm negative pregnancy test for female participants ≥12 years old or post-menarche.

[9] Treatment Day (D)0. For all participants in Arms 1, 2 and 3. Treatment dose will be given under direct supervision.

[10] Treatment D1. Only for participants in treatment Arm 3. Close observation for 3 hours post-treatment for safety evaluations

[11] Treatment D2. Only for participants in treatment Arm 3. Close observation for 3 hours post-treatment for safety evaluations.

[12] Population PK (2 timepoints per participant). for ALB arm at 1, 2, 3, 4, 5, 6, 7, 8, or 24h; for the single dose FDC arm at 1, 2, 3, 4, 5, 6, 7, 8, 24h, 48 or 72h; for three-dose FDC arm at pre-dose Day 3, 1, 2, 3, 4, 5, 6, 7, 8, 24h, 48 or 72h post-administration.

[13] Adverse events will be assessed by direct observations of the study physician, or reported by the participant or parent/guardian.

**Table 2. Schedule of Activities Phase III component (all sites)**

| Procedures | Pre-screening (up to 3 months prior scree.) | Study Visit 0 Screening Day -7 to -1 | Study Visit 1 Enrolment/Baseline Day 0 | Study Visit 2 Day 1 | Study Visit 3 Day 2 | Study Visit 4 Day 3 | Study Visit 5 Day 7 | Study Visit 6 Day 21+/-7 day post treatment | Unscheduled | Withdrawal |
|---|---|---|---|---|---|---|---|---|---|---|
| Informed consent[14] | X | X | | | | | | | | |
| Informed assent[15] | X | X | | | | | | | | |
| Stool collection kit delivered | | X | | | | | | X | | |
| Stool collection[16] | | X | | | | | | X | | X |
| Stool analysis (Baermann and Kato-Katz)[17] | | X | | | | | | X | | X |
| Inclusion and Exclusion criteria | | X | X | | | | | | | |
| Demographics[18] | | X | | | | | | | | |
| Physical exam[19] | | X | X | | | | | X | X | X |
| Vital signs[20] | | X | X | X | X | X | X | X | X | X |
| Height | | X | X | | | | | | | |
| Weight | | X | X | | | | | | | |
| Urine Pregnancy test[21] | | X | | | | | | | | |
| Haematuria Strip test as proxy for *Schistosoma*[h] | | X | | | | | | | | |
| Serum HIV test[22] | | X | X | | | | | | | |
| Randomization and treatment assignation | | | X | | | | | | | |
| Study drug administration | | | X[23] | X[24] | X[25] | | | | | |
| Study drug accountability | | | X | X | X | | | X | | X |
| Concomitant medication & disease review | | X | X | X | X | X | X | X | X | X |
| Adverse event monitoring[26] | | X | X | X | X | X | X | X | X | X |
| Complete Case Report Form (CRF) | | X | X | X | X | X | X | X | X | X |

### 2.2.2 Secondary outcomes

**Treatment efficacy:** Anti-helminthic efficacy, measured through egg reduction rate (ERR), will be determined by analysing a stool sample taken 21(+/-7) days after completing treatment with Kato Katz[2]. Egg reduction rate (ERR) will be calculated by using geometric means, calculated for hookworms, *T. trichiura* and *A. lumbricoides*. In addition, Anti-helminthic efficacy, measured by cure

[14] Informed consent will be obtained from parents or guardians of all participants <18years. Informed consent/assent can be performed up to 3 months prior to screening visit as part of the pre-screening activities OR during screening visit

[15] Assent to participate in the trial will be obtained from participants 12-17 years old.

[16] A stool collection kit will be provided to participants for the collection of stool samples after providing assent and consent. The stool should be collected <24 h before examination for eligibility check. An aliquot of the collected stool sample will be stored for QC by PCR to confirm microscopic results from Kato-Katz and Baermann test if microscopy positive at screening and for all follow-up stool samples. All samples will be tested at site. Should technical issues arise or in case of discrepancy in results, an aliquot of the stool sample will be tested abroad for QC purposes.

[17] Lab tests will be performed on a fresh stool sample collected <24 hours before examination.

[18] Age, Date of Birth, Sex, Participant ID, Guardian relationship, age and contact details

[19] Physical exam should include clinical diagnosis of scabies (rush/lesions on skin) before and after treatment. During unscheduled visits Physical exam will be performed only if necessary.

[20] Vital signs should Include heart rate, respiration rate, body temperature and blood pressure.

[21] A urine test kit will be provided in order to collect urine sample from all participants which will be examined by haematuria strip test as a proxy *for* Schistosoma eggs and to confirm negative pregnancy test for female participants ≥12 years old or post-menarche.

[22] Serum HIV test will be offered only to eligible participants at the Manhiça site in Mozambique because of high HIV prevalence.

[23] Treatment Day 0. For all participants in Arms 1, 2 and 3. Treatment dose will be given under direct supervision.

[24] Treatment Day 1. Only for participants in treatment Arm 3. Close observation for 3 hours post-treatment for safety evaluations.

[25] Treatment Day 2. Only for participants in treatment Arm 3. Close observation for 3 hours post-treatment for safety evaluations.

[26] Adverse events will be assessed by direct observations of the study physician, or reported by the participant or parent/guardian.

rate will be determined by analysing a stool sample taken 21(+/-7) days after completing treatment with qPCR.

At pre- and post-treatment visits a duplicate Kato-Katz test will be performed on a fresh stool sample collected <24 hours before examination. Baermann method will also be performed in parallel to Kato-Katz for the search of *S. stercoralis*. Evaluation by qPCR will be performed in pre- and post-treatment samples.

**Pharmacokinetic outcome measures (only phase II):** Two blood samples for population PK analysis will be collected for every participant by finger prick using the mitra devices [3] in the phase II component to adjust a population pharmacokinetic model. Additionally, a NCA will be performed to obtain (Cmax), time to reach Cmax (Tmax) and area under the curve (AUC) of ALB and IVM.
A complete description of the pk analysis can be found in the statistical analysis plan for pharmacokinetics.

**Participant acceptability/palatability outcome measures (only phase II):**
Facial hedonic scale and implied numerical rating scale (NRS) will be included in the Phase II component of the trial to measure attributes for the FDC orodispersible formulation in terms of taste, aftertaste; mouth feel; smell; time needed to dissolve/disperse (duration of administration). Secondary aspects will include visual aspects: embossing, surface aspects and colour. Refer to the protocol Section 9.5.2 for details.

