ISGlobal - Barcelona Institute for Global Health

Broad One Health Endectocide-based Malaria Intervention in Africa BOHEMIA

SPONSOR: ISGlobal – Barcelona Institute for Global Health

SPONSOR ADDRESS: Hospital Clínic - Universitat de Barcelona, Carrer Rosselló 132, 4a planta, E-08036 Barcelona, Spain

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WORK PACKAGE: Safety and Efficacy

WORK PACKAGE PROTOCOL NAME: A Phase III cluster-randomized, open-label, clinical trial to study the safety and efficacy of ivermectin mass drug administration to reduce malaria transmission in two African settings**.**

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Work Package: Safety and Efficacy

Approval Page

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PRODUCT MANUFACTURING AND PACKAGING

Ivermectin: Stromectrol® from Merck (3 mg tablets)

Albendazole: GMP certified product bioequivalent to Albenza® from GSK (400 mg tablets)

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Protocol Principal Investigator Signature page

The PI is responsible for ensuring that all study site personnel, including sub-investigators and other staff members conduct this study according to this protocol, International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines E6 (R2), the current version of the Declaration of Helsinki and the pertinent individual country laws/regulations and to comply with its obligations, subject to ethical and safety considerations during and after study completion. The PI also agrees not to disclose the information contained in this protocol or any results obtained from this study without written authorization from the Sponsor.

Investigational Product:	
Reference material:	
Protocol code:	
Date of Issue:	
Prepared by	

I have read and approve the protocol specified above and agree on its content:

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Signature **Date**

For the participating Study Site:

_____ Principal Investigator
Name **Designation**

_____ _____
Signature **Date**

Table of contents

Figure index

Table index

Abbreviations

ABBREVIATIONS	
ADR	Adverse Drug Reaction
AE	Adverse Event
AL	Artemether-Lumefantrine
AUC	Area Under the Curve
CDC	Centers for Disease Control and Prevention
CIBS	Ethics Committee of CISM
CISM	Centro de Investigação em Saúde De Manhiça
CNS	Central Nervous System
CRA	Clinical Research Associate
CRF	Case Report Form
DEC	Diethylcarbamazine
DOT	Directly Observed Treatment
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMA	European Medicines Agency
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GSK	GlaxoSmithKline
HFW	Health Facility Workers
ICH	International Conference on Harmonisation
IHI	Ifakara Health Institute
IP	Investigational Product
IRB	Institutional Review Board
IRS	Indoor Residual Spraying
LF	Lymphatic Filariasis
LFU	Lost to Follow Up
LLIN	Long Lasting Insecticidal Nets
LMP	Last Menstrual Period

ABBREVIATIONS	
MDA	Mass Drug Administration
MoH	Ministry of Health
MUAC	Mid-Upper Arm Circumference
NDA	New Drug Application
NMCP	National Malaria Control Programme
NTD	Neglected Tropical Disease
PCR	Polymerase Chain Reaction
PID	Participant Identifiers
PI	Principal Investigator
PK	Pharmacokinetics
pLDH	Parasite Lactate Dehydrogenase
PMI	President's Malaria Initiative
PPC	Preferred Product Characteristics
RA	Regulatory Authority
RDT	Rapid Diagnostic Test
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Seasonal Malaria Chemoprevention
STHs	Soil-Transmitted Helminths
SUSAR	Suspected Unexpected Serious Adverse Reaction
VCAG	Vector Control Advisory Group
WHO	World Health Organization

Protocol structure

The BOHEMIA program consists of a combination of studies organized around a central community prevention mass drug administration protocol and four sub-studies (i.e.; social science, entomology, health economics, and environmental), each written as an individual protocol. The protocol is central but used in two separate, individually powered trials in Mozambique and Tanzania. The trials have been powered on the efficacy outcome, which is described in this central protocol, whereas the remaining aspects are described in the corresponding sub-study protocols (Figure 1).

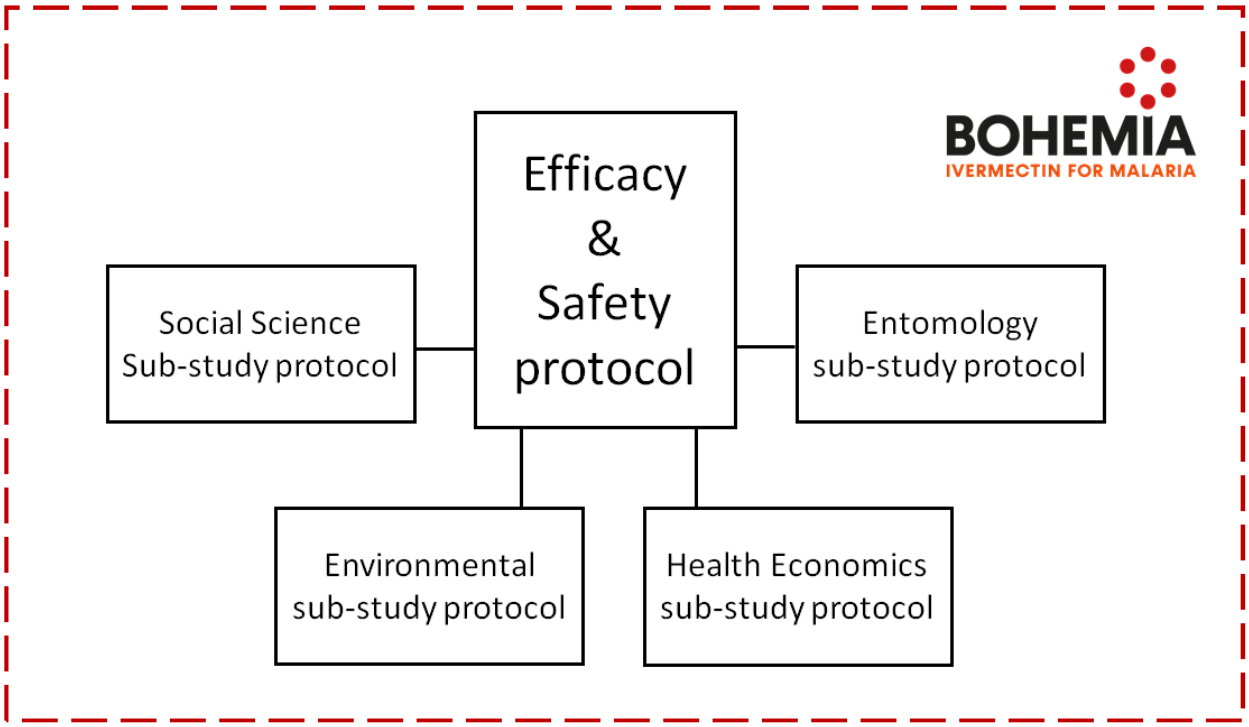


Figure 1.BOHEMIA protocol structures (environmental is only conducted in the Tanzania site)

Expected reviewers

This protocol will be reviewed by the following committees and authorities:

Technical reviews

- Internal Scientific Committee of the Centro de Investigação em Saúde de Manhiça (CISM)
- BOHEMIA Advisory Council
- BOHEMIA Data and Safety Monitoring Board
- WHOs Vector Control Advisory Group (VCAG)

Ethical reviews

- Ethics committee of CISM (CIBS)
- Ifakara Health Institute's Institutional Review Board (IHI's IRB)
- ISGlobal's IRB (CEIm)
- Kantonale Ethikkommission Bern (KEK Bern)
- National ethics committee of Mozambique
- National ethics committee of Tanzania
- WHO's Research Ethics Committee (WHO-ERC)

Regulatory reviews

- National regulatory authority (RA) of Mozambique
- National RA of Tanzania

Protocol synopsis

Title

A Phase III cluster-randomized, open-label, clinical trial to study the safety and efficacy of ivermectin mass drug administration to reduce malaria transmission in two African settings.

Principal Investigator

Dr. Regina Rabinovich

Phase of Development

Phase III

Number of Study Sites

Two BOHEMIA cluster randomized trials will be carried out in Mozambique (Southern Africa) and Tanzania (Eastern Africa). These sites encompass different transmission dynamics that increase the generalizability of results, namely: (a) a gradient of malaria transmission: moderate in Tanzania and very high in Mozambique, (b) different species composition of vector population, (c) different rain patterns and environmental conditions, and (d) different livestock species and human/livestock ratios.

Study purpose and objectives

Purpose

To evaluate the safety and efficacy of ivermectin MDA as a complementary malaria vector control tool in two cluster-randomized trials in Tanzania and Mozambique.

Primary objective

- To determine the safety (in humans) and efficacy of ivermectin MDA (administered to humans or humans and livestock simultaneously) for the prevention of malaria.

Secondary objectives

- To assess the efficacy of the intervention using complementary methods (efficacy)
- To assess the safety of the intervention with complementary methods (safety)
- To assess the pharmacokinetics (PK) of the proposed ivermectin dose/regime in its relationship with efficacy and safety outcomes (efficacy and safety)
- To assess the impact of ivermectin MDA at the proposed regimen on the prevalence of selected ectoparasitic neglected tropical diseases (NTDs) (scabies, headlice, tungiasis, and bed bugs)
- To assess the relationship between malaria incidence in children under 5 and community prevalence one month post last dose (this serves for prevalence outcomes and paves the way for future studies using prevalence as outcome which is much less resource-consuming than incidence)
- To assess the relationship between active and passive surveillance for malaria at health facility (this serves as validation of passive surveillance and paves the way for future studies using passive surveillance as outcome which is much less resource-consuming than active surveillance, ensuring no key safety events are missed)

- To assess the accuracy for malaria diagnosis of two different malaria rapid diagnostic tests (RDTs) used in comparison to polymerase chain reaction (PCR) (this is directly linked to efficacy as determined by RDTs)

Exploratory objectives

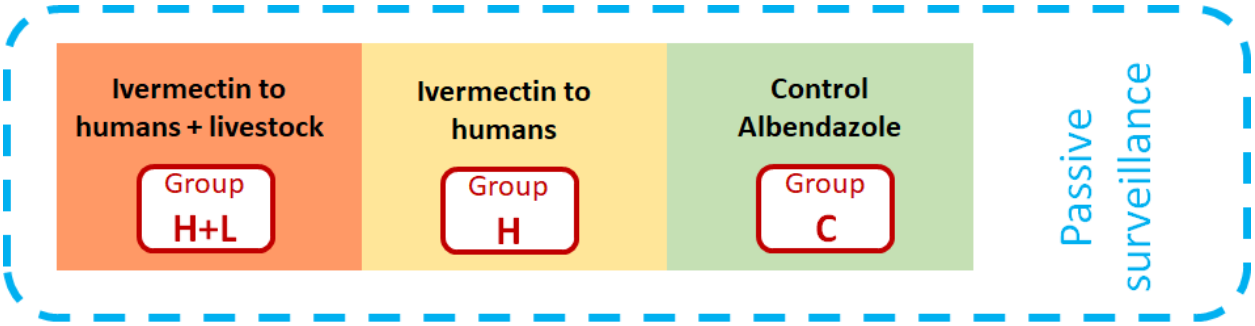
- COVID RESPONSE: to assess the serological prevalence and incidence of COVID-19 in the study area (Mozambique only)
- To assess the potential direct or indirect effect of ivermectin on immune response to the malaria parasite.
- To assess the relationship between self-reported pregnancy status, last menstrual period (LMP), and the results of pregnancy tests in women of reproductive age participating in the study.
- To assess all-cause and malaria-related mortality in intervention and control arms.
- To estimate the prevalence of relevant CYP and P-gp variations in the target population and subjects suffering from CNS-AEs

Overall Design

The study has been designed to meet the requirements of WHO´s preferred product characteristics (PPC) for endectocides.

Trial type

The BOHEMIA clinical trials are open label, cluster randomized, controlled, parallel arm, and will be conducted during the malaria transmission season in Mozambique and Tanzania.



Study groups

Population

Co-primary objectives are determined in different populations. Efficacy is primarily determined in a pediatric active cohort (children under 5 years of age in Mozambique and 5-15 years in Tanzania), pediatric active cohort, and safety is determined in anyone who receives the drug. Pregnant women and children under 15 kgs are not eligible for treatment.

Number of Participants

Each country has a calculated sample size to support the evaluation of the primary endpoint.

The sample size is based on the primary outcome (malaria incidence in a cohort of children followed prospectively in the cluster's core area for six months post first MDA round, an outcome chosen based on WHO's PPC for endectocides).

Based on the demographic data obtained in the pre-trial census, each cluster will include 300-400 people (depending on site), of which 240-300 will be eligible for treatment and at 80-150 will be children eligible for follow-up. There will be 53-54 clusters per arm in each country, resulting in inclusion of 45,000-48,000 participants to receive treatment or control and 2,000-3,000 children to be followed for six months in each country. The total participants across both trials will be 85,000-95,000 in treatment/control and 4,000-6,000 children followed for the primary outcome.

Treatment Groups

Three groups of clusters will be randomized to receive (a) ivermectin in humans, (b) ivermectin in humans + livestock, or (c) albendazole control, and each study district will be subject to enhanced passive surveillance for malaria.

Inclusion criteria

For human treatment/safety cohort: residents of the study area, weight over 15kg, ability to consent/assent, negative pregnancy test for women aged between 13 and 49.

For pediatric active cohort: children in the age of highest burden at the time of enrollment (under 5 years of age in Mozambique or 5-15 in Tanzania), resident of the study area, parent/guardian's consent.

For cross sectionals: all ages residents of the area prior to enrolment, consent/assent.

For livestock treatment: owner/guardian able to provide written consent, animal is expected to spend at least one week every trial month inside the cluster border.

Exclusion criteria

For human treatment/safety cohort: known hypersensitivity to ivermectin or albendazole, risk of *Loa loa* as assessed by travel history, pregnancy, lactating in the first week postpartum, weight under 15 kg, currently participating in another clinical trial, unwilling to consent or adhere to study procedures, severely ill, currently under treatment with inhibitors of CYP3A or P-gp, unable to consent.

For pediatric active: nonresidents, unable to obtain consent.

For cross-sectionals: nonresidents, unable to obtain consent

For livestock treatment: intention to milk or slaughter the animal during the withdrawal period of ivermectin treatment (as defined in product´s package insert)

Dosage

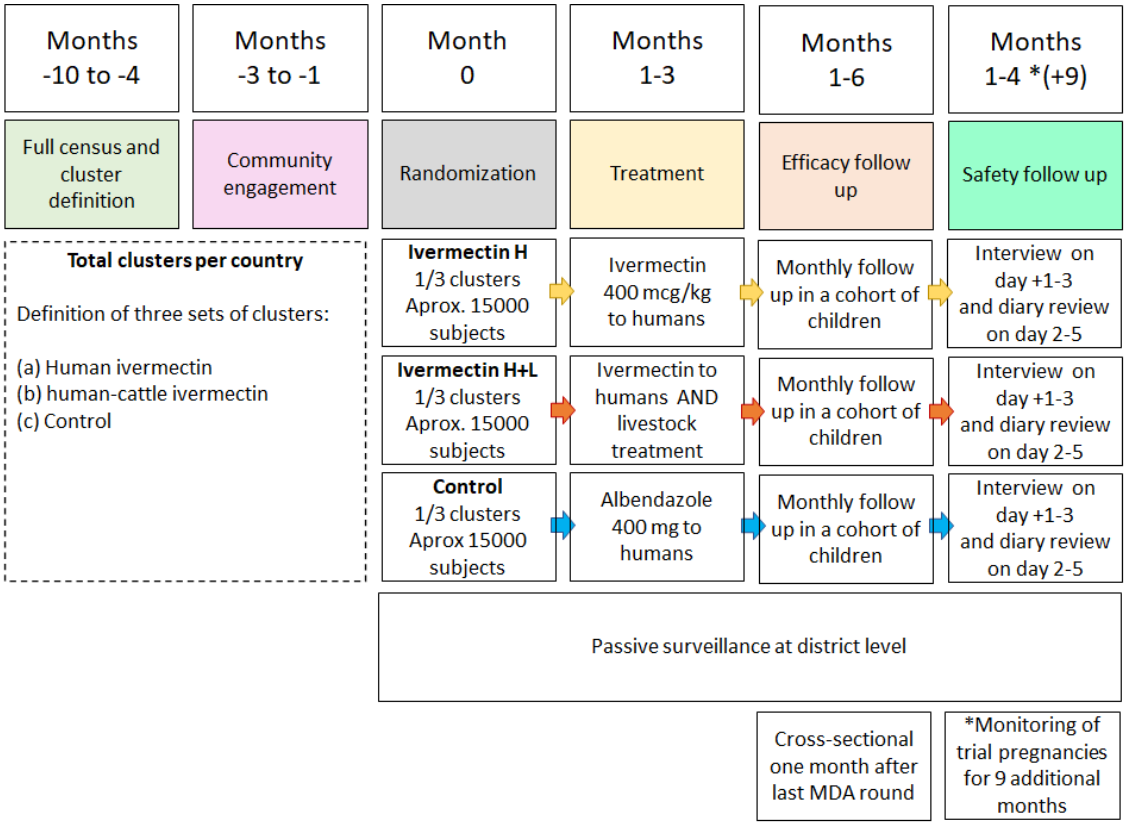
Eligible individuals in the ivermectin arm will receive a single dose of 400 mcg/kg, orally, given once a month for three months.

The control group will receive a single dose of albendazole of 400 mg, orally, given once a month for three months.

Estimated duration of study

Throughout the study there will be two different groups of participants enrolled, the ones receiving the treatment (> 15 kg) and the ones in the active pediatric cohort for the main outcome of malaria incidence (the ages of highest incidence in each country, under 5 years of age in Mozambique and 5-15 years in Tanzania). Each group will participate for different periods of time.

Only the group enrolled in the active pediatric cohort will contribute to the primary efficacy outcome and this group will participate from date of first dose for six months. The group receiving treatment (>15 kg) will contribute to the primary safety outcome and several secondary outcomes (PK, NTDs) and this group will participate for four months. Women of child-bearing age will be visited one month after the third dose for a final pregnancy test, any pregnancy occurring during the trial will be followed for 9 months. The cross-sectional survey will enroll participants of all ages one month after the last MDA round.



Summary of procedures

Safety and efficacy evaluation

Efficacy parameters

Primary outcome measure: infection incidence in children (<5 years in Mozambique and 5-15 years in Tanzania)

Secondary:

- Time to first positive RDT in children in the active pediatric cohort
- Molecular force of infection in a subgroup of children in the active pediatric cohort
- Malaria case incidence in all ages at health facility (passive surveillance)
- Malaria infection prevalence one month after the last MDA round in all ages (cross-sectional)
- Multiplicity of infection one month after the last MDA round in all ages

Safety Assessment

Participants will be asked by the field worker to record signs and symptoms associated with known Adverse Drug Reactions (ADRs) in the diary for review during the post-dosing safety visit occurring 2-5 days post dose.

The participant will be asked to provide information on any other events, physician visits or hospitalizations, and treatment taken during Day 0 through Day 6 during the post dosing safety visit.

Participants will be asked about any AEs occurring after day 6 day and in the interim to the next dosing day. BOHEMIA will use MedDRA coding.

Statistical methods

All analysis will be conducted in an intention-to-treat manner. Differential incidence between intervention and control clusters will be compared with a Poisson regression model using generalized estimating equations (GEE) with exchangeable correlation structure that considers the cluster design effect.

Sample size has been corrected to account for cluster-randomization.

Adjusted analysis will be controlled for cluster size, socio-economic status, and livestock-human ratio.

Procedures in treatment/safety cohort	**Census & clustering** Months -10 to -4	**Treatment enrollment visit** 1-30 days before dosing	**Study visit 1(S)** Day 1	**Study visit 2(PK)** Day 2	**Study Visit 3(S)** Day 3 +/-1 day	**Study Visit 4(S)** Day 6 +/- 1 day	**Study Visit 5(S)** Day 31 +/-3 days	**Study Visit 7(S)** Day 36 +/-1 day	**Study Visit 8(S)** Day 61 +/-3 days	**Study visit 10(S)** Day 66 +/-1 day	**Final safety visit 11(S)** Day 91 +/-1 day	**End of passive safety surveillance** Day 365+/-3 days	**Final pregnancy safety visit(S)** [d] Day 371 +/-3 day
Informed consent census *(separate protocol)*	X												
Demographic data collection	X												
Cluster randomization	X												
Informed consent for treatment		X					X[e]		X[e]				
Complete visit template		X	X	X	X	X	X	X	X	X	X		X
Anthropometry		X					X		X				
Assessment of selected NTDs [a]		X[a]					X[a]		X[a]		X[a]		X[a]
COVID response[a]		X					X		X		X		X
Review of infant vaccination card (if applicable)		X					X		X		X		
Collection of pharmacology samples [a]			X	X	X								
Collection of parasite response samples [b]			X				X		X				
Urine Pregnancy test [c]			X				X		X		X		
Administer study intervention			X				X		X				
Review of side effect personal diary						X		X		X			
Passive surveillance of side effects				X----------							----------X		
Adverse event review and evaluation				X----------							----------X		
Active pregnancy surveillance							X		X		X	X	X
Passive pregnancy surveillance (health facility)							X----------						----------X

[a]randomly selected sub-sample
[b]randomly selected sub-sample of children under 5 that also receive treatment (>15 kg)
[c]in participating female participants aged 13-49
[d]only pregnancies occurring post-treatment
[e]only subjects not previously enrolled

Procedures in the treatment/safety cohort

Procedures in efficacy (active pediatric cohort)	**Census & clustering** Months -10 to -4	**Enrollment/Baseline(E)** Visit 1, Day 1	**Study Visit 2(E)** Day 31 +/-3 days	**Study Visit 3(E)** Day 61 +/- 3 days	**Study Visit 4(E)** Day 91 +/-3 days	**Study Visit 5(E)** Day 121 +/-3 days	**Study visit 6(E)** Day 151 +/-3 days	**Study visit 7(E)** Day 181 +/-3 days
Informed consent for census	X							
Demographic data collection	X							
Randomization	X							
Informed consent		X						
Anthropometry		X			X			X
Review infant vaccination card		X			X			X
Complete visit template		X	X	X	X	X	X	X
Malaria RDT		X	X	X	X	X	X	X
Malaria treatment [a]		X	X	X	X	X	X	X
Assessment of selected NTDs		X	X	X	X	X	X	X
COVID response[b]		X	X	X	X	X	X	X
Malaria parasite immune response samples [b]		X	X	X	X	X	X	X
Plasmodium PCR for quality control and molFOI[c]		X	X	X	X	X	X	X
Passive incidence surveillance at health facilities (all ages)		X----------						----------X

[a]only if RDT is positive (see case definition)
[b]only in a randomly selected sub-sample
[c]only in a randomly selected 10% sub-sample

Procedures in the efficacy/incidence cohort

1. Introduction

1.1. Background

Malaria is preventable and treatable, and yet it remains a significant public health problem around the world. In 2018, there were 228 million cases and 405,000 malaria deaths globally. Around 93% of the cases and 94% of the deaths occurred in the WHO Africa Region [1]. Malaria disproportionally affects the livelihood of the rural poor and has a deep economic impact, since it both thrives in and perpetuates poverty [2].

Based on substantive progress in the 2000-2015 period, the World Health Organization (WHO), through the Global Technical Strategy (GTS) [3], proposed ambitious goals for malaria by 2030. These include the reduction of malaria cases by 90% as compared with the 2015 numbers and elimination in at least 35 countries. Achieving these goals could have a cumulative additional economic output of US$ 4.1 trillion [2].

However, the global fight against malaria is at a crossroads [4]. The 2017-2019 World Malaria Reports show that the decrease in cases and deaths had stalled, and the global program was off track to reach the 2030 milestones of the GTS. New and improved ways to fight malaria, particularly in the countries with the highest burden, are needed to get back on track. Moreover, the COVID-19 pandemic threatens to disrupt control programs and set back malaria to the levels of 20 years ago [5].

One critical challenge to achieving the 2030 goals is residual transmission, which is defined as "*persistence of malaria transmission following the implementation in time and space of a widely effective malaria programme*" [6]. This is driven by mosquito behavioral adaptations that allow transmission to continue even in presence of good coverage with core vector control tools such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Feeding outdoors, feeding early in the evening before bedtime, or leaving houses quickly after feeding, without resting indoors, are all behaviors that allow mosquitoes to avoid home-centered insecticides [7]. Additionally, temporarily feeding on livestock can allow mosquitoes to thrive and feed opportunistically on humans when available, contributing to sustain transmission [8, 9].

Endectocides are drugs that kill endo- and ectoparasites. Ivermectin is an endectocide licensed for human use in the 1980's. It has been a key drug in the treatment and control of onchocerciasis and lymphatic filariasis (LF) [10]. More than 3.7 billion treatments have been donated by Merck and mass-distributed at population level over the last 30 years [11]. Ivermectin also kills mosquitoes that feed on treated humans or animals. This has led to the suggestion that MDA with ivermectin can contribute to killing mosquitoes regardless of their behavior and location, and thus to address residual transmission [12].

The capability of ivermectin to kill mosquitoes has been evaluated in dozens of insectary-based experiments of colony or wild mosquito survival after feeding on ivermectin-containing blood [13-39]. Although a variety of methods, doses, hosts and vector species have been evaluated, common results are:

a) Ivermectin increases the mortality of *Anopheles* mosquitoes that ingest it in a blood meal.
b) Mosquito mortality is directly related to the ivermectin-blood concentration (i.e. dose-response gradient).
c) The lethal effect is driven by time the drug is present in the blood and concentration reached (area under the curve, AUC); the time-in-blood above certain threshold is the most important factor.

d) The drug causes a series of sublethal effects that can contribute beyond the killing effect to reduce transmission (mosquito knock-down, reduced fertility, reduce motility).
e) It appears that different mosquito species have different degrees of susceptibility to the drug, some species such as *Anopheles arabiensis* or *Anopheles minimus* die quickly after imbibing low concentrations, while others such as *Anopheles dirus* or *Anopheles darlingi* can tolerate higher concentrations in their bloodmeal.

Additional evidence has emerged from mosquito collections conducted in onchocerciasis-endemic areas before and after ivermectin MDA administered at the onchocerciasis dose of 150-200 mcg/kg [22, 23, 29]. These mosquito studies have shown three major effects:

a) A reduction of 33% in the 3-day mosquito survival lasting for about one week after MDA
b) A shifting of the mosquito age population structure towards younger, less infectious, ages lasting for about three weeks, and
c) A reduction of 77% in the proportion of mosquitoes carrying malaria parasites in their salivary glands (the sporozoite rate) lasting for about two weeks after MDA.

The only published cluster-randomized trial of ivermectin MDA for malaria to date, concluded that in Burkina Faso, six doses of ivermectin (150-200 mcg/kg) given three weeks apart reduced malaria incidence by 20% in children under five years of age who did not receive ivermectin, reflecting decreased transmission [40].

Several modeling exercises have also assessed the potential impact of ivermectin MDA on malaria transmission [36, 41, 42] and concluded that it can reduce malaria transmission in different settings, with the critical variables being:

a) The duration of the drug in the blood of treated subjects, reflecting both the dose and the regimen used
b) The coverage achieved in both human and livestock blood, and
c) The timing of the intervention in relation to the transmission season

Importantly, modeling suggests that adding ivermectin to other drug-based interventions, such as seasonal malaria chemoprevention (SMC) or MDA with antimalarials, could significantly enhance the effect and potentially reduce the number of rounds or coverage needed to achieve impact with the strategy.

BOHEMIA will conduct two cluster-randomized, individually powered trials in parallel in different eco-epidemiological settings [43]. The goal of these trials is to generate solid evidence to support the evaluation of ivermectin as a complementary vector control strategy for malaria prevention.

The BOHEMIA trials will be carried out in two sites in East and Southern Africa: Tanzania and Mozambique. These sites encompass different transmission dynamics that increase the generalizability of results, namely: (a) a gradient of malaria transmission: moderate-to-high in Tanzania and very high in Mozambique, (b) different species composition of vector population, (c) different rain patterns and environmental conditions and, (d) different livestock species and human/livestock ratios (Table 1).

Country	Region	Malaria	Most prevalent Livestock species	Key vectors
Tanzania	Rufiji	Mesoendemic Prevalence: 12-49% [44, 45] Estimated incidence: 1.5 infections/child/year*	Cattle	*An. arabiensis* *An. funestus*
Mozambique	Mopeia	Holoendemic Prevalence: 47-75%† Incidence: 4.2† infections/child/year*	Pigs and cattle	*An. gambiae* *An. arabiensis* *An. funestus*

Table 1. Key malaria indicators in the selected sites

*In Tanzania the highest incidence age group is 5-15 years of age, in Mozambique it is under 5 years old, †Chaccour et al. Unpublished.

The BOHEMIA clinical trials will be conducted according to the International Conference on Harmonization (ICH)- Good Clinical Practice (GCP) standards, with all required ethical and regulatory reviews and approvals. There will be appropriate clinical monitoring and a single independent Data and Safety Monitoring Board (DSMB).

ISGlobal is the funding recipient and trial sponsor for BOHEMIA.

In each country, BOHEMIA has partnered with established research organizations with a proven track record of successful projects conducted in-country; each site has a supporting letter from the Ministry of Health (MoH) and institutional leadership. In order to conduct BOHEMIA, we will implement relevant clinical trial monitoring, trial specific training, as well as obtaining ethical and regulatory approvals.

The identified country trial partners are:

- In Mozambique: Centro de Investigaçao em Saúde de Manhiça (CISM)
- In Tanzania: the Ifakara Health Institute (IHI)

Beyond the differences in malaria transmission and vector characteristics, the two sites also represent different languages and cultural contexts, which will also add to the learnings and support ultimate generalizability.

1.2. Investigational product and justification of dose and regimen

1.2.1. Ivermectin

The product under investigation is ivermectin in 3mg tablets as manufactured and distributed by Merck under the trademark Stromectrol®.

The human ivermectin regimen proposed for BOHEMIA is a single dose of 400 mcg/kg repeated monthly for three months. Note that this dose is included in the European Medicines Agency (EMA) approved ivermectin label for the Merck product in the Netherlands and for the Infectopharm Scabioral® label in Germany.

The dose will be administered using scales in the field for weight-adjusted administration. Given that dosing is limited by the size of the tablet (3mg) the individuals will receive a discrete number of tablets according to their weight band as shown in **Table 2.** The individual dose will be a mean of 400 mcg/kg for every 10-kg weight band (range 300-484).

	Number of 3mg tablets →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Weight [kg] ↓	**Dose [mg] →**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**	**39**	**42**	**45**
15-20	**Maximum dose per Kg**	200	400	600	800	1000	1200	1400	1600	1800	2000	2200	2400	2600	2800	3000
21-30		143	286	429	571	714	857	1000	1143	1286	1429	1571	1714	1857	2000	2143
31-40		97	194	290	387	484	581	677	774	871	968	1065	1161	1258	1355	1452
41-50		73	146	220	293	366	439	512	585	659	732	805	878	951	1024	1098
51-60		59	118	176	235	294	353	412	471	529	588	647	706	765	824	882
61-70		49	98	148	197	246	295	344	393	443	492	541	590	639	689	738
71-80		42	85	127	169	211	254	296	338	380	423	465	507	549	592	634
81-90		37	74	111	148	185	222	259	296	333	370	407	444	481	519	556
91-100		33	66	99	132	165	198	231	264	297	330	363	396	429	462	495

Table 2. Discrete doses based on table size and weight

Yellow are number of tablets resulting in doses bellow 200 mcg/kg for a particular weight range, green is number of tablets resulting in doss between 200 and 600 mcg/kg and in red are number of tablets resulting in doses above 600 mcg/kg. The numbers of tablets selected for BOHEMIA are marked with black borders.

According to population pharmacokinetic (PK) modeling (1000 repetitions, non-mixture model, 30% variability) [Felix Hammann modeling for BOHEMIA on Monolix software v2018R1, Lixoft, Antony, France], the proposed dose would render the PK parameters presented in **Table 3** and the curve presented in **Figure 2**.