**Resistance:** Whole genome sequencing, next-generation DNA sequencing and omic approaches will be used to assess underlying factors associated with low treatment response including assessment and evaluation of new protocols for sample processing and sequencing.

# 3. STUDY DESIGN

## 3.1 Design

Adaptive phase II/III trial to compare safety and efficacy of the active control arm (current standard of care) against 2 experimental arms.

**Phase II component**
Uni-centric, 3-arm, parallel, open-label, randomised, phase II trial to determine the safety and tolerability of FDC in paediatric population in different weight strata. Stratification by weight thus recruiting sequentially for safety and tolerability.

- Group 1 (38 participants): with body weight of [23-<30) Kg will receive 300-391 µg/Kg (FDC 400mg-9mg) or ALB.
- Group 2 (38 participants): with body weight of [30-45] Kg will receive 400-600 µg/Kg (FDC 400mg-18mg) or ALB.
- Group 3 (50 participants): with body weight of [15-23) Kg will receive 391-600 µg/Kg (FDC 400mg-9mg) or ALB.

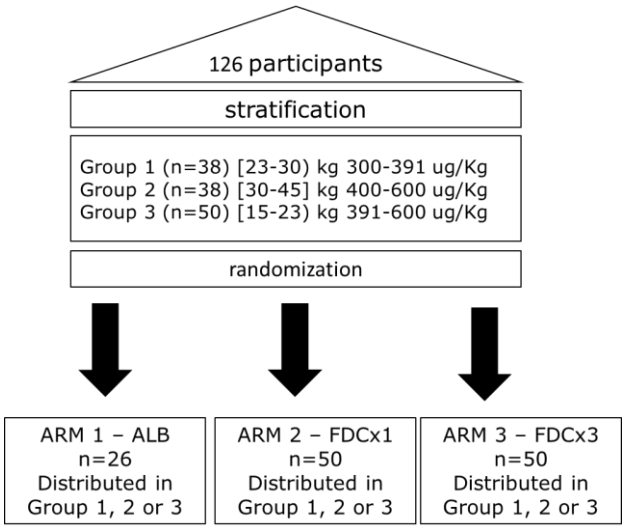

Figure 2. Phase II design (Kenya)

**Phase III component**

A Single-Blinded, Randomized, Multi-Centre, Parallel-Group, Active-Controlled, Superiority Study to Evaluate the Efficacy and Safety of a Single Day or 3-day Single Dose of FDC for the treatment of *Trichuris trichiura*, hookworm, *Strongyloides stercoralis* in paediatric and young adult population.

a. Arm 1: Single dose of a tablet of ALB 400 mg (active control arm)
*b.* Arm 2: Single dose of a tablet of FDC 400mg-18mg (≥45 kg of body weight at baseline) or 400mg-9mg (<45 kg of body weight at baseline).
c. Arm 3: Daily dose of a tablet of FDC 400mg-18mg (≥45 kg of body weight at baseline) or 400mg-9mg for 3 days (<45 kg of body weight at baseline).

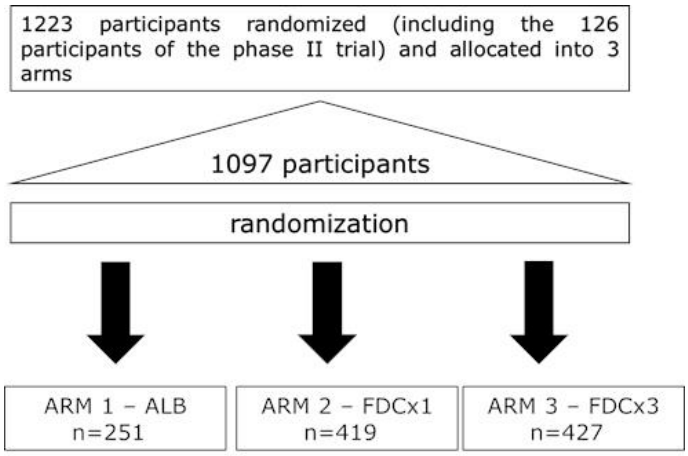

Figure 3. phase III design (Kenya, Ethiopia, Mozambique)

## 3.2 Trial sites

This study will be conducted in primary and secondary schools in Kenya (phase II and phase III), Ethiopia (phase III) and Mozambique (phase III).

## 3.3 Treatment

**Phase II component**

Participants will be randomly allocated with unequal probability to receive either:

Treatment Arm 1: Single dose of a tablet of ALB
Treatment Arm 2: Single dose of a tablet of FDC
Treatment Arm 3: Daily dose of a tablet of FDC for 3 days.

and will be recruited by µg/Kg of IVM to be received in a sequential manner starting from Group 1 through to Group 3 as shown below:

- Group 1: receiving 300-391 µg/Kg or ALB 400mg- 38 participants (body weight: 23-<30 Kg) will be recruited in this group. (FDC 400mg-9mg).
- Group 2: receiving 400-600 µg/Kg or ALB 400mg– 38 participants (body weight: 30-45 Kg) will be recruited in this group. (Participants <45 kg: FDC of 400mg ALB-9mg IVM, participants ≥45 kg: FDC of 400mg ALB-18mg IVM).
- Group 3: receiving 391-600 µg/Kg or ALB 400mg- 50 participants (body weight: 15-23 Kg) will be recruited in this group. (FDC 400mg-9mg).

**Phase III Component**

Participants will be randomly allocated with unequal probability (Arm 1 p=0.2, Arm2 p=0.4, Arm3 p=0.4), according to the specific expected cure rate by specie, to receive either:

Treatment Arm 1: Single dose of a tablet of ALB 400 mg (active control arm).
Treatment Arm 2: Single dose of a tablet of FDC 400mg-18mg or 400mg-9mg.
- For participants <45 kg of body weight at baseline: FDC of 400mg ALB-9mg IVM.
- For participants ≥45 kg of body weight at baseline: FDC of 400mg ALB-18mg IVM.

Treatment Arm 3: Daily dose of a tablet of FDC 400mg-18mg or 400mg-9mg for 3 days.
- For participants <45 kg of body weight at baseline: FDC of 400mg ALB-9mg IVM.
- For participants ≥45 kg of body weight at baseline: FDC of 400mg ALB-18mg IVM.