	Monthly		Cumulative in 3 months	
	Median	**Percentiles 2.5-97.5%**	**Median**	**Percentiles 2.5-97.5%**
Cmax	63.8 ng/ml	44 - 88.5 ng/ml	63.8 ng/ml	44- 88.5 ng/ml
AUC	2353 ng·h/ml	1313 - 4169 ng·h/ml	7059 ng/ml	3939 – 12507 ng·h/ml
Tmax	5.3 h	3.9 – 7 h	5.3 h	3.9 – 7 h

Table 3.PK parameters of the ivermectin dose proposed for BOHEMIA [concentration in whole blood]

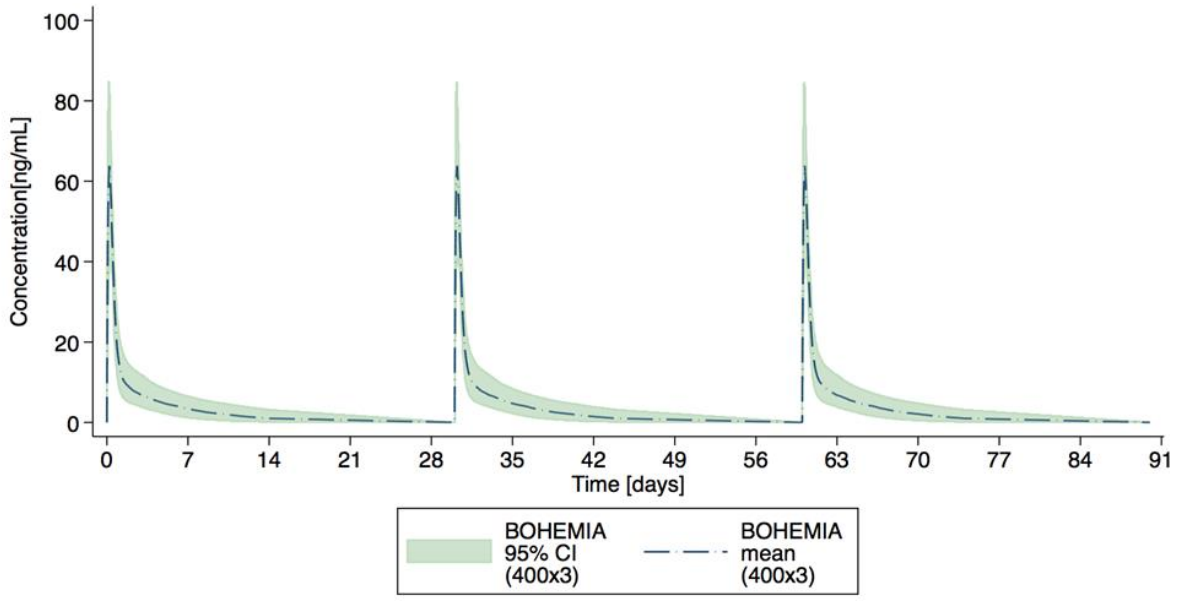


Figure 2.Predicted PK of the BOHEMIA dose
median values and 95% CI. [concentration in whole blood]

1.2.2. Albendazole

The investigational product (IP) to be used as control is 400 mg chewable tablet. The gold standard for bioequivalence testing will be the GlaxoSmithKline (GSK) product under the Albenza® name. Existing data suggest albendazole does not affect mosquito survival [21]. Previous trials or case reports assessing the effect of albendazole in blood-feeding insects such as scabies or lice have provided no evidence of effect [46, 47].

1.3. Preclinical experience

1.3.1. Ivermectin

Merck's original new drug application (NDA) contains reports of a large number of toxicology studies on ivermectin conducted between 1977 and 1995. The most relevant studies are summarized in supplementary tables 1-3. Merck's full safety report is fully available under the U.S. Freedom of Information Act and has been reviewed as part of a BOHEMIA funded independent safety review.

1.4. Clinical experience

1.4.1. Relevant PK aspects of ivermectin

The elimination of ivermectin is biliary and via metabolic processes, presumably by Cytochrome P_{450} 3A4 (CYP3A4). In healthy volunteers, the reported half-life after oral administration ranged between 12 and 36 hours. This may increase for up to 54 hours in onchocerciasis patients [48]. In addition, it is conceivable that elimination is prolonged when the relevant metabolic pathways are inhibited, such as with co-administration of CYP3A4 inhibitors such as ritonavir (this is incorporated into exclusion criteria).

1.4.1.1. How does the BOHEMIA regimen compare with currently approved ones?

The ivermectin label has been extensively modified over 30 years of use for treatment and for public health campaigns. The FDA-approved ivermectin dosing regimen for onchocerciasis MDA is 150-200 mcg/kg (every 12 months), although the possibility of quarterly use in individual patients is also included in the label [49]. The 400 mcg/kg dose, once a year, is included in the Mectizan label and in the Stromectol label in Europe [50], although it is recommended by public health authorities twice a year in some areas [51]. For moderate to severe crusted scabies, three doses of 200 mcg/kg within two weeks are recommended in the Australian label [52].

The main PK parameters of the dose and regimen proposed dose for BOHEMIA are compared with actual or modelled parameters for the currently label-approved doses of ivermectin in **Table 4.**

	BOHEMIA **400 mcg/kg monthly x 3 months**	**Onchocerciasis** **150-200 mcg/kg single [53]**	**Moderate to severe scabies** **200 mcg/kg, 3 doses within 2 weeks [modelled]**
Cmax	**63.8** [44- 88.5]	**38** [35 - 41]	**38.3** [27.8 - 52.1]
AUC	**2353** [1313-4169]	**1032** [874-1210]	**3532** [1970 – 6266]
Tmax	**5.3** [3.9 – 7]	**5.6**	**29** [27.8 - 30.3]

Table 1.Main PK parameters of the ivermectin dose proposed for BOHEMIA as compared with the same parameters for currently approved doses for other indications.

PK model by Hammann. All parameters in median [range]. Cmax: ng/ml, AUC ng·h/ml, Tmax: hours, [concentration in whole blood]

The 400 mcg/kg dose is included in the EMA-approved Stromectol® and Mectizan® labels in the Netherlands [54] and the Scabioral® label in Germany [55]. The safety of the 400 mcg/kg dose is well established as more than 80,000 independent drug exposures have occurred in clinical trials. See supplementary file for additional data and a summary of the trials where ivermectin 400mcg/kg was used.

[Note: these 80,000 independent exposures do not include additional exposures to ivermectin 400 mcg/kg when combined with other drugs like albendazole or Diethylcarbamazine (DEC) which significantly increases the human experience with this dose]. None of the studies reported drug-related SAEs; only minor adverse events (AEs) were observed, which disappeared within one week and were mostly related to the death of filaria parasites in the skin causing dermal reactions and inflammation.

See supplementary file for comparative PK curves of the BOHEMIA dose with dose recommended for the treatment of NTDs under different labels as well as with the highest out of label doses used to date.

1.5. Study rationale

Using MDA of endectocides such as ivermectin to reduce malaria transmission offers several potential advantages:

1. It would target malaria vectors feeding on treated humans regardless of the place and time of biting, effectively targeting residual transmission driven by mosquitoes that feed outside times and spaces protected by core vector control tools.
2. It would target malaria vectors that feed on livestock and that are not exposed to insecticide within the home, effectively targeting zoophagic-driven residual transmission.
3. It holds additional potential to tackle insecticide resistance given that ivermectin's mechanism of action differs from public health insecticides currently in use.
4. It offers an opportunity for the integrated prevention of malaria and NTDs [56, 57], which may help achieve efficiencies.
5. Broader ivermectin delivery to livestock would have positive externalities on households, by reducing the burden of intestinal helminths and ecto-parasites in dominant domesticated herds; this can in turn increase income, food security, and liberate resources for re-investment in household improvement and human healthcare.

To date, one field randomized trial (RIMDAMAL) [40] has published the impact of ivermectin MDA on malaria transmission. In this small trial, a group of 8 villages (total population 2,700) was randomized to one of two interventions (a) single dose of ivermectin 150-200 mcg/kg given to all eligible people (>90 cm height) and albendazole 400 mg or (b) the single dose of ivermectin and albendazole at baseline plus five additional doses of ivermectin 150-200 mcg/kg given every three weeks. The main finding was a 20% reduction in the cumulative uncomplicated malaria incidence in children under five living in the villages that received six doses of ivermectin (statistical methods debated) [58, 59]. Additionally, the authors of RIMDAMAL suggest there might be a direct effect of ivermectin on the malaria parasite by inhibiting the development of liver stages, based on the larger impact seen in children under five years of age that also received the drug and one previous animal model [60]. The hypotheses include a direct effect on liver stages of the parasite or ivermectin-mediated immunomodulation.

There are five other relevant trials ongoing, assessing the efficacy of ivermectin alone or in combination with antimalarials to reduce malaria transmission.

Advancing the development of this repurposed drug (pivoting endpoint from treatment to vector control) will require the following:

a) Fulfilling the minimally accepted criteria of WHO's PPC for ivermectin for malaria (PPCs) [61],
b) A data package that includes the safety, efficacy and acceptability of the dose, regimen and application via MDA for indirect impact on malaria;
c) Availability of pre-qualified product with possibility of procurement with support of UN agencies or multilateral funds.
d) Country uptake which can be facilitated by a WHO policy recommendation and inclusion in the national strategic plan.

WHO´s PPC states that the desired efficacy of an endectocide as stand-alone insecticide in areas of high to moderate transmission is at least 20% reduction in the incidence of clinical malaria (as primary outcome) and incidence of infection (as secondary outcome) in children under 5 (the highest incidence age-group in areas with high-transmission), lasting for at least 1 month following a single regimen. Assessing this effect requires a cluster-randomized design with meticulous follow-up of a cohort of children for efficacy and the whole exposed population for safety outcomes, which is the design selected for the BOHEMIA trials.

The primary outcome measure is proposed as incidence in a six-month period given that ivermectin is a short-acting intervention with <1% residual drug at 30 days after each dose. Efficacy assessment will continue 4 months post last dose of ivermectin, and this will include analysis of the vector population. We have proposed the appropriate duration of impact evaluation relevant to the biology of the intervention, and geared towards being able to clean the data, lock the database, and conduct the primary efficacy and safety analysis efficiently.

WHO guidance states that trial design and duration should reflect the nature of the intervention and are left at the discretion of researchers. These trials are robustly powered and are being conducted in moderate to high burden areas, so we believe the risk of failing to find an effect is low and if so, it would throw the utility of the intervention into question. Given the pressure to identify an additional intervention for high burden areas, urgency to complete the primary analysis would offer data for consideration of a policy recommendation a full year earlier. Finally, it is quite likely, given the other trials ongoing, that ultimately ivermectin, if successful, would be delivered in one of several co-administration strategies with an antimalarial (or a vaccine), and that is the regimen that could be evaluated following the seminal BOHEMIA trial.

Advancing the development of this new tool towards implementation in the field can be accomplished in a time frame to contribute to the 2030 GTS goals.

COVID response (Mozambique only): given limited testing capacity, as of October 2020, the state of the pandemic in Mozambique has been extrapolated from point-estimates of seroprevalence conducted in three cities. There is uncertainty on how the pandemic will progress over 2021, so we propose a descriptive exploratory objective that uses the operational scaffold of BOHEMIA to provide more accurate data on the sero-prevalence and sero-incidence of COVID-19 in the district of Mopeia. This data will allow for the analysis of potentially important confounders (co-circulating febrile disease) as well as to inform local policy and help adapt field operations and procedures to the state of the local transmission. Monthly serology testing in a sub-group will allow for a dynamic estimate of incidence and transmission rate to complement prevalence estimates.

Ivermectin is given yearly to over 300 million people in NTD endemic areas. In the context of NTDs, for which there is a clearly stablished risk-benefit. Women of child bearing age are screened for pregnancy based on self-reported status. Women reporting being pregnant are excluded from treatment.

This trial is designed to assess the efficacy of ivermectin for a potential new indication: malaria. Should this trial produce positive results, implementation in the field will require clarity on the inclusion of women of child bearing age with the intention to reduce malaria transmission. A risk-benefit for the new indication requires data on safety of ivermectin during pregnancy.

Given uncertainty regarding the efficacy of ivermectin to reduce malaria transmission and insufficient data regarding safety during pregnancy, in this trial, when women are invited to participate, pregnancy tests are offered as part of the screening process previous to enrollment. That is, women declining the test or those with positive results are not eligible for enrollment. Hence, this trial will generate limited evidence on the safety of ivermectin treatment during pregnancy.

In consequence, additional data regarding the safety of ivermectin during pregnancy will be needed before future implementation. If this trial establishes the efficacy of ivermectin against malaria, the required pregnancy data can be obtained in a second study that Unitaid has agreed to support.

1.6. Risks and benefits

1.6.1. Risks

The ivermectin dose chosen for the BOHEMIA trials is included in the EU approved label of Stromectol [54]. Albendazole will also be used as per EU and country label. The only differences in the ivermectin administration are the repeated monthly dosing during the malaria season, and the indication for indirect, community benefit for malaria, rather that the direct treatment of other infections. Thus, the key anticipated risks for participants taking ivermectin are related to potential bioaccumulation given the experimental regimen (one dose a month for three months). The theoretical risks for teratogenicity are contemplated in the label for both drugs.

1.6.1.1. Drug accumulation

a. Ivermectin

The 400 mcg/kg ivermectin dose is currently included in the EU label (The Netherlands), and there are records of more than 80,000 safe, independent administrations facilitating regulatory considerations (see table S4). The proposed regimen (i.e.; 400 mcg/kg single dose monthly x 3 months), although experimental, is predicted to result in a measurable impact as well as minimum drug accumulation based on pharmacological modeling.

When ivermectin is given repeatedly at fixed intervals (multiple dosing), plasma concentrations will increase until absorption is balanced by elimination. The accumulation ratio (AR) is a measure of how average steady-state concentrations relate to the first administration. For all practical purposes, steady-state conditions are considered to be reached in multiple dosing after 3-5 half-lives of the drug have

passed. The AR can be calculated from observed data taken from non-compartmental analyses, such as from the ratios of maximum concentrations (C_{max}) at steady state (ss) and single dose (single):

$$AR = \frac{C_{max,ss}}{C_{max,single}}$$

AR can also be calculated from the ratio of area under the concentration-time curves (AUC):

$$AR = \frac{AUC_{ss}}{AUC_{single}}$$

It may also be calculated analytically with knowledge of the elimination rate constant (k_{el}) and the dosing interval (τ):

$$AR = \frac{1}{1 - e^{(-k_{el}*\tau)}}$$

where k_{el} and τ are required to be on the same time scale (e.g.; hours).

Figure 3 shows the predicted accumulation of ivermectin accounting for different half-lives.

b. Albendazole

The 400 mg dose of albendazole is recommended by WHO for annual or bi-annual mass treatment of children up to 14 years of age as a public health intervention in areas where the baseline prevalence of any soil-transmitted infection is 20% or more among children, in order to reduce the worm burden [62].

Although the BOHEMIA regimen will be slightly higher than once or twice a year (i.e.; 400mg monthly x 3 months), much higher doses are already SRA label-approved for the treatment of other diseases such as neurocysticercosis (800 mg daily for 8-28 days) and cystic hydatid disease (400mg daily for 28 days).

Several clinical trials have safely tested experimental, more frequent, albendazole regimens for NTDs. This includes three annual single doses [63-65] as well as a daily dose for seven [66] and twelve days [67] for LF and three-monthly treatment for Soil-Transmitted Helminths (STHs) [68].

Figure 3.Accumulation of ivermectin in once-monthly dosing (30 days)

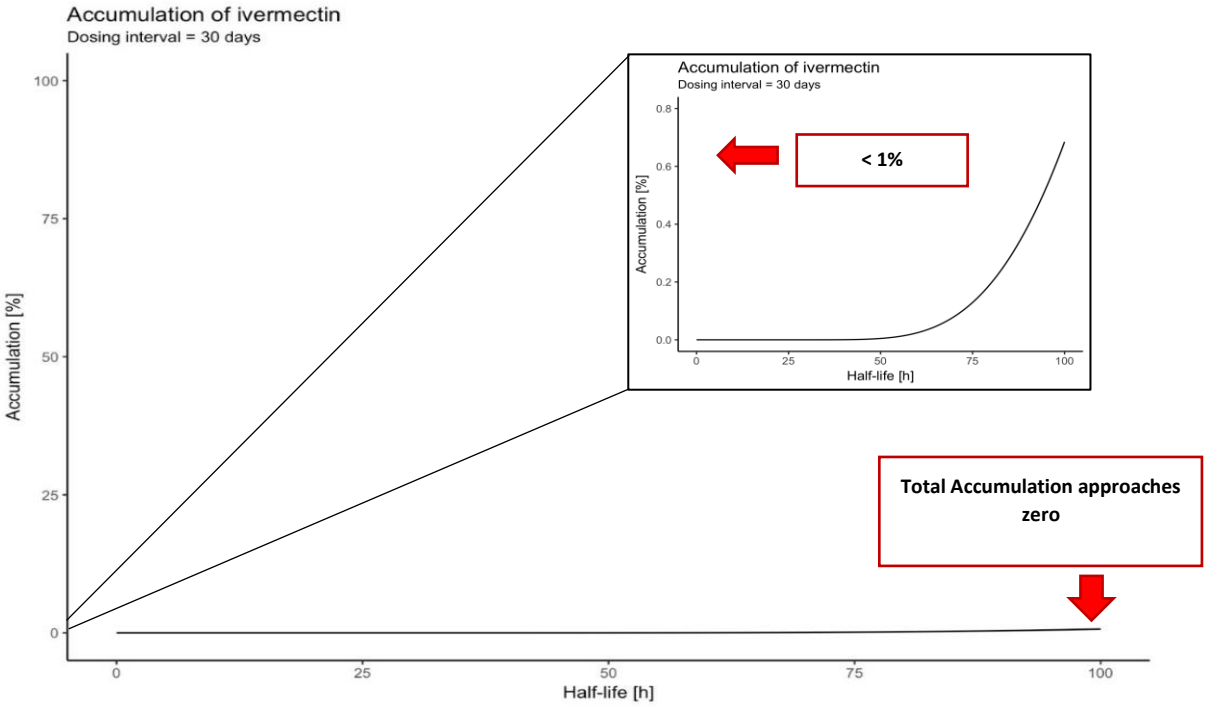
Assuming a dosing interval of $\tau = 30d$, we can visualize the influence of half-life on AR. The accumulation is given as a percentage. Modeling indicates that there is no relevant accumulation of ivermectin to be expected even with a (8-fold) prolonged half-life of up to 100h, which results in expected accumulation of <1% of the dose.

1.6.1.2. Adverse reactions

a. Ivermectin

All data in this section is taken from the FDA-approved Merck Stromectrol® 2009 label [49].

An acceptable safety profile was observed in an open study in pediatric patients ages 6 to 13 [49].



When ivermectin is used in patients with *strongyloidiasis* (dose of 200 mcg/kg) the following side effects classified by body system have been described:

- Body as a Whole: Asthenia/fatigue (0.9%), abdominal pain (0.9%)
- Gastrointestinal: anorexia (0.9%), constipation (0.9%), diarrhea (1.8%), nausea (1.8%), vomiting (0.9%)
- Nervous System/Psychiatric: dizziness (2.8%), somnolence (0.9%), vertigo (0.9%), tremor (0.9%)
- Skin: pruritus (2.8%), rash (0.9%), and urticaria (0.9%).

When ivermectin is used in patients with *onchocerciasis* (dose 150-200 mcg/kg) the following side effects have been described:

- Mazzoti reactions during the first 4 days after treatment including arthralgia/synovitis (9.3%), lymph node enlargement or tenderness (up to 13.9%), pruritus (27.5%), urticarial rash (22.7%) and fever (22.6)
- Worsening of pre-existing ophthalmological conditions: limbitis (5.5%), punctate opacity (1.8%).
- Additionally, the following clinical adverse reactions were reported as possibly, probably, or definitely related to the drug in ≥1% of the patients: facial edema (1.2%), peripheral edema (3.2%), orthostatic hypotension (1.1%), and tachycardia (3.5%). Drug-related headache and myalgia occurred in <1% of patients (0.2% and 0.4%, respectively).

In patients receiving cumulative doses above 800 mcg/kg in a week, unspecific, mild (not impeding daily activities), transient (lasting less than 24 hours), visual disturbances (blurred vision, tunnel vision) have been described.

When ivermectin is used in patients with concomitant *Loa* with high parasite burden (> 15.000 mf/ml) the following side effects have been described:

- Encephalopathy in 0.01-0.11% of the treated population [69]
- The syndrome includes confusion, lethargy and coma

Laboratory test findings reported as possibly, probably or definitely associated with ivermectin:

- Elevation in liver enzymes (ALT and/or AST) (2%)
- Decrease in leukocyte count (3%).
- Leukopenia and anemia (<1%).

The following adverse reactions have been reported since FDA marketing authorization:

- Hypotension (mainly orthostatic hypotension)
- Worsening of bronchial asthma
- Toxic epidermal necrolysis
- Stevens-Johnson syndrome
- Seizures
- Hepatitis
- Elevation of liver enzymes
- Elevation of bilirubin
- CNS toxicity in individuals with nonsensical mutation of the P-gp

b. Pharmacogenetic considerations

Ivermectin is metabolized by CYP3A enzymes and effluxed over various organ barriers (e.g. the blood-brain barrier, intestinal mucosa) by the drug transporter P-glycoprotein (P-gp, ABCB1). While no genetic variants with a clearly characterized functional impact on enzyme activity are known for the CYP3A isoform CYP3A4, the second isoform expressed in adults, CYP3A5, is well known for its genetic polymorphism. Of particular importance is the variant *3 (rs776746) that affects mRNA splicing, and results in mRNA degradation and thus no production of a functional protein. While a majority of individuals of European ancestry are homozygous carriers of the *3 variant and thus have no CYP3A5 activity (“nonexpressers”), the functional allele, *1, is more common in African populations, resulting in an increased frequency of CYP3A5 “expresser” phenotypes. In addition, two missense variants, *6 and *7,

occur at a relevant frequency in African populations. Given this extensive polymorphism, interindividual differences in the CYP3A5 phenotype could affect the metabolism of ivermectin and potentially also the susceptibility to adverse effects or individual dose requirements. Indeed, a small study in patients from Ghana suggested an impact of *CYP3A5**1/*3 on response to ivermectin [70]. However, the impact of *CYP3A5* genetic variation on ivermectin PK and ADRs has not been assessed.

Similarly, several genetic polymorphisms are known in the gene encoding P-gp, *ABCB1*. Given the neurological adverse effects observed with ivermectin and the important role of P-gp in drug transport across the blood-brain barrier, *ABCB1* genetic variation may impact the susceptibility to ivermectin-induced neurological adverse effects, but has not been investigated yet in this context except for a single small study including only four patients with adverse effects and nine control patients. In this study [71], two of the four patients with adverse effects were homozygous for an *ABCB1* haplotype that was also associated with CNS adverse effects from codeine [72]. There is also a recent case report of a child with severe CNS toxicity after ivermectin treated in association with nonsense mutations in the *ABCB1* gene [73].

The current project with ivermectin use in a large study population for malaria prevention and with its sub-study on ivermectin PK thus provides a unique opportunity to perform a pharmacogenetic study on ivermectin metabolism and neurological adverse effects, in order to gain novel insights into the underlying mechanisms. Furthermore, identification of genetic determinants of ivermectin distribution and metabolism provide insight into potential benefits of dose individualization, e.g. in individuals not achieving effective systemic concentrations at standard doses to obtain the desired detrimental effect on malaria-carrying mosquitoes.

c. Albendazole

All data in this section is taken from the FDA-approved GSK Albenza® 2009 label [74]

The following AEs were observed during clinical studies. The causality has not necessarily been established for these events.

Common (≥1%)

- Abdominal pain was the most frequently reported symptom (1%) during short term dosing, however this frequency was not significantly different from that in placebo-treated patients.

Uncommon (>0.1% and <1%)

- Diarrhea
- Nausea
- Vomiting
- Dizziness
- Itchiness and/or skin rashes were reported.

There was no significant difference in the percentage of patients experiencing diarrhea, compared to placebo-treated patients.

Rare (< 0.1%)

- Bone pain
- Proteinuria

- Low red cell count
- Leukopenia
- Transiently raised hepatic enzymes
- Hypersensitivity reactions including rash, pruritis and urticaria have been reported very rarely.

The following adverse reactions have been reported since marketing authorization:

- Headache (≥ 0.1% and <1%).
- Erythema multiforme and Stevens-Johnson syndrome have been reported very rarely (<0.01%)

During prolonged higher dose albendazole therapy (>28 days of continuous dosing) for hydatid disease, blood disorders such as pancytopenia, aplastic anemia and agranulocytosis have been associated very rarely with albendazole treatment. Patients with liver disease, including hepatic echinococcosis, appear to be more susceptible to bone marrow suppression. In this context of prolonged treatment, there have also been reports of severe hepatic abnormalities, including jaundice and hepatocellular damage which may be irreversible. These reactions are not anticipated in the BOHEMIA clinical trials in which a single dose is used once for three months, as opposed to continuous high dosing for 30 days which is the context in which these issues have been described.

1.6.1.3. Pregnancy

a. Ivermectin

Information from the Stromectol® label [49].

At high doses, well above (ranging from 10-80-fold human therapeutic doses and regimens), ivermectin is a known teratogen in mammals and is classified by FDA as a category C drug (these categories are no longer in use, as FDA now recommends considering risk/benefit on an individual basis). During onchocerciasis MDA campaigns with lower dose within the EMA label, visually pregnant women are excluded but no formal testing is routinely performed on women of childbearing age. There are several reports of inadvertent treatment during pregnancy, a recent metanalysis by Nicolas et al. analyzed data from 893 inadvertently exposed pregnancies (97 in the first trimester) and concluded there is insufficient evidence to understand the safety of ivermectin during pregnancy.

b. Albendazole

Information from the Albenza® label [74]

There are no adequate and well-controlled studies of albendazole administration in pregnant women. Pregnancy Category C (these categories are no longer in use, as FDA now recommends considering risk/benefit is considered on an individual basis). Albendazole has been shown to be teratogenic (causing embryotoxicity and skeletal malformations) in pregnant rats and rabbits. The label warns against using albendazole in pregnant women except in clinical circumstances where no alternative management is appropriate. Patients should not become pregnant for at least 1 month following cessation of albendazole therapy.

1.6.1.4. Blood sampling

In selected study groups (children enrolled in the active pediatric cohort, participants in the population PK sub-study and COVID response) blood will be collected via finger prick. All collections will be from skin capillaries using a sterile technique and done by trained personnel. This sampling via finger prick has a small risk of infection, temporary pain from the finger prick (and squeezing for the blood drops) and possible bruising/sensitivity lasting for a few days. In order to reduce discomfort, personnel will try obtain all samples from a single prick.

1.6.1.5. Social risks

Women of child bearing age will be asked about their pregnancy status and pregnancy tests will be offered and performed privately before any dose with study product. If the test is positive or test consent declined, or if the woman is visibly pregnant, the woman will be excluded from the trials.

For minors (aged 12-17 years), in order to minimize the risk of social harm, assent will be obtained privately prior to MDA regardless of gender. This is to maintain participant confidentiality whilst at the same time avoiding singling out the female minors and the requirement for pregnancy tests.

The Social Science team will start formative research from July 2021 and will be gathering additional data from the field. As the core team receive this ongoing feedback, the most appropriate methodologies are explored, and implemented in the relevant study specific procedures.

1.6.1.6. Animal handling

There is a minimal risk that livestock owned by households may be injured when they are restrained for treatment. To reduce this risk, fieldworkers will be trained in safe restraint techniques and will stop the process if there is any concern for the safety of the animal. Animals proving difficult to restrain will be left un-visited. Animals missing two consecutive visits will be considered lost to follow up.

1.6.2. Benefits

The BOHEMIA trials have a design in which a group of participants receive the drug (weight above 15 kg, non-pregnant, not taking excluded medications) but the main outcome is measured in another population, an active pediatric cohort (under 5 years in Mozambique and 5-15 years in Tanzania) representing a portion of all children in these age ranges in the area. There are potential benefits for all groups, those taking the drug, those in the active pediatric cohort and the rest of the community which receive ivermectin.

In the intervention group, those taking ivermectin will benefit from its known effects on ectoparasites such as scabies [75], head lice [76], tungiasis [77] as well as STH including partial efficacy against *Ascaris* [78] and *Trichuris* [78] and full efficacy against *Strongyloides* [79]. The sites selected for BOHEMIA are not currently considered endemic for onchocerciasis or LF but filariae in the intervention group, should there be any, would also be treated. There is a potential indirect benefit to the ivermectin group in terms of reduced malaria transmission, which is the primary objective of the trials.

Participants in the control group will receive albendazole single dose. The main rationale for this is ethical. While there is clear *equipoise* (that is, **lack** of evidence whether ivermectin works as intended) for the potential benefit of ivermectin regarding malaria transmission, ivermectin has proven efficacy against *Ascaris lumbricoides* and partial efficacy against *Trichuris trichura,* two of the most frequent STHs. Additionally, the effect of helminth infection on the human immune response is well established [80], for

malaria there is limited evidence in this regard [81, 82]. Having a degree of deworming in both groups can help reduce confounding due to potential helminth-related immunoregulation.

Albendazole serves three purposes in the design of these trials:

a. It provides participants in the control group with the benefit of deworming
b. It helps with acceptability as participants in both groups will pass worms
c. It increases comparability given some deworming in both groups

Members of the community that do not participate in the study will also receive an indirect benefit. This includes those ineligible for drug administration (pregnancy, children under 15 kg, concomitant medication) or children under 5 that are not eligible for treatment and are not part of the active pediatric cohort. These groups will receive the indirect benefit of a reduced transmission of NTDs in their communities given the above-mentioned effects.

Accuracy of clinical diagnosis and recording as well as treatment access at health system will be facilitated by placing study personnel in all health facilities (health facility workers, HFW), who will ensure that: (a) all visits to the health facility and all RDT results are accurately recorded, (b) all study visits are associated with a village code, allowing for matching the patient results with the treatment group, and (c) that antimalarial stock levels are reported in a timely manner to the district authorities in an attempt to prevent stock-outs.

In the three arms, children that are enrolled in the active pediatric cohort will contribute to the primary outcome measure (malaria infection incidence) and will benefit from a monthly visit from a trained study team member that will test and treat any malaria infection regardless of symptoms. These children will also receive prompt referral to the health facility should any sign of other severe illness be detected during the visits.

Livestock in the corresponding group will receive ivermectin treatment in accordance to the approved package insert. These animals will be dewormed which has been proven to increase yield of meat and milk [83] and provide owners with a direct economic benefit [84].

1.7. Study sites

BOHEMIA cluster randomized trials will be carried out in two sites in East and Southern Africa: Mozambique and Tanzania. These sites encompass different transmission dynamics that increase the generalizability of results, namely: (a) a gradient of malaria transmission: moderate in Tanzania and very high in Mozambique, (b) different species composition of vector population, (c) different rain patterns and environmental conditions, (d) different livestock species and human/livestock ratios and (e) different housing and practices (**Table 1**). These differences will also play an important role in assessing the role of seasonality given the proposed one-year duration of the trial.

The clinical trials will be conducted by an established research organization in each country with a track record of conducting successful research projects and a population that meets the trials' requirements.

1.7.1.1. Main selection criteria for trial sites

a) Characterized malaria transmission and control measures
Baseline estimates of malaria transmission, particularly incidence in the most vulnerable population, was of key importance for calculating a sample size that can match the WHO recommended epidemiological outcomes. Both sites have LLINs as primary vector control method, coverage is high (ownership of at least one LLIN per household was 94% in Mopeia in 2018 and 89-95% in Rufiji in 2017 [45, 85]). Mopeia receives IRS from President's Malaria Initiative (PMI) (structure coverage 79% in 2017 and 87% in 2018); Rufiji additionally received larviciding from the National Malaria Control Program (NMCP) (1,500 households) in October 2018 and January 2019 with currently no plan for additional larviciding.

b) Diversity
Two distinct geographies (east and southern Africa), two transmission intensities (meso- and holoendemic) and vectors including important dominant species (*An. gambiae*, *An. arabiensis*, *An. funestus* in Mozambique and *An. arabiensis*, *An. funestus* in Tanzania) and secondary species (*An. rivulorum* in Mozambique and *An. merus* in Tanzania).

c) Absence of *Loa loa* in the area of the study site
There is no reported *Loa loa* in Mozambique or Tanzania.

d) Clinical research capacity and partnership
Each BOHEMIA site has already established clinical trial capacity in country with active work streams in malaria and investigators are linked to national systems for research and to the country MoH, as evidenced by letters of support. Each member of BOHEMIA has been actively engaged in formulating the research strategies of this protocol and agreed to the proposed data management, assays (including centralization where relevant), commitment to working to ICH-GCH principles, and governance structure of the consortium.

e) Smallholder farming
Broader livestock ivermectin delivery will not only impact on the mosquito vector and thus human malaria, but also reduce the burden of intestinal helminths and ectoparasites in dominant domesticated herds. The households to be randomized to the clinical trials must meet the protocol requirements for livestock ownership.

2. Study objective and purpose

The BOHEMIA program consists of a combination of studies organized around a central community prevention mass drug administration protocol and four sub-studies (i.e.; social science, entomology, health economics and animal health, and environmental), each written as an individual protocol. The safety and efficacy protocol is central and used in two different trials implemented in Mozambique and Tanzania. The trials have been powered on the efficacy outcome, which is described in this central protocol, whereas the remaining aspects are described in the corresponding sub-study protocols

2.1. Primary research question

Does ivermectin MDA *to the eligible human population in three monthly doses of 400 mcg/kg at the start of the rainy season (with or without including livestock) result in a relevant reduction of malaria transmission (as shown by a 20% reduction in infection incidence and supported by entomological measurements) with an acceptable safety profile?*

The BOHEMIA clinical trials aim to provide the necessary evidence on safety and efficacy to contribute towards a WHO analysis and policy recommendation on the complementary use of ivermectin as a first-in-class endectocide to reduce malaria transmission.