## 3.4 Randomisation

In the phase II component, participants with *T. trichiura* infection (confirmed by Kato-Katz technique in a fresh stool sample) will be enrolled in a sequential manner per weight group in order to administer the desired dose, starting from 300-391 µg/kg ivermectin. Participants in the phase II trial will be stratified in different weight groups: Group 1 (23-<30 kg) 38 participants, group 2 (30-45 kg) 38 participants and group 3 (15-23 kg) 50 participants in order to gradually increase the dose of ivermectin in the FDC. Then, participants will be allocated by simple randomization to one of the three study arms with unequal probability (ALB: p=0.2, n=26; FDCx1: p=0.4, n=50; FDCx3: p=0.4, n=50)

In the phase III component, allocation of participants to study arms will be done by block randomization and stratified by STH species. We will ensure balanced allocation to the three arms in the three study countries. Treatment allocation for each study participant will be concealed in opaque sealed envelopes that will be opened only after enrolment. Study participants will be assigned a unique study number linked to the allocated treatment group. Participants will be randomly allocated with unequal probability, according to the specific expected cure rate of each species.

## 3.5 Determination of sample size

Sample size was calculated based on available data from peer-review publications complemented with reasonable estimates of efficacy for those experimental groups that have not been previously tested. For these calculations, the efficacy of the control arm (Albendazole 400mg in a single dose), was obtained for *T. trichiura* and hookworms from a systematic review and meta-analysis where

temporary trends in efficacy (with the corresponding confidence intervals) were incorporated (Table 3)[4]. For the efficacy of the control arm against *S. stercoralis*, an alternative source was used [5], since the systematic review by *Moser et al* did not include this species; for that reason, a clinical trial that included an arm of Albendazole 400mg for 3 consecutive days was used, assuming a "best case scenario" for the efficacy of the control group; this estimated efficacy is also in the range of a systematic review assessing the efficacy of Albendazole at various (but not single) doses[5]. For the FDC at single dose, the calculations were based on the estimated efficacies (and their corresponding confidence intervals) in a systematic review that calculated the Relative Risks of cure of diverse drug regimens against Albendazole 400mg single dose (Table 3)[6,7]. For. *S. stercoralis*, the estimated efficacy of FDC was calculated based on a recent clinical trial using Ivermectin single and multiple-dose regimens [8]. Finally, for the FDC in 3-dose regimens, considering its use in public health, deployment logistics in MDA campaigns and expected impact of the FDC, we estimated that an improvement of at least 15 percentage points would be the minimum improvement in efficacy to be demonstrated in order to make the FDCx3 regimen worth considering.

Sample size was calculated estimating the efficacy of the different experimental drug or combinations for each of the STH of interest [9,10], and gathering the individual samples sizes for the study. The sample size was calculated for pairwise comparisons of the expected Cure Rates for three study groups with an overall significance level of 5% adjusted for multiple tests by Bonferroni's correction, 80% power and inflated for 10% lost-to-follow-up.

The estimated total number of participants for the adaptive design is 1223 (*T. trichiura* 625, *S. stercoralis* 286 and hookworm 312). This sample size is powered to be able to measure efficacy for all three species in the phase III component. The sample size for the phase II component is 20% of the total participants for *T. trichiura* (126 participants). The remaining 80% of the *T. trichiura* participants will be randomised in the phase III component. Table 4 details the stratification of the participants per species with a 10% lost-to-follow-up.

The total number of participants to be included in this clinical trial is 1223 (126 in phase II and 1097 in phase III). Following randomisation, 251 participants will be allocated to the ALB treatment arm, 427 will be allocated to the FDC treatment arm and 419 in the FDCx3 treatment arm. Table 4 details the stratification of the participants per species with a 10% lost-to-follow-up.

The 10% lost-to-follow-up inflated sample sizes of the ALB-group for *T. trichiura*, *S. stercoralis* and hookworm are 129, 47 and 101 respectively, and the corresponding sample sizes of the individual experimental treatment groups are 248, 120, and 101 for FDC-group and 248, 119 and 110 for FDCx3-group. The corresponding powers of each of the tests are 91%, 100% and 80% to compare the CRs of *T. trichiura* in ALB *vs* FDC, ALB *vs* FDCx3 and FDC *vs* FDCx3 respectively; 95%, 100% and 80% to compare the CRs of *S. stercoralis* in the above-mentioned comparisons and 82%, and 80% to compare the CRs of hookworm respectively for ALB vs FDCx3 and FDC vs FDCx3 comparisons. We do not compare CRs of hookworm in ALB and FDC as they are known to be the same. These powers were computed assuming that the expected CRs for *T. trichiura* are 23.7%, 43.7% and 59% in the ALB, FDC and FDCx3 groups respectively; 45%, 79% and 94% for *S. stercoralis* and 79.5%, 79.5% and 95% for hookworm. **Table 3** and **Table 4** show the sample size calculation according to the expected Cure Rate for each drug and helminth of interest, and the resulting sample size inflated by 10% due to the estimated lost-to-follow-up. Assumptions used for the sample size calculations (e.g. expected CRs, lost-to-follow-up, etc) should be checked during the interim analysis.

### 3.6 Study stopping guidance

The following stopping rules will apply at relevant key instances in the ALIVE study:

1. The occurrence of ≥1 serious adverse reaction (where causality is at least possible) or SUSAR in any of the weight groups in the Phase II will result in suspension of dose escalation in the FDC arm pending review by the DSMB (keeping in mind the sequential recruitment strategy in phase II).
2. The occurrence of ≥2 serious adverse reactions or SUSARs at the end of phase II will result in suspension of progress to phase III pending review by the DSMB.

Overall the occurrence of serious adverse reactions or suspected unexpected serious adverse reactions in 2% of the study populations at interim analysis (50% recruitment) will result in suspension of further dosing of the Investigational Product pending review by the DSMB. This is equivalent to 12 safety events which meet these criteria in 612 participants recruited at the point of the interim analysis.

**Table 3. Sample size and power calculation according to the expected efficacy (Cure Rate) of the different treatment arms**

| Comparison | Target Power | Actual Power | Sample Size | Expected Cure Rate | Overall Alpha | Bonferroni adjusted Alpha |
|---|---|---|---|---|---|---|
| *Trichuris trichiura* | | | | | | |
| ALB | | | 116 | 23.7% | | |
| vs IVM-ALB | 80% | 91% | 223 | 43.7% | 0.05 | 0.0167 |
| vs (IVM-ALB)x3 | 80% | 100% | 223 | 59% | 0.05 | 0.0167 |
| IVM-ALB vs (IVM-ALB)x3 | 80% | 80% | | | 0.05 | 0.0167 |
| *Strongyloides stercoralis* | | | | | | |
| ALB | | | 42 | 45% | | |
| vs IVM-ALB | 80% | 95% | 108 | 79% | 0.05 | 0.0167 |
| vs (IVM-ALB)x3 | 80% | 100% | 107 | 94% | 0.05 | 0.0167 |
| IVM-ALB vs (IVM-ALB)x3 | 80% | 80% | | | 0.05 | 0.0167 |
| Hookworm | | | | | | |
| ALB | | | 91 | 79.5% | | |
| vs IVM-ALB | 80% | -- | 91 | 79.5% | 0.05 | 0.0167 |
| vs (IVM-ALB)x3 | 80% | 82% | 99 | 95% | 0.05 | 0.0167 |
| IVM-ALB vs (IVM-ALB)x3 | 80% | 80% | | | 0.05 | 0.0167 |

**Table 4. Final sample size calculation taking into account 10% lost-to-follow-up**

| | Phase II | | PHASE III | | PHASE II/III |
|---|---|---|---|---|---|
| **Group** | N | N total Phase II[1] | N | N total Phase III[1] | **N total** |
| ***Trichuris trichiura*** | | | | | |
| ALB | 23 | **26** | 93 | **103** | **129** |
| FDCx1 | 45 | **50** | 178 | **198** | **248** |
| FDCx3 | 45 | **50** | 178 | **198** | **248** |
| **Total** | **113** | **126** | **449** | **499** | **625** |
| ***Strongyloides. stercoralis*** | | | | | |
| ALB | | | 42 | **47** | **47** |
| FDCx1 | | | 108 | **120** | **120** |
| FDCx3 | | | 107 | **119** | **119** |
| **Total** | | **0** | **257** | **286** | **286** |
| **Hookworm** | | | | | |
| ALB | | | 91 | **101** | **101** |
| FDCx1 | | | 91 | **101** | **101** |
| FDCx3 | | | 99 | **110** | **110** |
| **Total** | | **0** | **281** | **312** | **312** |
| **TOTAL** | | **126** | | **1097** | **1223** |

[1]Calculated by inflating the sample size using considering 10% lost-to-follow-up

# 4. ANALYSIS POPULATIONS

## 4.1 Study population data sets

**Safety population**

The safety analysis will be based on the intention-to-treat (ITT) population, defined as all participants who were randomized in the trial.

**Efficacy population**

The efficacy analysis will be based on the ITT population. For the handling of missing data, the worst-case scenario will be considered, considering all individuals without efficacy data as treatment failures.

**Population PK analysis (phase II)** A complete description of the pk analysis can be found in the statistical analysis plan for pharmacokinetics.

For population pharmacokinetic analysis, the 126 participants included in the 3 arms of phase II (26 in arm 1, and 50 in the arms 2 and 3) will be randomly assigned to different subgroups (7 subgroups for arm 1, and 10 subgroups for arm 2 and 3) with different sampling times (2 blood samples per subgroup for arm 1 and 2, and 2 blood samples plus predose on Day 2 per subgroup for arm 3). The two sampling times has been randomly obtained from two different post-dosing ranges covering from 1 to 7 hours (range I) and from 8 to 72 hours (range II). Thus, the overall sampling times describes the entire pharmacokinetic profile for the two drugs (Ivermectin and Albendazole) for each of the 3 groups of body weight (Group 1: 23-30kg, Group 2: 30-45kg, and Group 3:15-23kg).

For the random assignment of the blood capilar extractions (with Mitra (40) Clamshell), a randomization of the times of PK extraction (9 points for arm 1 and 11 and 12 points for arms 2 and 3

respectively) will be carried out carried out for each arm of treatment: [+1h, +2h, +3h, +4h, +5h, +6h, +7h, +8h and +24h] for the arm 1 (albendazole treatment); and [+1h, +2h, +3h, +4h, +5h, +6h, +7h, +8h, +24h, +48h and +72h] for the arm 2 (coadministration IVM+Albendazole); and [baseline pre-administration on Day 2 and +1h, +2h, +3h, +4h, +5h, +6h, +7h, +8h, +24h, +48h and +72h] for arm 3 (coadministration IVM+Albendazole)x3 consecutive days)..
For Arm 1 (albendazole treatment), the 9 points (+1h, +2h, +3h, +4h, +5h, +6h, +7h, +8h and +24h) has been divided in 2 groups:
i) One group of 5 points comprising the points from +1h until +5h
ii) One group of 4 points comprising the points from +6h until +24h
For assigning the 2 points we randomize 1 time from each group
For Arm 1 (5 subgroups of 4 participants + 2 subgroups of 3 participants):

SubGroup 1 (n=4): +3h, +6h
SubGroup 2 (n=4): +4h, +24h
SubGroup 3 (n=4): +2h, +8h
SubGroup 4 (n=4): +5h, +7h
SubGroup 5 (n=4): +1h, +7h
SubGroup 6 (n=3): +2h, +24h
SubGroup 7 (n=3): +4h, +8h

For Arm 2 (albendazole + ivermectin treatment), the 11 points (+1h, +2h, +3h, +4h, +5h, +6h, +7h, +8h, +24h, +48h, +72h) has been divided in 2 groups:
i) One group of 6 points comprising the points from +1h until +6h
ii) One group of 5 points comprising the points from +7h until +72h
For assigning the 2 points we randomize 1 time from each group
For Arm 2 (10 groups of 5 participants):

SubGroup 1 (n=5): +4h, +72h
SubGroup 2 (n=5): +5h, +48h
SubGroup 3 (n=5): +2h, +8h
SubGroup 4 (n=5): +1h, +8h
SubGroup 5 (n=5): +3h, +72h
SubGroup 6 (n=5): +4h, +24h
SubGroup 7 (n=5): +3h, +7h
SubGroup 8 (n=5): +2h, +48h
SubGroup 9 (n=5): +5h, +7h
SubGroup 10 (n=5): +1h, +24h

For Arm 3 (10 groups of 5 participants), (albendazole + ivermectin treatment x 3 consecutive days), we follow the same schedules as for Arm 2, but adding predose on Day 2 and starting the extractions from that point, as follows

SubGroup 1 (n=5): predose Day 2, +4h, +72h
SubGroup 2 (n=5): predose Day 2, +5h, +48h
SubGroup 3 (n=5): predose Day 2, +2h, +8h
SubGroup 4 (n=5): predose Day 2, +1h, +8h
SubGroup 5 (n=5): predose Day 2, +3h, +72h
SubGroup 6 (n=5): predose Day 2, +4h, +24h
SubGroup 7 (n=5): predose Day 2, +3h, +7h
SubGroup 8 (n=5): predose Day 2, +2h, +48h
SubGroup 9 (n=5): predose Day 2, +5h, +7h

SubGroup 10 (n=5): predose Day 2, +1h, +24h

**Sensitivity analysis populations**

Safety sensitive populations: A per-protocol analysis will be performed including all randomized participants who received at least one dose of medication.