Each trial is powered to fulfil the specifications of the WHO PPCs for endectocides to reduce malaria infection incidence by at least 20% in children in the highest burden age group (under 5 years of age [61] in Mozambique and 5-15 years in Tanzania). Each country will have a sample size that allows for a stand-alone analysis.

Demonstrating an impact of ivermectin as complementary vector control tool will be key to design or understand the effect of other potential combinations with currently available or future tools. The primary epidemiologic endpoint will be supported by entomological assessments and by the monitoring of transmission in other age groups to improve understanding of the mechanisms affecting transmission.

2.2. Primary objective

To determine the safety (in humans) and efficacy of ivermectin MDA (to humans or human and livestock simultaneously) for the prevention of malaria.

Note these co-primary objectives are determined in different populations. The efficacy endpoint is primarily measured in children (under 5 years of age in Mozambique and 5-15 in Tanzania) and safety (see section 5.1) is determined by anyone who receives the drug. Given inclusion criteria there might be a small overlap in the two populations.

2.3. Secondary objectives

- To assess the efficacy of the intervention using complementary methods (efficacy)
- To assess the safety of the intervention with complementary methods (safety)
- To assess the PK of the proposed ivermectin dose/regime in its relationship with efficacy and safety outcomes (efficacy and safety)
- To assess the impact of ivermectin MDA at the proposed regimen on the prevalence of selected ectoparasitic NTDs (efficacy on NTDs and acceptability)
- To assess the relationship between malaria incidence in children under 5 and community prevalence at the peak of the malaria season (this serves for prevalence outcomes and paves the way for future studies or future evaluation using this outcome which could require fewer resources).
- To assess the relationship between active and passive surveillance for malaria at health facility (this serves as validation passive surveillance and paves the way for future studies using this outcome which is much less resource-consuming while ensuring no key safety events are missed)
- To assess the accuracy for malaria diagnosis of two the different malaria rapid diagnostic tests (RDTs) used in comparison to PCR (this is directly linked to efficacy as determined by RDTs)

2.4. Exploratory objectives

- To assess the potential direct or indirect effect of ivermectin on immune response to the malaria parasite.
- To assess the relationship between self-reported pregnancy status, the LMP and the results of pregnancy tests in women of reproductive age participating in the study.
- To assess all-cause and malaria-related mortality in intervention and control arms.
- Estimate prevalence of relevant CYP and P-gp variations in the target population and subjects suffering from CNS AEs
- COVID RESPONSE: to assess the serological prevalence and incidence of COVID-19 in the study area (Mozambique only)

3. Study design

The BOHEMIA clinical trials have been designed in accordance with VCAG recommendations for conducting Phase III trials of traditional vector control tools in two different epidemiological settings [43]. The trials will use ivermectin as first-in-class complementary vector control product to target residual transmission.

The clinical trials will be open label, cluster randomized, controlled, parallel arm trials (see section 3.2 for groups and sequence where extensive rationale is provided) and will last one year. Given the nature of endectocides, which provide indirect protection through a community effect, the intervention must be randomized in clusters.

Independent analysis for the primary endpoints will be conducted at each site; the trials will implement the same design, with sample size adapted to local epidemiology. There will be subsequent cross-trial analysis to explore variations in efficacy across the sites, in the context of varying endemicity, vectors and other variables, as well as site-specific safety issues should these exist.

BOHEMIA will rely on control measures already present in both sites (see section 1.7 for details on measures and coverage). The trials will not distribute LLINs or apply IRS but will evaluate the added value of ivermectin to nationally implemented programs.

3.1. Outcome measures

3.1.1. Primary

3.1.1.1. Efficacy

- Infection incidence in the most vulnerable age group (children under 5 years of age in Mozambique and 5-15 years in Tanzania) for 6 months from the moment of the first MDA round in their community.

Infection incidence has been chosen as primary endpoint based on the WHOs PPC for endectocides, that states that in areas of moderate to high transmission, the minimally acceptable efficacy criterion would be “at least 20% reduction in the incidence of clinical malaria (as primary outcome) and incidence of infection (as secondary outcome) in children under 5 (5-15 in Tanzania), lasting for at least 1 month following a single regimen”. The monthly frequency of visits has been chosen given that infected children will receive treatment with Coartem (artemether-lumefantrine), which has an established post-treatment

prophylaxis period of two weeks [86, 87], monthly visits will allow for at least 14 days -at-risk between visits.

Modeling for the BOHEMIA trials has predicted a reduction of up to 40% in yearly malaria incidence, and thus a 20% target is conservative (see section 3.5). In addition, the main finding of the RIMDAMAL (the first field randomized trial of ivermectin MDA against malaria) was a 20% reduction in the cumulative uncomplicated malaria incidence in children under 5 living in the treated villages, so there is a precedent for such a reduction.

Infection incidence will be determined in a cohort of children in the target age that is followed prospectively for six months post receipt of first dose in the community by a dedicated field team. Each child will be tested at home using RDTs (Parasite Lactate Dehydrogenase (pLDH) and HRP2 based). Two different tests will be used given the known persistence of HRP2 antigen after treatment with up to 50% residual positivity after six weeks while LDH-based tests become negative in just 48 hours [88]. Treatment for children with positive tests will be provided according to **Table 5**. Quality control of RDT results will include PCR assessment of 10% of the samples.

c. Case definition

Given the use of two different RDTs, there are four possible results combinations at each visit. The potential implications for treatment and inclusion as an incident case are detailed in **Table 5**. In summary, any positive RDT will result in treatment but not all will be considered an incident case.

HRP2	pLDH	Treatment	Interpretation	Inclusion as incident case
Negative	Negative	No	Negative	No
Positive	Positive	Yes	Incident case	Yes
Positive	Negative	Yes	Potential false positive[1]	Yes if enrolment visit, then onwards only if HRP2 negative in previous visit
Negative	Positive	Yes	Potential false negative[2]	Yes[3]

Table 2: Case definition by RDT results

[1]If this child received full treatment in the last month, the most likely outcome is that this is the result of residual false-positive HRP2 given two weeks of prophylaxis by Coartem and incubation period of at least 10 days. [2]This result is unlikely given higher sensitivity of HRP2 but could occur in the rare event of an infection with a parasite with HRP2 gene deletion. [3]if this event occurs more than once during follow-up it will warrant PCR analysis for potential HRP2 deletion screening.

d. Selection of tests as primary trial outcome

Although PCR assay is more sensitive to detect infections, it can have remarkable variations across laboratories, adds considerable costs and needs for standardization/validation with little added benefit in

the context of detecting malaria in a cohort of children with relatively high parasitemia given no or limited acquired immunity. Additionally, it cannot be used for treatment decisions in the field.

PCR will however be used to determine parasite prevalence in the cross-sectional surveys done to the whole population one month after the last MDA round given that a high proportion of sub-microscopic and sub-RDT infections can be expected among adults in moderate-high transmission areas.

3.1.1.2. Safety

- Rate of AEs and serious adverse events (SAEs) and difference between ivermectin and albendazole

3.1.2. Secondary

3.1.2.1. Complementary efficacy analysis methods

- Time to first positive RDT in children in the cohort
  - *This is derived from the RDTs performed in the cohort of children*
- Molecular force of infection in a subset of children in the cohort
  - *This is derived from the PCR performed for quality control of the RDTs in the cohort of children*
- Malaria case incidence in all ages presenting at health facility
  - *This is the result of the passive surveillance data collected at health facilities and supports the active surveillance results.*
- Malaria prevalence in all ages one month after the last dose
  - *This is derived from the cross-sectional studies*
- Multiplicity of infection in all ages one month after the last dose
  - *This is derived from the cross-sectional study*

3.1.2.2. Complementary safety variables

- Observed tolerability of the dose
- Rate of SUSARs
- AEs and SAEs by organ system

3.1.2.3. Pharmacokinetics

- Whole blood concentrations of ivermectin in dried blood spots.

For BOHEMIA it is assumed that whole blood ivermectin concentrations are approximately 70% of the plasma concentration based on previous work from Duthaler *et al.* [89]. This will facilitate interpretation of results and extrapolation of supporting studies based on either plasma or whole blood concentrations. The dry blood spot method to be used in BOHEMIA has been validated according to EMA standards [89].

3.1.2.4. Impact of ivermectin against selected ectoparasites and NTDs in the dosage regimen chosen

- Serial prevalence of scabies using a simplified version of the algorithm described by Mahe *et al*. [90] which consists of two questions and evaluation of exposed skin on hands, knees, elbows, armpits, wrists, and feet.
- Serial prevalence of head lice by visual inspection over three minutes in scalp sites of predilection for head lice: the back of the ears, temples and neck [91].
- Serial prevalence of *Tunga penetrans* via visual inspection of the skin in both feet and applying the Fortaleza scale [92].
- Serial prevalence and severity of bed bug infestation by direct questions about bedbugs in the house, and visual inspection of exposed skin and sleeping rooms for evidence of bed bug bites or bed bug infestation.

3.1.2.5. Prevalence - incidence correlation

- Malaria infection incidence in children (under 5 years in Mozambique and 5-15 years in Tanzania) in the pediatric active cohort at community and health facility levels
- Malaria prevalence at all ages

3.1.2.6. Relationship between active and passive surveillance

- Infection incidence in children at community level (active surveillance)
- Infection incidence in children at health facility level (passive surveillance)

3.1.2.7. Performance of RDTs vs PCR

- Results correlation between HRP2 and pLDH-based RDTs
- Results correlation between RDTs and PCR
- Proportion of RDT negative but PCR positive infections

3.1.3. Exploratory

3.1.3.1. Effect of ivermectin on the immune response to the malaria parasite

- Comparison of infection incidence between children of the same age that receive ivermectin (>15 kg) vs. those who did not receive ivermectin (<15 kg)
- Changes in the innate or acquired immune response against the malaria parasite

3.1.3.2. Accuracy of self-reported pregnancy status and LMP

- Proportion of pregnant women among those self-reporting as non-pregnant
- Proportion of pregnant women among those reporting LMP more than 2 weeks before the test.

3.1.3.3. All-cause mortality

- Rate of all-cause and malaria-related mortality as determined by health facility records and BOHEMIA safety reporting during the trial
- Verbal autopsies of deaths of participants on both cohorts

3.1.3.4. Pharmacogenomics

- Case-control association study of ivermectin-associated neurological adverse effects with genetic variation in *CYP3A5* and *ABCB1* and assess the association of the same genetic variants with ivermectin PK.

3.1.3.5. COVID RESPONSE: descriptive serological prevalence and incidence of COVID-19 in the study area

- (Mozambique only) Prevalence of COVID-19 antibodies using a rapid serologic test during the cross-sectionals
- (Mozambique only) Sero-incidence of COVID-19 using rapid serologic tests for 6 months in a subsample of the study population

3.2. Study type

The two BOHEMIA clinical trials are open label, cluster randomized, controlled, parallel arm trials that will be conducted during the peak malaria season in Mozambique and Tanzania respectively.

3.2.1. Clusters

The rationale for cluster randomization is based on (a) the intervention will be applied to communities, and (b) the impact will be measured as reduction of rates, which are best assessed at community level. Additionally, by using clusters as units of randomization instead of individuals, we reduce contamination that could occur if close neighboring participants were assigned different treatments. To help reduce contamination, clusters are divided into a central core and a buffer zone, and outcomes will be assessed under a 'fried-egg' design. This means that while the intervention is administered throughout the cluster, sampling will only occur in a central / core region.

Clusters will be defined according to the following key definitions and methods:

Household: a house (or compound) and its occupants are regarded as a unit. In many areas, there is more than one household in a single compound. In this case, households are considered as people who consistently share meals.

Village: a self-contained group of households regarded as a community. This can include sub-groups such as neighborhoods.

Cluster: the randomization unit in which groups of subjects or households are randomized.

Core: central area of a cluster. The population living here receive the interventions or control, and is eligible for participating in the active cohort for outcome measurement (Figure 4)

Buffer: the area surrounding a cluster´s core. Its size is in direct relationship with contamination, larger buffers are associated with less contamination. A minimum size will be defined according to the level of contamination risk deemed acceptable according the resulting proportion of concordant households in a radius resembling mosquito flying range around core households. The population living here receives the interventions or control, but is not considered eligible for primary outcome measure.

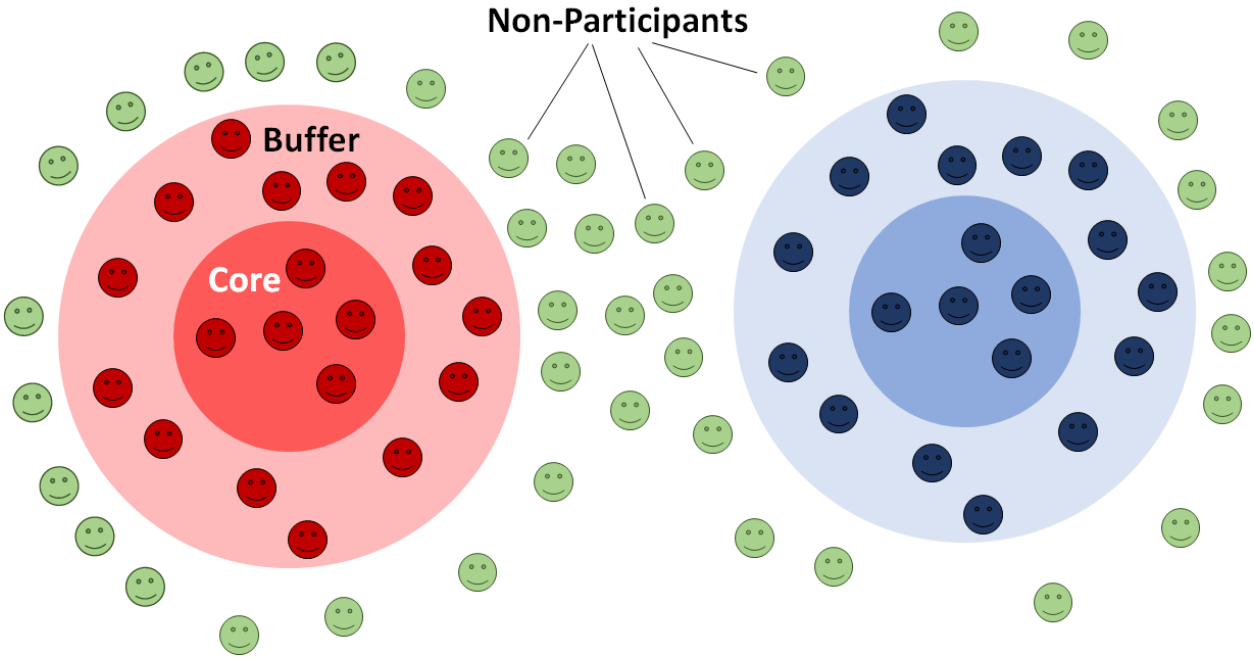


Figure 4. Clusters, core and buffers

Contamination: a state of heterogeneous or impure roll out of the intervention in any given cluster or group of clusters. This implies a risk of over- or under estimating the effect size. Given that the maximum recorded dispersal of Anophelines in East Africa is 661 meters [93], contamination risk will be assessed by calculating the mean proportion of households with equal assignment in a 1 km radius around every core household. For control households only ivermectin-treated households within this 1 km radius may cause contamination. For ivermectin-treated households both control and non-participating households may cause contamination (Figure 5).