Efficacy sensitive population: A per-protocol analysis will be conducted, including all randomized participants who received at least one dose of medication, did not withdraw, without major protocol deviations, and were not lost to follow-up. Also, participants who did not meet the inclusion or exclusion criteria, received an incorrect treatment, or were administered an incorrect dose will be excluded from the per-protocol analysis.

## 4.2 Analysis close date

The analysis closing date is September 2023 after all data has been checked and cleaned.

**Data cleaning**

The data will be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any change will be made on the source documents (if applicable) and updated in the database accordingly.

## 4.3 Data download

The database will be locked once that all trial data are complete and verified according to the Monitoring Plan. When locked, the data will be downloaded into .csv, and .dta formats for statistical analyses.

# 5. STATISTICAL ANALYSES

## 5.1 General considerations

### 5.1.1 Data summary

Unless otherwise specified, summaries for continuous variables will include the descriptive statistics for number of participants (n), mean, standard deviation (SD), minimum (min), median, and maximum (max). The min and max values will be presented to the same number of decimal places as the raw data. The means and medians will be presented to one more decimal place than the raw data. The SDs will be presented to two more decimal places than the raw data. If the raw data has 3 decimal places or more, 3 decimal places will be presented for mean, median, min and max, and SDs.

Summaries for categorical (discrete or dichotomous) variables will include the number and/or percentage of participants in a particular category. Percentages will be presented to one decimal place, unless otherwise specified. Population counts (either number of participants or number of time points at the assessment) for each treatment group will be used as the denominator in the calculation of percentages unless otherwise specified.

### 5.1.2 Baseline

Unless otherwise specified, the baseline will be defined as the last non-missing value prior to the first dose of study drug.

Change from baseline is defined as the difference between the post-baseline assessment value and the baseline value, i.e., Post-baseline Assessment Value – Baseline Value.

### 5.1.3 Study day

Study day is relative to the day of first dose of study drug. Day 1 is defined as the day of the first dose of study drug. Study days after Day 1 are calculated as: Assessment date – Date of Day 1 + 1.
Study days prior to Day 1 are calculated as: Assessment date – Date of Day 1. The day prior to Day 1 is Day -1.

### 5.1.4 Unscheduled visits

Unscheduled visit value may be used to provide a measurement for a scheduled time point, a baseline, a last or a worst value, if appropriate according to their definitions. The unscheduled visit values will not be summarized by unscheduled visit but will be presented in the listings chronologically as the visit occurs.

### 5.1.5 Handling of missing data

Every effort will be made to minimise the amount of missing data in the trial. Information on the reason for missing data will be obtained, whenever possible.

**Baseline demographic data**

For missing data on age, gender, height and weight at baseline, multiple imputation will be performed.

**Efficacy data**

For the efficacy analysis, the worst-case scenario will be considered, where participants with missing data will be considered as treatment failures. In such a case it will be assumed that all participants with no egg count data at day 21 will have the same egg count as at baseline.

**Adverse Events**

The following imputation rules for missing or partial AE start dates are used:

- If only Day is missing, the start day will be the first day of the month or the date of study drug first dose if the AE end date is on/after the date of study drug first dose or is missing/partial AND the start year and month of the AE are the same as year and month of the study drug first dose date;
- If Day and Month are both missing, the start date will be January 1 of the year or the date of study drug first dose if the AE end date is on/after the date of study drug first dose or is missing/partial AND the start year of the AE is the same as year of the study drug first dose date;
- If Day, Month, and Year are all missing, the date will not be imputed. However, if the AE end date is on/after the date of study drug first dose, then the AE will be considered a TEAE.

The missing or partial AE stop data will not be imputed.

If the severity of an AE is missing, it will be classified as “severe” in the summary tables by severity. If the assessment of relationship of study drug is missing, it will be classified as “related” to the study drug.

**Concomitant Medications**

The same imputation rule as AE start date imputation will be used for missing or partial start dates of medications.

The missing or partial medication stop data will not be imputed.

### 5.1.6 Analysis visit window

In general, the analysis visit window will not be derived, and the visits will be used as reported on the electronic case report form (eCRF). If the derivation of analysis visit window is needed, the following analysis visit window approach will be applied:

- If the assessments are collected at a scheduled visit, the collected data will be mapped to the nominal scheduled visit;
- If the assessments are not collected at a scheduled visit but are collected at an unscheduled visit, the collected data will be mapped based on the analysis window derived as lower bound (exclusive) at the midpoint between the given scheduled visit and preceding scheduled visit, and upper bound (inclusive) at the midpoint between the given scheduled visit and next scheduled visit according to the scheduled visits for corresponding assessments as outlined in Table 1 and Table 2;
- If more than one assessments (not replicate/triplicate assessments) occur within a given analysis visit, the assessment closest to the target study day will be used. In case of ties between assessments located on different sides of the target day, the later assessment will be used. In case of ties located on the same side of the target day, the value with the later entry date/time will be used.

## 5.2 Participant characteristics

### 5.2.1 Participant disposition

Participant disposition will be summarized for the ITT population using the number and percentage of participants who are randomized, who are randomized but do not receive the study treatment, who are treated, who complete the study, and who early withdraw from the study and primary reason for early withdrawal from the study.

A separate summary will be provided for the number and/or percentage of participants who are screened, and who screen failures and reason for screen failures.

Participant disposition will be listed for all randomized participants. Participants who are screen failures as well as the eligibility status for the study will be listed for all screened participants as well.

### 5.2.2 Protocol deviations

Protocol deviations will be identified and categorized in the eCRF, and designated as major/critical or minor.

The major/critical protocol deviations will be summarized by category for the ITT population.

All protocol deviations will be presented in a listing.

### 5.2.3 Demographics and baseline characteristics

Demographics and other baseline characteristics will be summarized using the ITT population. The demographics characteristics will include age groups (school-age children (5 to 14 years-old) and young adults (15 to 18 years-old), gender, weight, height, body mass index (BMI). Other baseline characteristics will include photophobia, blurred vision, visual impairment, pruritus, skin lesions compatible with scabies, serum HIV test (Mozambique only), body temperature, blood pressure, heart rate, respiratory rate.
The baseline parasitological testing results, including number and percentage of participants who are positive for *Trichuris trichiura*, *Ascaris lumbricoides*, Hookworm, Haematuria, *Strongyloides stercoralis* will be presented.

Bivariate analysis of baseline demographics characteristics between screen failure participant and randomized participant may be presented.

Individual demographics and baseline characteristics will be listed.

### 5.2.4 Concomitant medications

Concomitant medications are defined as any medications that were ongoing or started on or after the first dose of study treatment.

Medications will be recorded and coded using the latest version of World Health Organization (WHO) Drug Enhanced Dictionary. Concomitant medications will be summarized by Anatomical Therapeutic Chemical (ATC) classification pharmacological or therapeutic subgroup (Level 2) and preferred drug name for the safety population. At each level of summarization, a participant is counted once if he/she reported 1 or more medications at that level.

The by-participant listing will be provided for prior and concomitant medications.

### 5.2.5 Concomitant diseases

The concomitant diseases will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA).

The concomitant diseases will be summarized by system organ class (SOC) and preferred term (PT) in the safety population.

The by-participant listing will be provided for concomitant diseases.

### 5.2.6 Study drug administration

For the single dose treatment arms, the number and percentage of participants who receive study drug will be summarized. For the repeat dose treatment arm, the number and percentage of participants who receive 1, 2, or 3 doses of study drug will be provided.

Study drug administration will be presented in a data listing.

## 5.3 Efficacy analysis

### 5.3.1 Hypothesis

The main hypothesis is that FDC (either at single or 3-day regimens) will be more effective against STH than the current strategy (single dose ALB alone).

Specific hypotheses:

- Primary Efficacy Endpoint: Higher estimated CR for *T. trichiura* 21 days after treatment, using microscopy in participants allocated to arms 2 and 3 than to arm 1.
- Secondary Efficacy Endpoint(s):
    - Higher estimated CR for hookworm and *S. stercoralis* 21 days after treatment using microscopy in participants allocated to arms 2 and 3 than to arm 1.
    - Higher estimated ERR for *T. trichiura* 21 days after treatment using microscopy in participants allocated to arms 2 and 3 than to arm 1.
    - Higher estimated CR for hookworm, *T. trichiura* and *S. stercoralis* 21 days after treatment, using qPCR in participants allocated to arms 2 and 3 than to arm 1.

## 5.4 Primary efficacy outcomes analysis

The primary efficacy endpoint for the efficacy phase III trial is the cure rates for *T. trichiura* 21 days after treatment using microscopy. The analysis will be conducted on the ITT population and in the sensitivity population (per protocol). The analysis will include both Phase II and Phase III participants. The Cochran–Mantel–Haenszel (CMH) test, controlling the effect of site if that is appropriate (sufficient participants), will be used to compare the cure rates for the 3 treatment groups. A participant is considered cured if the baseline egg count or larval count is not 0, and the post-treatment egg count or larval count is 0. Similarly, a participant is considered to have treatment failure if the baseline egg or larval count is not 0, and the egg or larval count after treatment is not 0.

A total of 2 stool samples (1 pre-treatment and 1 post-treatment) will be obtained from each participant. The primary efficacy analysis will be based on ITT population. Efficacy for each type of infection will be analysed separately. A participant with multiple infections will be included in the analysis of each target species that the participant is infected with.

Stool sample collected pre-treatment will be used for the baseline information, and stool sample collected post-treatment will be used for the post-treatment information in the statistical analysis.

### 5.4.1 Secondary efficacy outcomes analysis

The analysis will be conducted on the ITT population and in the sensitivity population (per protocol)

The secondary efficacy endpoints for the phase II and phase III are the following:

- CR for *T. trichiura* 21 days after treatment, as determined by microscopy (phase II).
- CR for hookworm and *S. stercoralis* 21 days after treatment, as determined by microscopy (phase II and phase III).
- ERR for *T. trichiura* 21 days after treatment, by microscopy (phase II and phase III).
- ERR for hookworm 21 days after treatment, by microscopy *(*phase II and phase III*)*.
- CR for *T. trichiura*, hookworm and *S. stercoralis*, by PCR (phase II and phase III).
- Parasite burden decrease after 21 days for *T. trichiura*, hookworm, and *S. stercoralis*, by PCR (phase and phase III).

The first 2 endpoints above regarding the CR will be analyzed using the same approach as the primary endpoint analysis.

Egg reduction rate (ERR) for each species (for *T. trichiura* and hookworms) at end of treatment period will be estimated. *S. stercoralis* will not be evaluated by this analysis.

$$ERR\ (geometric\ mean) = 1 - \frac{e^{\frac{1}{n}\sum \log(EPG\ follow\ up+1)} - 1}{e^{\frac{1}{n}\sum \log(EPG\ baseline+1)} - 1}$$

The logarithmic transformation will be conducted. As there may be participants with a count of zero eggs in the post-treatment stool sample, a value of 1 will be added to total egg count at baseline and total egg count at post-treatment so that the logarithmic transformation can be applied. The geometric mean will be used to summarize the mean egg count at pre-treatment and post-treatment visits and in the calculation of the mean percent egg reduction. Treatment differences will be evaluated by an analysis of covariance (ANCOVA), in which the logarithm of the egg count at post-treatment is the dependent variable, site (if appropriate), and treatment as fixed effect, and the logarithm of the egg count at pre-treatment is the covariate. Baseline egg count will also be analysed as an independent variable that conditions treatment response [11].

Cure rates will be also calculated by qPCR. For this, a participant is considered cured if the mean Ct-values are less or equal than 40 at baseline, and greater than 40 after treatment. When there are non-detectable levels of DNA in the sample, it will be categorized with a Ct-value equal to 50. Cochran–Mantel–Haenszel (CMH) test, controlling the effect of site if that is appropriate (sufficient participants), will be used to compare the cure rates for the 3 treatment groups. In addition, Kappa test and the Fisher 's exact test or chi - square test will be used to compare the CR obtained by Kato-Kats and PCR for the same treatment arm.