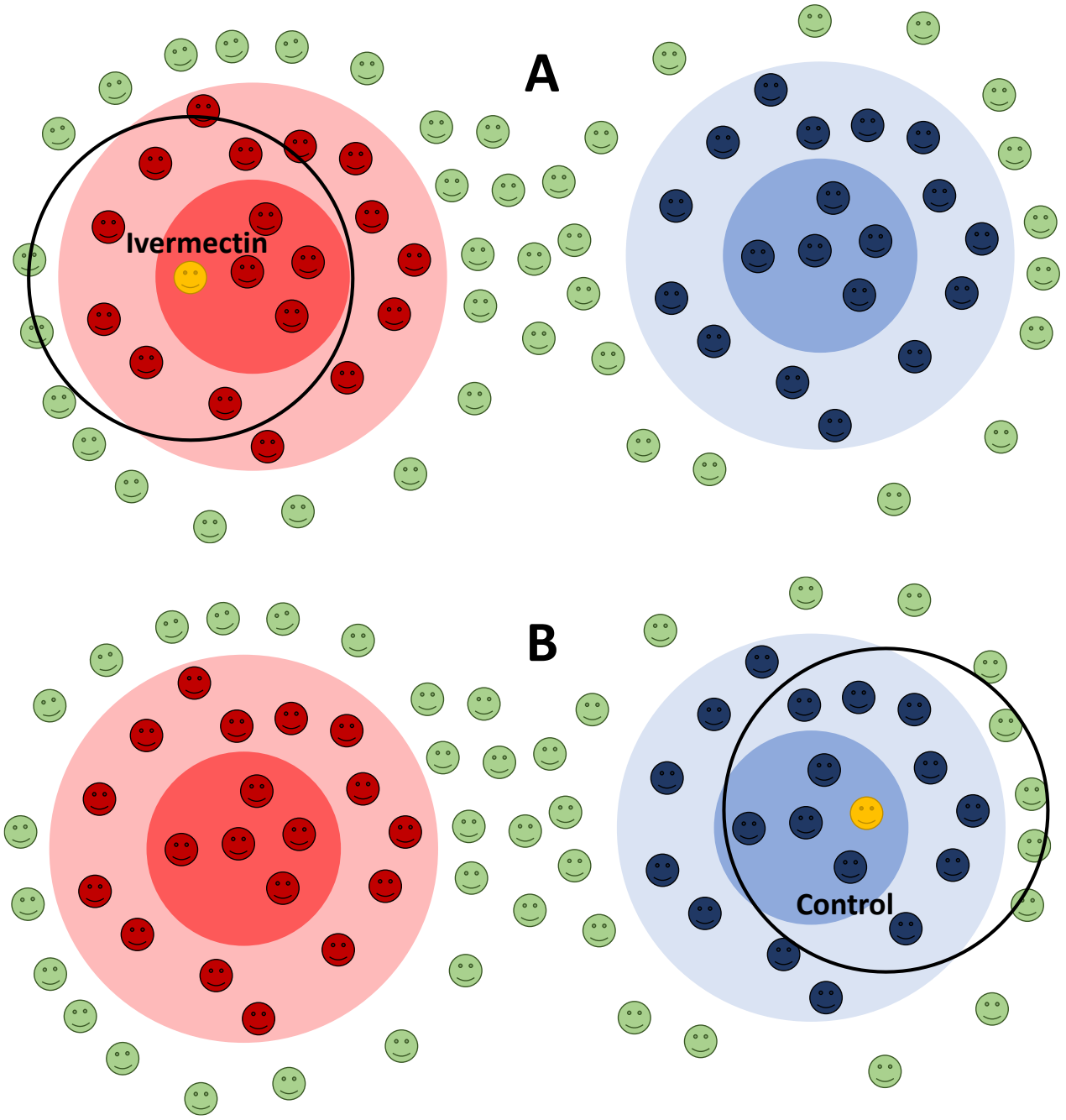


Figure 5. Buffers and contamination.
Panel A, note that the proportion of participants with equal assignment in the radius around the marked household is 12/14 (85%) this is because non-participants may contaminate ivermectin clusters. On the contrary, in panel B, the proportion of participants with equal assignment in the radius around the marked household is 100%, this is because non-participants do not contaminate control clusters as the treatment of controls has no effect on mosquitoes.

Cluster delineation: clusters will be defined following a baseline census (described in the BOHEMIA census protocol). Clusters will be delineated to maximize feasibility and efficiency of recording the

main outcome: malaria incidence in a cohort of children followed prospectively. These clusters have no size restrictions in terms of households or population, but are subject to the following constraints:

- Each cluster must contain a minimum buffer of 400 meters. The final size of this buffer will be defined to optimize the proportion of individuals with identical assignment status in a radius (500-1000 m) around each household in the core.
- Clusters may not overlap (effectively making the minimum buffer size 800 m between two cores).
- A range of cluster numbers and cluster sizes will be created to sustain the same power but adapting to the local distribution of the population as mapped during the activities under the demography protocol.
- Core children should ideally be from different households.

3.2.2. Sequence and groups

Three groups of clusters will be randomized to receive (a) ivermectin in humans, (b) ivermectin in humans and livestock or (c) control (i.e.; albendazole), and all the area will be subject to enhanced passive surveillance for malaria (**Figure 6**).

The nomenclature for these groups will be as follows

- Ivermectin in humans: [H]
- Ivermectin in humans and livestock: [H+L]
- Control (albendazole): [C]

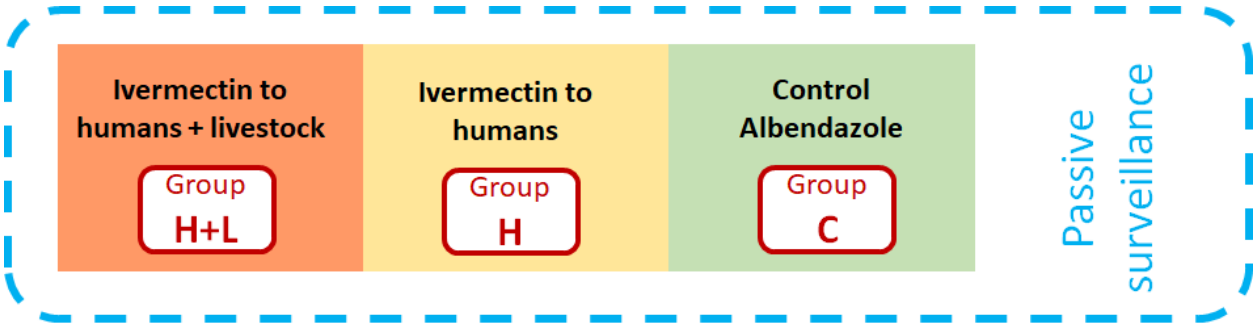


Figure 6. Study groups and intervention sequence

3.3. Assignment of intervention

3.3.1. Randomization

Cluster randomization implies that all individuals in the same cluster will receive the same intervention. Once all clusters in the district are defined, the target sample will be randomized using a computer-generated algorithm.

3.3.2. Allocation

Clusters will be randomized 1:1:1 into three study arms. The treatment status of each cluster will be assigned during cluster delineation and then transmitted to the population during a randomization ceremony

3.3.3. Blinding

The study will be open label but outcome assessor-blinded Participants in the ivermectin and control arms will receive products that differ in aspect and numbers. Participants will be informed that they are taking one of two antihelminthics. The sponsor and the study statistician will remain blinded to the assignment of each cluster group. All unblinded DSMB reports will be prepared by an independent statistician.

3.4. Sample size

The sample size for each country is based on the main outcome (malaria incidence in a cohort of children followed prospectively in the cluster's core area for six months), an outcome chosen based on WHO's PPC for endectocides. This sample size has been corrected for cluster effect following Hayes and Bennett formula for unmatched rates [94].

The magnitude (20% reduction) was selected based on WHO's recommended PPC for endectocides [61]. Additionally, the results of the RIMDAMAL trial suggest that a 20% reduction in malaria incidence can be achieved using a dosing regimen with a similar cumulative dose [40], these results although not statistically significant [58] do point towards an expected effect size. Mathematical modeling based on the Imperial College malaria model incorporating ivermectin [42] was conducted for this project and predicts a 40% reduction in malaria incidence in both countries. The model is described in detail in section 3.5 below.

The final sample size is dependent on the population distribution as identified in the demography protocol.

The number of children per cluster can vary in order to streamline the cluster generation once the demographic data is available. The power, significance level and target effect size will remain unchanged. The final number of clusters depends on the cluster size (the number of children in the active pediatric cohort recruited in the core of all clusters). Smaller cluster sizes require a higher number of clusters to render the same power. Figure 7)

The target number of children to be included in every cluster is defined by the expected enrollment rate and loss to follow up rate. We have estimated these as 15% and 20% respectively. So, for a cluster size to be 24 i.e., 24 children in the cohort finishing the study, the cluster must include 35 *eligible* children in the beginning (35 eligible of which an estimated 5 [15%] do not consent, 30 consent, of which 6 [20%] are lost to follow up)

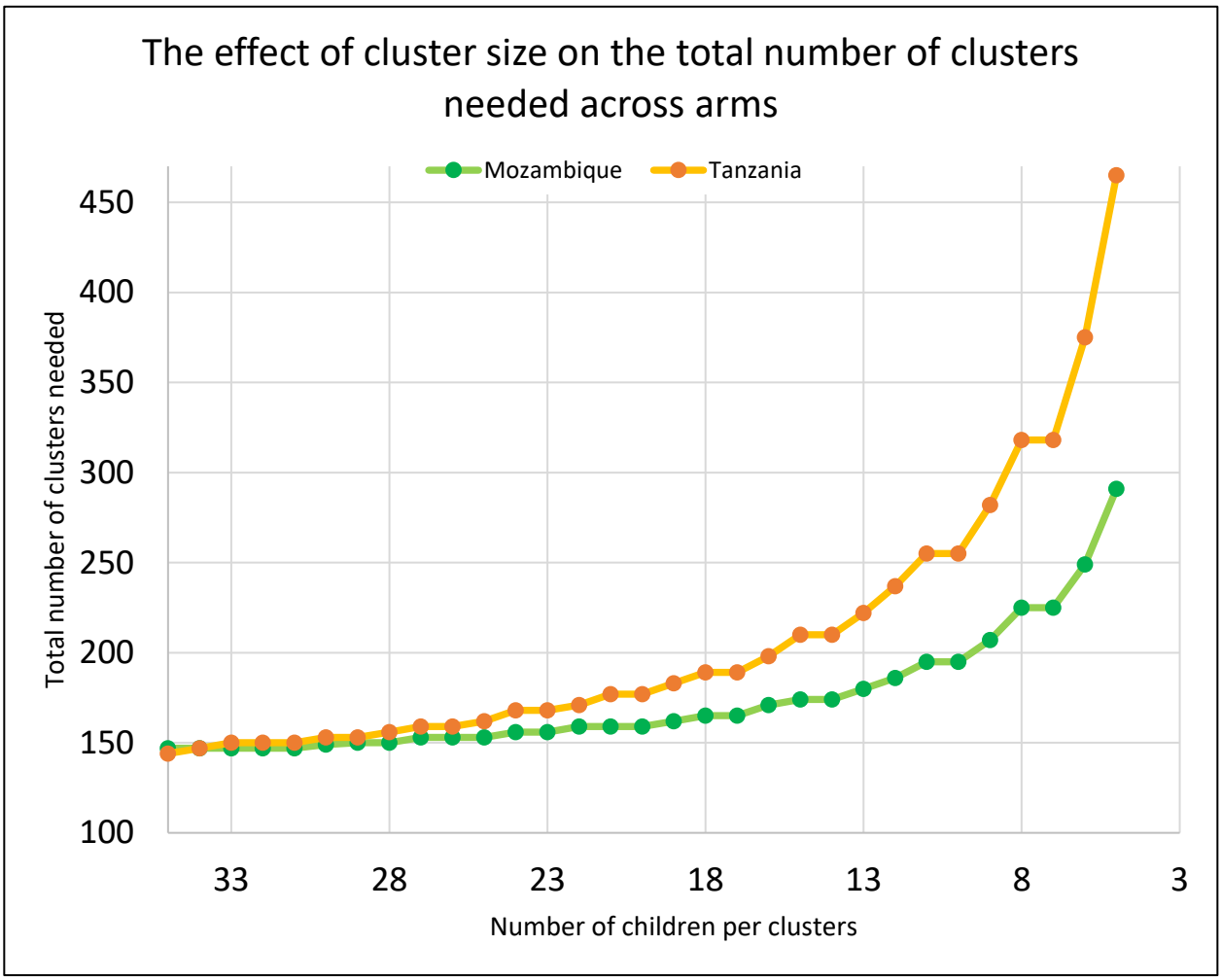


Figure 7.Cluster size and total clusters needed

The number of clusters required increases exponentially as the number of children per cluster (cluster size) decreases. This is particularly marked below 13 children per cluster. The number of children represented is the number of *eligible* children required at the beginning, not accounting for 15% non-consenting and up to 20% LFU.

3.4.1. Mozambique

Peak incidence age: < 5 years

Power: 80%

Significance level: 5%

Expected incidence in control group (λ_0): 4.20

Expected incidence in intervention (λ_1): 3.36

Incidence reduction: 20%

Optimal cluster size: 20 children (assuming 3 non consenting and 3 LFU)

Follow up time: 6 months starting post community dosing

Coefficient of variation (k_m): 0.35

People treated per cluster: approximately 300

Optimal total clusters required: 159 clusters (53 per arm)

The final sample size may have to be adjusted as by the table below. However, the power and affect size will remain constant as does the ceiling of treatable adults (not to exceed 48,000 eligible adults)

Total clusters needed	Total children eligible per cluster	Non-consenting 15%	Included	LFU 20%	Final size	Total children in clusters	Total children enrolled	Total children completing trial
147	35	5	30	6	24	5145	4373	3499
149	30	5	26	5	20	4470	3800	3040
153	25	4	21	4	17	3825	3251	2601
159	20	3	17	3	14	3180	2703	2162
174	15	2	13	3	10	2610	2219	1775
195	10	2	9	2	7	1950	1658	1326
291	5	1	4	1	3	1455	1237	989

3.4.1.1. Preliminary data

The baseline incidence and the coefficient of variation used for the sample size calculation for Mozambique are considered robust as they have been obtained from a recently finished cluster-randomized trial in Mopeia, the very same district where BOHEMIA will be conducted. The previous trial assessed the cost-effectiveness of combining LLIN and IRS [95].

3.4.1.2. Calculations

Based on the demographic data to be confirmed in the preceding census, the clusters are planned to include approximately 420 people, of which 280 will be above 4-5 years of age and potentially eligible for **treatment** and 140 will be children under 5 potentially eligible for **follow-up**. There will be 53 clusters per arm in Mozambique.

Clusters in Mozambique will be randomized in three arms as per **Figure 6**, resulting in inclusion of approximately 45,000 participants to receive treatment or control and approximately 2,000-4,000 children to be followed for six months.

With this sample size the study has 80% power at 5% significance to detect a 20% reduction in malaria incidence infection, from 4.20 cases/child-year at risk to 3.36 for the human-only intervention, using a coefficient of variation (k_m) of 0.35 for Mopeia/Mozambique. This value for k_m has been back-calculated from a cluster randomized trial recently-conducted in the same study site [96].

For the human and livestock intervention, this sample size has 80% power to detect a further 20.5% reduction in malaria incidence infection between the human and human + livestock intervention arms, from 3.36 cases/child-year at risk (the lower incidence expected in the human only arm) to 2.67.

The total number of clusters needed per arm has been increased by one for all scenarios to account for potential LFU of a whole cluster (withdrawal of community consent, displacement, etc.)

3.4.2. Tanzania

Peak incidence age: 5-15 years

Power: 80%

Significance level: 5%

Expected incidence in control group (λ_0): 1.50

Expected incidence in intervention (λ_1): 1.16

Incidence reduction: 22.5%

Cluster size: 25 children (assuming 4 non-consenting and 4 LFU)

Follow up time: 6 months starting post community dosing

Coefficient of variation (k_m): 0.35

People treated per cluster: approximately 300

Total number of clusters required: 162 (54 per arm)

The final sample size may have to be adjusted as by the table below. However, the power and affect size will remain constant as is the ceiling of treatable adults (not to exceed 45,000 eligible adults)

Total clusters needed	Total children eligible per cluster	Non-consenting	Included	LFU	Final size	Total children in clusters	Total children enrolled	Total children completing trial
144	35	5	30	6	24	5040	4284	3427
153	30	5	26	5	20	4590	3902	3121
162	25	4	21	4	17	4050	3443	2754
177	20	3	17	3	14	3540	3009	2407
210	15	2	13	3	10	3150	2678	2142
255	10	2	9	2	7	2550	2168	1734
465	5	1	4	1	3	2325	1976	1581

3.4.2.1. Preliminary data

The baseline incidence for Tanzania has been extrapolated from a recent study data that characterized malaria prevalence in a range of ages in Rufiji [44, 45], the very same district where BOHEMIA will be conducted. According to this recent data, the highest burden groups are children aged 5-15 years (prevalence of 21.5% at season's peak ranging from 9.4 to 29.7%). An incidence of 1.5 cases per child-year-at-risk was extrapolated from this prevalence using the ensemble model prediction of Cameron et al [97]. This extrapolation was corroborated against the expected incidence given climate zone by the modeling conducted to estimate the intervention's effect size (section 3.5).