Correlation between Kato-Katz counts and qPCR Ct-values will be explored through Pearson´s or Spearman´s (according to the underlying distribution) correlation tests. Different regression methods to predict EPGs based on Ct-values will be explored.

Increases in Ct-values after treatment will be considered as a reduction in parasite load. To measure whether the increase in ct is significant in each treatment arm, the "Wilcoxon signed-rank test" will be used.

The qPCR will allow the identification of hookworm species (*N. americanus* and *A. duodenale*), so it will be evaluated if there are differences in the response to treatment for these species. Kappa test will be used for the evaluation of both tests in the calculation of CR.

Relevant covariates will also be included in the data analysis. Particularly, HIV infection will be assessed as a covariate in participants recruited in Mozambique.

### 5.4.2 Exploratory efficacy outcome analysis

The exploratory efficacy endpoints for the phase III are the following:

- CR and ERR for *A. lumbricoides* in co-infected participants.
- Prevalence of scabies before and after treatment administration of ALB, FDC and FDCx3.

The summary statistics will be presented for the above exploratory endpoints.

### 5.4.3 Sub-group analyses

Subgroup analyses will be performed for the primary efficacy outcome on the ITT population. The variables for the subgroup analysis will include:

- Ivermectin drug exposure: categorized by >400 µg/Kg vs ≤400 µg/Kg
- Age: categorized by school-age children (5 to 14 years-old) & young adults (15 to 18 years-old).
- Co-infection: categorized by mono-infected vs co-infected
- Infected with HIV vs no infected with HIV
- Worm burden: categorized by WHO categories of egg burden categories measured by Eggs Per Gram (EPG) through Kato-Katz method [14]:

| Species | Light | Moderate | Heavy |
|---|---|---|---|
| *T. trichiura* | 1 – 999 | 1000 – 9999 | ≥10000 |
| Hookworms | 1 – 1999 | 2000 – 3999 | ≥4000 |
| *A. lumbricoides* | 1 - 4999 | 5000 - 49999 | ≥50000 |

### 5.4.4 Multiplicity

Multiplicity adjustment will not apply to the primary and secondary outcome analyses.

## 5.5 Safety analysis

Safety analysis in the phase II corresponds to the primary outcome/endpoint, i.e, the frequency, type, severity and relationship to study drug for all adverse events and severe adverse events. For the safety analysis in phase III, the safety population will be used for the analysis and participants will be considered by arm and by both FDC arms pooled (overall) and by number of doses of FDC received to explore dose-responses.

### 5.5.1 Adverse events

All AEs will be coded using the latest version of MedDRA at the time of the database lock. The actual version of the MedDRA coding dictionary used will be noted in the AE tables and listings.

Treatment-emergent adverse events (TEAEs) are defined as adverse events that occur or worsen on or after the first dose of study treatment.

The severity of the adverse events is assessed by the investigator as mild, moderate, or severe. The relationship to the study drug is determined by the investigator as definite, probably, possibly, unlikely, or unrelated. The categories “unrelated” and “unlikely” will be mapped to “unrelated”, the categories “possibly”, “probably” and “definite” will be mapped to “related”.

An adverse event is considered a serious adverse event (SAE) if it results in any of the following outcomes: 1) results in death, 2) is a life-threatening adverse event, 3) requires hospitalization or prolongation of existing hospitalization, 4) results in a persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions, or 5) a congenital anomaly/birth defect.

An overall summary of TEAEs will be presented showing the number and percentage of participants with: any TEAE, any study drug-related TEAE, any severe TEAE, any treatment-emergent SAE, any study drug-related treatment-emergent SAE, any TEAE leading to study discontinuation, and any TEAE leading to death.

The following TEAEs will be summarized as frequency and percentage of participants by SOC and PT:

- TEAEs
- TEAEs by descending PT
- Severe TEAEs
- Study drug-related TEAEs
- Treatment-emergent SAE
- Study drug-related treatment-emergent SAE
- TEAEs leading to study discontinuation

All adverse events for each patient, including the same event on multiple occasions, will be listed, indicating the preferred term.

Drug-related TEAEs will be analysed using ordinal logistic regression with the untoward effect classified as absent, mild, moderate, or severe and the factorial treatment regimens (without interaction term) as predictor variables. For a count outcome such as the number of TEAEs or treatment-emergent SAEs incidence rate ratio (IRR) and its 95% CI will be computed using Poisson or Negative binomial regression.

The following by-participant listings will be provided:

- AEs
- SAEs
- TEAEs leading to study drug withdrawal
- TEAEs leading to study drug interruption
- TEAEs leading to study discontinuation

- TEAEs leading to death

### 5.5.2 Vital signs

Vital sign measurements of systolic and diastolic blood pressure, heart rate, respiratory rate, and body temperature will be summarized based on safety population. Descriptive statistics for observed values and change from baseline in the vital sign parameters will be calculated and presented by treatment arm and visit.

The by-participant listing for the vital signs results will be provided.

### 5.5.3 Physical examination

Physical examination includes photophobia, blurred vision, visual impairment, pruritus skin lesions compatible with scabies, and others.

The shift from baseline to post-baseline visit in the physical examination result (including “Yes”, “No”, “Not Determined”) by the above variable will be provided for safety population.

The by-participant listing for the results of physical examinations will be provided.

## 5.6 Additional analyses

### 5.6.1 Population pharmacokinetic analysis (phase II)

The population PK analysis for the phase II will be reported in a separate document.

### 5.6.2 Participant palatability/acceptability (phase II)

The NBRs for participant overall opinion of taste, overall opinion of smell, overall opinion of texture, and parent or caregivers overall opinion on their child’s acceptability of the formulation will be summarized using descriptive statistics.

### 5.6.3 Resistance analysis (phase III)

Analyses of anthelmintic resistance analysis will focus on *T. trichiura* and hookworms separately. In the case of hookworms (which comprise more than one species), anthelmintic resistance will be evaluated for each hookworm species if more than one species is present; we predicted that *Necator americanus* will be the predominant species. All samples determined to be positive (pre- and post-treatment) for these parasites by microscopy will be included in the resistance evaluation and grouped by treatment arm. The total number of samples included in resistance analysis has been calculated according to the efficacy estimations shown in Table 3 (see Table 5):

**Table 5. Estimations of number of microscopically positive samples pre- and post-treatment by treatment arm and by specie.**

| Species | Treatment arm | Pre-treatment samples (n) | Expected CR (%) | Post-treatment samples (n) | Total samples (n) |
|---|---|---|---|---|---|
| ***T. trichiura*** | ALB | 129 | 23.7 | 99 | 228 |
| | ALB+IVM | 248 | 43.7 | 140 | 388 |
| | ALB+IVM (x3) | 248 | 59 | 102 | 350 |
| | **Total** | **625** | **45.4** | **341** | **966** |
| **Hookworm** | ALB | 101 | 79.5 | 21 | 122 |
| | ALB+IVM | 101 | 79.5 | 21 | 122 |
| | ALB+IVM (x3) | 110 | 95 | 6 | 116 |
| | **Total** | **312** | **84.6** | **48** | **360** |
| **TOTAL** | | **937** | | **389** | **1326** |

DNA extracted from eggs collected from parasite-positive samples will undergo whole-genome sequencing, from which genetic variation within and between samples will be determined. To identify genetic variation associated to the treatment response and therefore, with anthelmintic resistance, we will measure distribution of genome-wide genetic diversity between treatment groups and throughout the genome. Genome-wide nucleotide diversity (pi), Waterson estimator, Tajima's D, Fu's and pairwise FST will be estimated in 50 kbp non-overlapping sliding windows. To improve the visualisation of the genome-wide comparisons, analyses will be restricted to scaffolds in chromosomal linkage groups. Statistical significance will be inferred by the distribution of data points, from which data points that lie great than three standard deviations from the genome-wide mean to be outliers of interest. We will identify genes in these outlier regions of genetic differentiation between parasite populations showing differences in treatment response (i.e. good responders vs poor responders, pre-treatment vs post-treatment) in the three treatment arms separately and in combination.

Principal component analysis (PCA) will be used to determine if there is population genetic structure present; if there is, we use the principal component data to correct our association testing to account for this potential bias. Two different association tests will be performed: (i) a logistic regression genome-wide association between samples from participants with good treatment response phenotypes, and post-treatment samples from children with poor treatment response phenotypes; (ii) a linear regression genome-wide association analysis with the ERR estimates as a continue variable for all samples collected before treatment. For both tests, top principal components of the PCA will be used as covariates, and a Bonferroni (or other, suitable multiple-testing methods) corrected threshold will be used to determine genome-wide level of significance. We will characterise the predicted effects of genetic variation we uncover on genes, and identify relationships between genes associated with the outlier variation by functional enrichment analyses.

Finally, considering the previous association between the β-beta tubulin gene variants and resistance, we will examine this gene and genetic variants within it for associations with resistance, and within the context of all variants identified in the genome-wide analyses.

Genomic data are collected for exploratory purpose and may not be reported in the clinical study report if the sample analysis can not be finished.

### 5.7 Interim analyses

Two interim analyses are planned: 1) after the end of the phase II component for decision making to move to the phase III component; 2) after 50% of the study participants are enrolled. Regular evaluation of the safety data every 3 months will be performed by the DSMB. The study is designed as a superiority trial, so all tests will be two-sided and an overall significance level will be 0.05 In the interim efficacy analysis, the expense function alpha with the O'Brien-Fleming approach will be used to determine the type I error rate for each interim and final analysis.

### 5.8 Statistical software

All statistical analyses will be performed with the statistical software Statistical Analysis System (SAS) for Windows Version 9.4 or later (SAS Institute, Inc., Cary, NC) and R software [15].

## 6. REFERENCES

[1] Anderson RM, Truscott JE, Hollingsworth TD. *The coverage and frequency of mass drug administration required to eliminate persistent transmission of soil-transmitted helminths.* Philos Trans R Soc Lond B Biol Sci. 2014;369(1645):20130435.

[2] Verweij JJ, Rune C. *Molecular Testing for Clinical Diagnosis and Epidemiological*. 2014;27(2):371–418.

[3] Protti M, Mandrioli R, Mercolini L. *Tutorial: Volumetric absorptive microsampling (VAMS)*. Anal Chim Acta. 2019;1046:32–47.

[4] Moser W, Schindler C, Keiser J. *Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis.* BMJ. 2017 Sep;358:j4307.

[5] Marti H, Haji HJ, Savioli L, Chwaya HM, Mgeni AF, Ameir JS, et al. *A comparative trial of a single-dose ivermectin versus three days of albendazole for treatment of Strongyloides stercoralis and other soil- transmitted helminth infections in children*. Am J Trop Med Hyg. 1996;55(5):477–81.

[6] Clarke NE, Doi SAR, Wangdi K, Chen Y, Clements ACA, Nery S V. *Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: A systematic review and network meta-analysis*. Clin Infect Dis. 2019;68(1):96–105.

[7] Gotuzzo E, Echevarria J, Ac WJ, Terashima A, Samalvides F, Ja P, et al. *Ivermectin versus albendazole or thiabendazole for Strongyloides stercoralis infection ( Review )*. Cochrane Database Syst Rev. 2016;(1).

[8] Buonfrate D, Salas-Coronas J, Muñoz J, Maruri BT, Rodari P, Castelli F, et al. *Multiple-dose versus single-dose ivermectin for Strongyloides stercoralis infection (Strong Treat 1 to 4): a multicentre, open-label, phase 3, randomised controlled superiority trial*. Lancet Infect Dis. 2019;19(11):1181–90.

[9] Chow, S.C.; Shao, J.; Wang H. *Sample Size Calculations in Clinical Research*. Chapman & Hall/CRC, editor. 2008. 99–100 p.

[10] StataCorp. *Stata Statistical Software*. 2017.

[11] Levecke B, Mekonnen Z, Albonico M, Vercruysse J. *The impact of baseline faecal egg counts on the efficacy of single-dose albendazole against Trichuris trichiura*. Trans R Soc Trop Med Hyg. 2012 Feb;106(2):128–30.

[12] Savic RM, Jonker DM, Kerbusch T, et al. *Implementation of a transit compartment model for describing drug absorption in pharmacokinetic studies.* J Pharmacokinet Pharmacodyn 2007; 34: 711-26

[13] Jonsson EN, Karlsson MO. *Xpose: an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM*. Comput Meth Programs Biomed. 1999; 58(1): 51–64.

[14] WHO. *Helminth control in school-age children. A guide for managers for control programmes*. Geneva: World …. 2011.

[15] R Core Team. R: *A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. 2013.