The value for k_m for Tanzania was set as 0.35 to align both sites and provide the trial with the power to detect variations across a wide range of incidences. Assuming a normal distribution and using this K_m and the previously extrapolated mean incidence [μ] of 1.5 we calculated the interval expected to cover 95% of the cluster rates (1.50 +/- 1.96*0.525, where 0.525 is the standard deviation [σB] associated with the

selected K_m of 0.35 [$K_m = \sigma B/\mu$]). This 95% confidence interval is 0.47-2.52, which coincides with the range extrapolated from prevalence and seems robust enough to encompass 95% of observations in Rufiji.

3.4.2.2. Calculations

Based on the demographic data to be confirmed in the census, the clusters are planned to include approximately 340 people, of which 250 will be above 15 years of age and potentially eligible for **treatment** and 90 are children 5-15 potentially eligible for **follow-up.** There will be 54 clusters per arm in Tanzania.

Clusters in Tanzania will be randomized in three arms as per **Figure 6**, resulting in inclusion of approximately 40,000 participants to receive treatment or control and 4,000 children to be followed for six months.

With this sample size the study has 80% power to detect a 22.5% reduction in malaria incidence infection, from 1.50 cases/child-year at risk to 1.16 for the human-only intervention, using a coefficient of variation (k_m) of 0.35 for Rufiji/Tanzania.

For the human and livestock intervention, this sample size has 80% power to detect a further 23.5% reduction in malaria incidence infection, from 1.16 cases/child-year at risk to 0.88.

The total number of clusters needed per arm has been increased by one for all scenarios to account for potential LFU of a whole cluster (withdrawal of community consent, displacement, etc.)

The Tanzania site has been powered to detect a slightly larger difference between arms (22.5-23.5%) in order to harmonize the number of clusters among sites.

3.4.3. Sample size scenarios

3.4.3.1. Drought

In this scenario the incidence at baseline is 50% lower than expected.

With the current sample size, the Mozambique site has 80% power to detect a 22% difference in incidence from 2.10 to 1.64 infections per child-year-at-risk. The Tanzania site has 80% power to detect a 26.5% difference in incidence from 0.75 to 0.55 infections per child-year-at-risk.

3.4.3.2. Flooding

In this scenario the incidence at baseline is 50% higher than expected and the coefficient of variation is set at 0.5 to account for displaced populations.

With the current sample size, the Mozambique site has 80% power to detect a 25.5% difference in incidence from 6.3 to 4.69 infections per child-year-at-risk. The Tanzania site has 80% power to detect a 26.5% difference in incidence from 2.25 to 1.65 infections per child-year-at-risk.

3.4.3.3. Conflict

In this scenario 20% of the total clusters are LFU due to population moving out or withdrawing community consent.

With 42 clusters per arm, the Mozambique site has 80% power to detect a 22.5% difference in incidence from 4.2 to 3.26 infections child-year-at-risk.

With 43 clusters per arm, the Tanzania site has 80% power to detect a 25% difference in incidence from 1.5 to 1.13 infections child-year-at-risk.

3.4.3.4. Rumors

In this scenario 50% of the children in every cluster withdraw consent or are LFU.

With cluster size 7, the Mozambique site has 80% power to detect a 24.5% difference in incidence from 4.2 to 3.17 infections child-year-at-risk.

With cluster size 9, the Tanzania site has 76% power to detect a 25% difference in incidence from 1.5 to 1.139 infections child-year-at-risk.

3.4.3.5. Weak ivermectin effect

In this scenario the effect size is 15% instead of the 20% required by WHO to consider as public health value, K_m remains unchanged at 0.35.

With the current sample size, the Mozambique site has 54% power to detect a 15% difference in incidence from 4.2 to 3.57 infections per child-year-at-risk. The Tanzania site would have a 45% power to detect a 15% difference in incidence from 1.5 to 1.28 infections per child-year-at-risk.

The susceptibility of the sample size to changes in the coefficient of variation are presented in **Figure 8.**

3.4.3.6. Probability of success with each sample size scenario

The probability of success (PoS) gives an overall estimate of the probability a trial is likely to succeed. The PoS is calculated by computing the statistical power of each scenario, multiplying this by the estimated probability of each scenario, which is then summed up. For the scenarios outlined below, the PoS for Mozambique and Tanzania are 79.2% and 78.6%, respectively **Figure 9.**

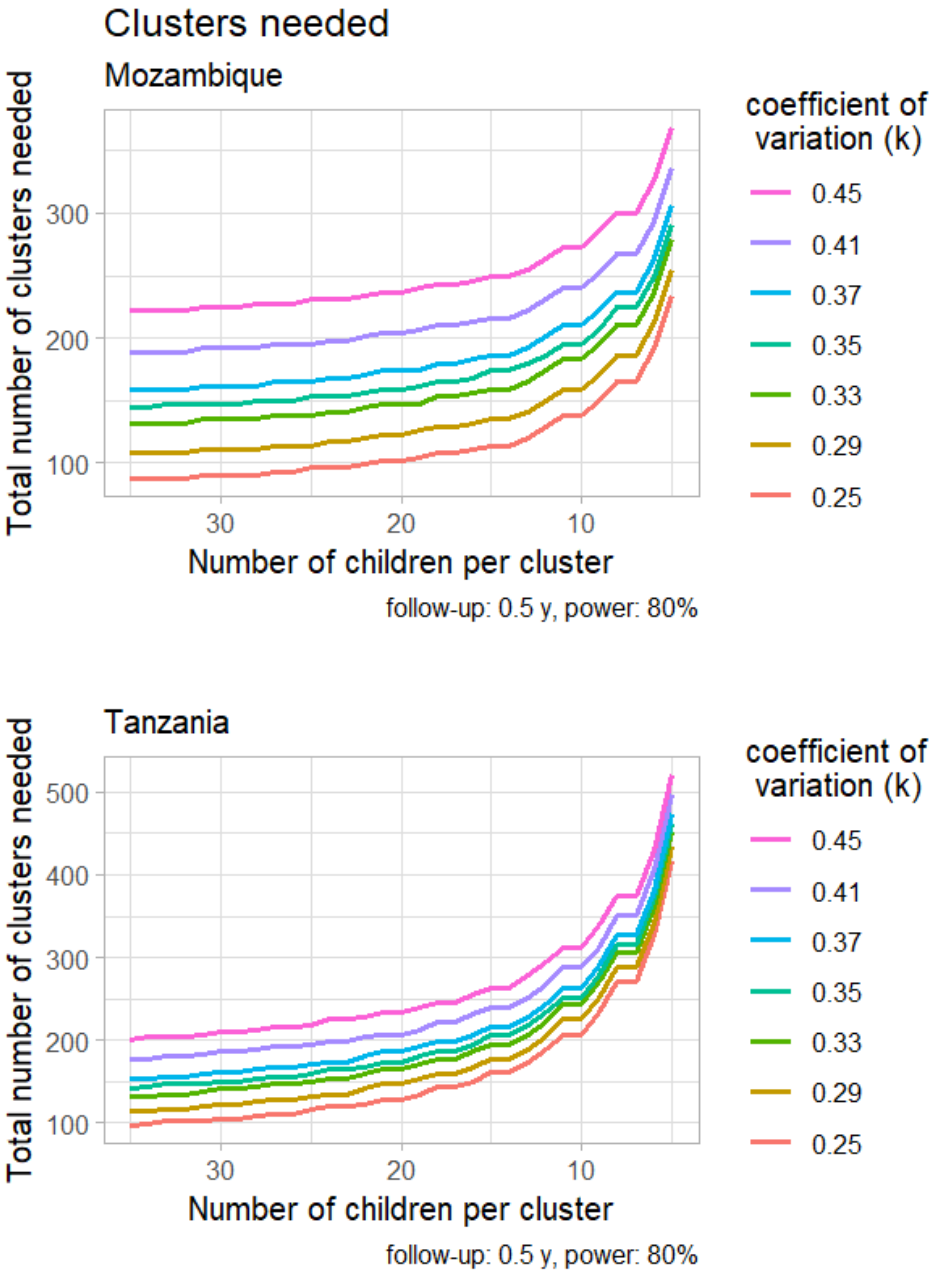


Figure 8.Susceptibility of the sample size to changes in the coefficient of variation (km)

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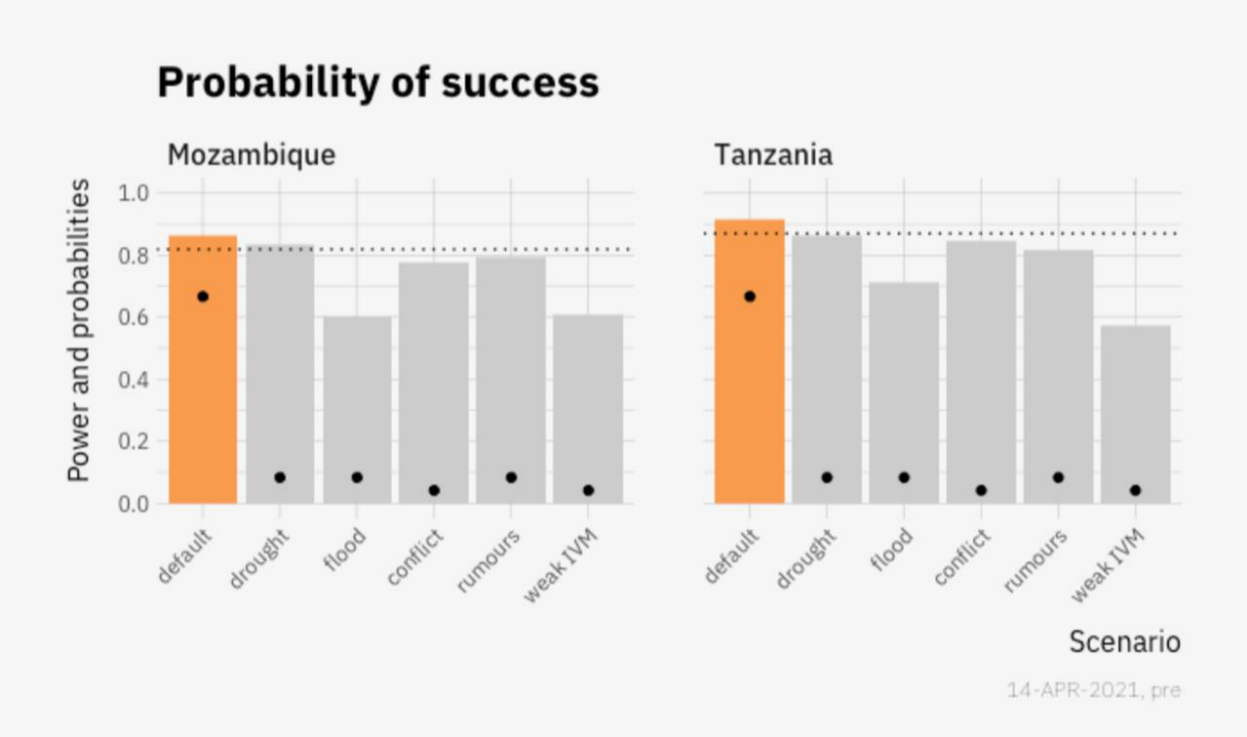

Figure 9.Probability of success by trial sites

Probability of success by trial sites (dotted lines). The grey bars indicate the statistical power the intervention would achieve for different scenarios compared to the default scenario (orange bar), for which the trials are powered. The dots show the assigned probabilities for each scenario.

3.4.4. Cross sectional

3.4.4.1. Mozambique

Accounting for cluster effect, a sample of 1590 participants (530 individuals per arm, 10 individuals per cluster), has 80% power to detect a 26.5% difference in overall prevalence from 45% to 33% using a K_m of 0.35 in Mozambique. The same sample can detect a further 30% reduction from 33 to 23% in the human + livestock arm.

3.4.4.2. Tanzania

Accounting for cluster effect, a sample of 3240 participants (1080 individuals per arm, 20 individuals per cluster), has 80% power to detect a 30% difference in overall prevalence from 20% to 14% using a K_m of 0.35 in Tanzania. The same sample can detect a further 34% reduction from 14 to 9.2% in the human + livestock arm.

3.4.5. Whole blood PK of ivermectin

The PK of ivermectin will be assessed in a random subset of the treated population using dried blood spot capillary sampling.

3.4.5.1. Randomization procedures for Population PK

106 clusters in Mozambique and 108 clusters in Tanzania will be randomized to one of the treatment arms to receive ivermectin (either with or without co-treatment of livestock). Approximately one fifth of these clusters will be included in the PK assessment, i.e. 20 clusters in Mozambique and 20 clusters in Tanzania, totaling 40. Note that an equal number of clusters will come from either ivermectin treatment arm.

Within each cluster where PK sampling is done, 14 participants will be randomly selected.

Participants will be assigned to one of two sampling schedules (A or B).

The intended sampling schedule is presented in Table 8.

Sample number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Time point	0.5 - 1h	2- 3h	4 +/- 1h	8 +/- 1h	12 +/- 2h	24 +/- 4h	72 +/- 6h

Table 8. Pk sampling times

3.4.6. Association of CYP 3A5 and ABCB1 pharmacogenetics with CNS AEs

The first 100 participants to report CNS AEs judged to be drug-related by the investigator will be asked to supply a blood sample for pharmacogenetic testing for CYP 3A5 and ABCB1 SNPs by finger prick and transfer to a DBS filter card (4 drops, 10-20 uL each). The frequency of the SNPs in this cohort will be compared to the prevalence within a subset of the PK sub study population. This subset will be selected by availability of unused filter cards with whole blood samples, in total n=400 blood samples.

3.4.7. Impact of ivermectin against selected ectoparasites and NTDs

Scabies is the condition used to calculate sample size of this outcome. The baseline prevalence of scabies will be determined more accurately during the census. For the purposes of this calculation, it is assumed to be 6% in both sites [98]. Sampling four random adults per cluster (approximate n: 600), the assessment would be powered to detect a 90% reduction [75] from 6 to 0.6% using a K_m of 0.35 in one year. All children in the efficacy cohort will be assessed for NTDs at enrollment and in visits 1-4 and 6. NTDs will be assessed in the safety cohort at enrollment and at visit 3 (see section 6 below).

3.4.8. Direct or indirect effect of ivermectin on the immune response against the malaria parasite

This is an exploratory objective that aims at collecting preliminary data on changes cause by ivermectin on the antibody response pattern to plasmodium infection as well as changes in the cellular immune response. As such, the sample is a convenience one.

In Mozambique: the subgroup of children under five in the active pediatric cohort that weigh more than 15 kg and hence also receive ivermectin treatment.

In Tanzania: the first 200 children 5-15 years old in the active pediatric cohort that weigh more than 15 kg and hence also receive ivermectin treatment.

Additionally, in both countries, random 200 participants from the treatment cohort eligible for the PK sampling.

3.4.9. COVID response

This is an exploratory objective aimed at informing decisions by local authorities. As it is difficult to foresee the COVID situation in rural Mozambique by Q1 2023, the sample proposed is a convenience one. The prevalence will be determined by including a COVID RDT in the cross-sectionals and the incidence by including the first 200 adults in the safety cohort and the first 200 children in the efficacy cohort.

3.5. Modelled predicted Impact using this design

Mathematical modeling is a useful tool for understanding complex transmission dynamics, and for predicting the impact of interventions. In this report, we simulated the proposed intervention for the following purposes. See supplementary material for modelling report.

3.6. Expected duration of participation

Throughout the study there will be two different groups of participants enrolled (section 5), the ones receiving the treatment (> 15 kg) and the ones in the active pediatric cohort for the main outcome of malaria incidence (the ages of highest incidence in each country, under 5 years of age in Mozambique and 5-15 in Tanzania). Each group will participate for different periods of time.

The group enrolled in the active cohort will contribute to the primary efficacy outcome and will participate for 6 months. The group receiving treatment (>15 kg) will contribute to primary safety outcome and some secondary outcomes (PK, NTDs) and will participate for four months. Women of child-bearing age will be visited one month after the third dose for a final pregnancy test. The cross sectionals will enroll participants in all ages. **Figure 10** shows a schematic of the activities conducted for the safety and efficacy assessment in each group.

Participants who become pregnant at any time will be immediately withdrawn from further treatment, but will be followed for evaluation to end of pregnancy. All appropriate withdrawal assessments may be performed at the discretion of the investigator.

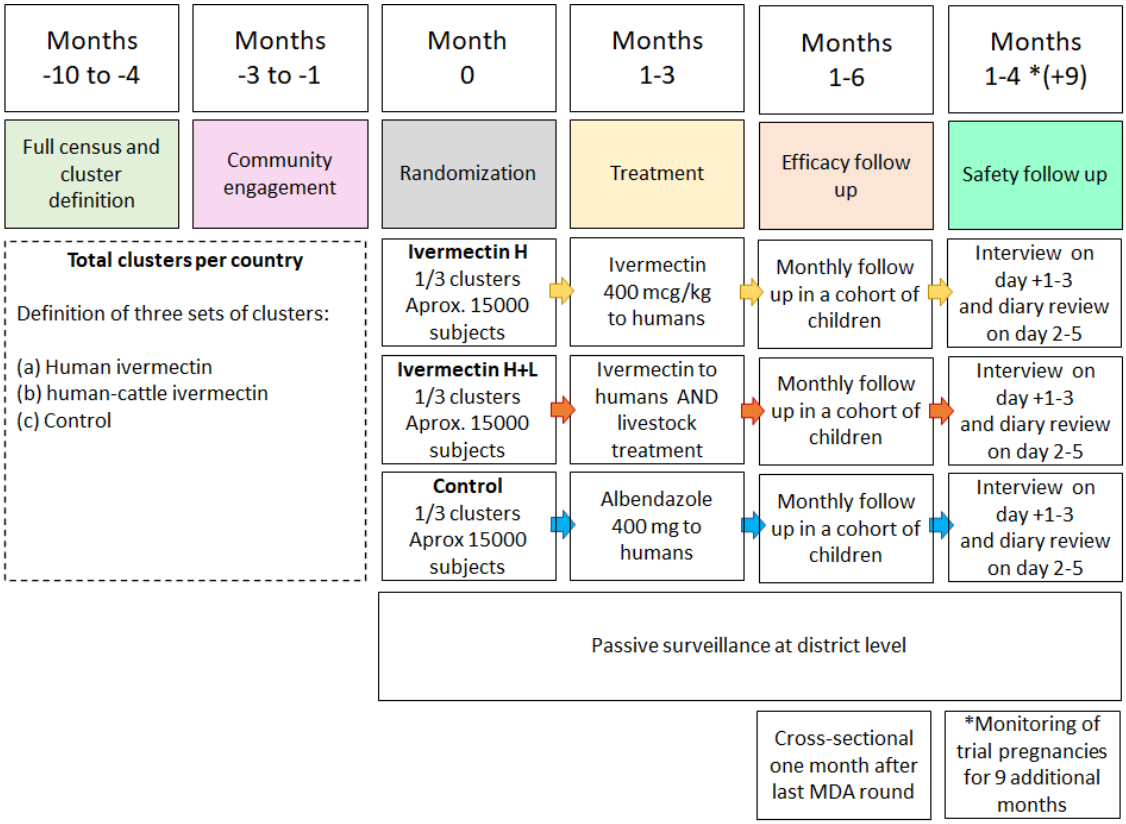

Figure 10. Simple schematic with sequence of activities.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.

The time period for collecting pregnancy information is identical to the time period for collecting AEs. Pregnancy information is collected from the signing of informed consent to 4 weeks after the last dose for non-pregnant participants.

The investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study (up to one month after the last dose). The investigator will record pregnancy information on a specific pregnancy notification form and submit it to the safety desk within 24 hours after knowledge of a participant's pregnancy. The participant will be followed to determine the outcome of the pregnancy, be it full-term or prematurely terminated. Information on the status of the mother and child will be forwarded to the safety desk. Follow-up will end at pregnancy outcome.

3.7. Target livestock coverage

There are no field studies that can inform the most appropriate target for livestock coverage with ivermectin MDA. However, a recent modeling study [99] investigated the impact of ivermectin treated

cattle in the control of vectors of malaria and reduction of malaria prevalence. The study evaluated the effect of a single dose of ivermectin in cattle during the rainy season, with a coverage of 70%, on malaria prevalence in humans the following year. The model found a maximum reduction in malaria prevalence of between 50-60%, depending on initial endemic malaria prevalence. Changing the percent coverage of livestock by 10% in either direction (i.e., 60% or 80%) only changed the reduction in malaria prevalence by ~+/-3%. The model presented did not include the use of ivermectin in humans simultaneously and did not account for livestock density or proximity to humans; however, it could be anticipated that these two factors will actually enhance the effectiveness of ivermectin treated cattle in reducing malaria incidence.

A target coverage rate for livestock of 60-70% will be reasonable logistically, given that there will be some households that refuse to consent to treatment, and that a proportion of livestock must be excluded from treatment due to milk and meat withdrawal times (i.e., a cow producing milk for human consumption or cow/pig intended for slaughter within regulatory withdrawal period will not receive treatment), and the possibility of migratory cattle has been considered for the Tanzania site. The animals left untreated due to lack of consent or withdrawal concerns will serve as a source of refugia for intestinal helminths. The concept of refugia means that untreated cows will release intestinal helminths into the environment that have not been exposed to ivermectin. It has been shown that when as few as 1-10% of animals are left untreated it will slow the rate of the development of resistance to anthelminthic drugs [100]. Further information is provided in the health economics protocol.

3.8. Data sources

Data will not be recorded directly on paper CRFs. Using remote data entry, data will be captured directly into electronic source documents with the relevant fields automatically updated in the CRF. Informed consents, diaries and SAE documentation will be paper based.

4. Investigational product

4.1. Product

All IP used in BOHEMIA will be procured by the Sponsor from a manufacturer with Stringent Regulatory Agency (SRA) approval or prequalification from WHO, as per funder requirements.

In the case of ivermectin, for which only one WHO prequalified product exists, the selected product is ivermectin in 3mg tablets as manufactured by Merck Stromectrol® SRA approved. In the case of albendazole, the final product will be GMP certified and fully bioequivalent with GSK's Albenza®.

4.1.1. Dosage and regimen

The ivermectin group will receive a single dose of 400 mcg/kg, given once a month for three months. The participant weight and related dosage will be confirmed using portable scales prior to administration.

The albendazole group will receive a single dose of 400 mg, given once a month for three months.

4.1.2. Dosage form

The following dosage forms will be used in the study:

- Ivermectin: 3 mg tablets
- Albendazole: 400 mg tablets
- Animal treatment: ivermectin injectable at 1%

4.1.3. Packaging

Ivermectin will be received in country in bottles of 500 tablets.

Albendazole will be received in country in bottles of 100 tablets.

Once in country, the BOHEMIA team will re-package the ivermectin in bottles of 150 tablets. Albendazole will be re-packaged in identical bottles of 30 tablets in accordance with good manufacturing and good pharmaceutical practices (GPP) and country regulations.

4.1.4. Labeling

The products will be pre-labeled by the selected SRA-approved manufacturers in English prior to shipment. After re-packaging, the label will be revised in order to meet GCP and GPP requirements for the labeling of clinical trial.

4.1.5. Livestock treatment

The veterinary product will be commercially available veterinary ivermectin for injection at 1%. The product to be used will be registered in each country and will be used at an approved dose for an approved indication (livestock deworming) with approved withdrawal periods for slaughter and milking.

4.2. Import

Upon receipt of regulatory approval and permission to import the IP, the manufacturer/s will ship the IP (ivermectin and albendazole) to Maputo in Mozambique and Dar es Salaam in Tanzania. The sites will be notified of the shipping date, number of boxes and waybill number so that the shipment can be tracked. Upon arrival, the site's agent will ensure efficient clearance and release by the local customs office. After the number of boxes and waybills have been verified to ensure that all IP is accounted for, IP will be transported via secure transport to the local packaging site.

4.3. Storage

All products will be stored in accordance with GCP and following the storage instructions in the label.

4.4. Procedures for monitoring participant compliance

All treatment will be administered under direct observation by the field worker. Immediate tolerability will be observed for 30 minutes after dosing. If the participant vomits within 30 minutes of taking the dose, the dose will be repeated. Participant dosing will be recorded by the fieldworker on the electronic visit template.

4.5. Accountability procedures

The site-PI will be responsible for establishing a system for the correct handling of study drugs to ensure that:

Deliveries of IP are correctly received and documented by an appropriately qualified responsible person.

Accurate records are maintained for the receipt of IP, for the dispensing of IP to fieldworkers, administration to participants and for performing daily accountability on issued and returned IP during the dosing period.

Certificates of delivery and return must be signed by the site PI or authorized pharmacist and copies retained in the investigator site file.

All IP is to be handled and stored safely and properly and in agreement with the given storage instructions for each product.

The IP is to be prescribed only by appropriately qualified and designated study site personnel.

The IP is dispensed only to trial participants in accordance with the protocol.

Field workers must return all unused IP and empty containers to the pharmacist for reconciliation with a frequency to be defined operationally. Discrepancies must be accounted for, and Corrective and Preventive Actions plans implemented to address any matters before the next month's dosing.

At the end of the study, delivery records must be reconciled with records of usage and returned stock. Any discrepancies must be accounted for in writing.

At the end of the trials any returned and unused investigational product and/or packaging at the site will be either donated to the local NTD program or destroyed upon agreement with the sponsor. Drug donation/destruction certificates will be issued that refer to document the bottle and batch number for traceability.

5. Selection of participants

5.1. Participant inclusion criteria

5.1.1. For human treatment/safety cohort

- Residents of the study area
- Male or female weighing more than 15kg
- Adult able to provide written consent
- Minors aged 12 to 17 able to provide assent
- Parent/guardian's able to provide consent for minors

- Negative pregnancy test for women aged between 13 and 49
- Agreement to adhere to study visits and procedures

5.1.2. For pediatric active cohort:

- Children in the age of highest burden at the time of enrollment (under 5 years of age in Mozambique or 5-15 in Tanzania)
- Residents of the study area
- Parent/guardian's able to provide consent for minors
- Minors aged 12 to 15 in Tanzania able to provide assent

5.1.3. For cross sectionals

- Residents of the area for at least 3 months prior to enrolment
- Parent/guardian's consent for minors
- Ability to provide assent for minors aged 12 to 17
- Written consent from adults

5.1.4. For livestock treatment:

- Owner/guardian able to provide consent
- Animal expected to spend at least one week every study month inside the cluster border

5.2. Participant exclusion criteria

5.2.1. For human treatment/safety cohort:

- Known hypersensitivity to ivermectin or albendazole
- Risk of *Loa* as assessed by travel history to Angola, Cameroon, Chad, Central African Republic, Congo, DR Congo, Equatorial Guinea, Ethiopia, Gabon, Nigeria or Sudan
- Pregnant women
- Lactating women in the first week postpartum
- Children < 15 kg
- Currently participating in another clinical trial
- Unwilling to provide informed consent or assent
- Unwilling to adhere to study visits and/or procedures
- Severely ill either self-reported or in the eyes of the investigator, e.g. defined as need for clinical care, or active or progressive disease interfering with activities of daily living. If in doubt, these criteria can be confirmed after a call with either the site PI/MD/safety officer against a pre-defined list.
- Currently under treatment with inhibitors of CYP3A or P-gp or other drugs that can interfere with the study (examples given in **Table 7**)

Class	Drug	Rationale
Antiarrhytmic/ Antihypertensive	Quinidine	
	Amiodarone	

	Diltiazem	May increase ivermectin exposure by inhibiting its metabolism and excretion or competing with CYPs or the P-gp.
	Spironolactone	
	Verapamil	
Antibiotic- Macrolides	Clarithromycin	
	Erythromycin	
Antifungal agents	Itraconazole	
	Ketoconazole	
Inmunosupressants	Cyclosporine	
	Tacrolimus	
Anti HIV therapy	Indinavir	
	Ritonavir	
Anticoagulant	Warfarin	
H-2 blockers	Cimetidine	May increase albendazole metabolite levels by inhibiting its metabolism and excretion
Steroids	Dexamethasone	

Table 3.Concurrent treatment used as exclusion criteria for the treatment/safety cohort

5.2.2. For incidence cohort:

- Non-residents
- Currently enrolled in other clinical trial

5.2.3. For cross sectionals

- Non-residents

5.2.4. For livestock treatment

- Received ivermectin three weeks than four weeks ago
- Intention to milk or slaughter the animal for human consumption during the withdrawal period
- Calves under 8 weeks and piglets under 6 weeks of age.

6. Study assessments and procedures

A graphic timeline for the study is presented in **figure 11**
Study procedures for year one and year two are summarized in **Tables 8 and 9.**

		Study months											
		1	2	3	4	5	6	7	8	9	10	11	12
Treatment & Safety cohort (>15 kg)	Mass Drug Adinistration												
	Active safety surveillance												
	Assessment of NTDs*												
	Assessment of parasite response*												
	Passive safety surveillance												
	Active pregnancy surveillance												
	COVID response*												
	Passive pregnancy surveillance												
Efficacy & incidence cohort (< 5 years)	Active incidence surveillance								★				
	Assessment of NTDs												
	Assessment of parasite response*												
	COVID response*												
	Passive incidence surveillance												
Cross sectional	Cross sectional												

Figure 11. Graphic study timeline. The star marks the availability of first efficacy data.

Procedures in treatment/safety cohort	**Census & clustering** Months -10 to -4	**Treatment enrollment visit** 1-30 days before dosing	**Study visit 1(S)** Day 1	**Study visit 2(PK)** Day 2	**Study Visit 3(S)** Day 3 +/-1 day	**Study Visit 4(S)** Day 6 +/- 1 day	**Study Visit 5(S)** Day 31 +/-3 days	**Study Visit 7(S)** Day 36 +/-1 day	**Study Visit 8(S)** Day 61 +/-3 days	**Study visit 10(S)** Day 66 +/-1 day	**Final safety visit 11(S)** Day 91 +/-1 day	**End of passive safety surveillance** Day 365+/-3 days	**Final pregnancy safety visit(S)** [d] Day 371 +/-3 day
Informed consent census *(separate protocol)*	X												
Demographic data collection	X												
Cluster randomization	X												
Informed consent for treatment		X					X[e]		X[e]				
Complete visit template		X	X	X	X	X	X	X	X	X	X		X
Anthropometry		X					X		X				
Assessment of selected NTDs [a]		X[a]					X[a]		X[a]		X[a]		X[a]
COVID response[a]		X					X		X		X		X
Review of infant vaccination card (if applicable)		X					X		X		X		
Collection of pharmacology samples [a]			X	X	X								
Collection of parasite response samples [b]			X				X		X				
Urine Pregnancy test [c]			X				X		X		X		
Administer study intervention			X				X		X				
Review of side effect personal diary						X		X		X			
Passive surveillance of side effects				X----------							----------X		
Adverse event review and evaluation				X----------								----------X	
Active pregnancy surveillance							X		X		X	X	X
Passive pregnancy surveillance (health facility)							X----------						----------X

[a]randomly selected sub-sample
[b]randomly selected sub-sample of children under 5 that also receive treatment (>15 kg)
[c]in participating female participants aged 13-49
[d]only pregnancies occurring post-treatment
[e]only subjects not previously enrolled

Table 4.Study procedures for the treatment/safety cohort.

Procedures in efficacy (active pediatric cohort)	**Census & clustering** Months -10 to -4	**Enrollment/Baseline(E)** Visit 1, Day 1	**Study Visit 2(E)** Day 31 +/-3 days	**Study Visit 3(E)** Day 61 +/- 3 days	**Study Visit 4(E)** Day 91 +/-3 days	**Study Visit 5(E)** Day 121 +/-3 days	**Study visit 6(E)** Day 151 +/-3 days	**Study visit 7(E)** Day 181 +/-3 days
Informed consent for census	X							
Demographic data collection	X							
Randomization	X							
Informed consent		X						
Anthropometry		X			X			X
Review infant vaccination card		X			X			X
Complete visit template		X	X	X	X	X	X	X
Malaria RDT		X	X	X	X	X	X	X
Malaria treatment [a]		X	X	X	X	X	X	X
Assessment of selected NTDs		X	X	X	X	X	X	X
COVID response[b]		X	X	X	X	X	X	X
Malaria parasite immune response samples [b]		X	X	X	X	X	X	X
Plasmodium PCR for quality control and molFOI[c]		X	X	X	X	X	X	X
Passive incidence surveillance at health facilities (all ages)		X----------						----------X

[a]only if RDT is positive (see case definition)
[b]only in a randomly selected sub-sample
[c]only in a randomly selected 10% sub-sample

Table 5. Study procedures for the efficacy/incidence cohort

6.1. Study-visit procedures

There will be an initial abbreviated census in 2020 for enumeration purposes. One year before the ivermectin MDA campaigns a census will be developed in both trial sites, Mopeia and Rufiji. During this process, all households eligible for the study will be mapped (geographical coordinates registered) and assigned a numerical code. In addition, all eligible individuals in each household will be surveyed and will receive a permanent identification number (i.e.; Ext_ID). Prior to launch in 2022, villages will be stratified and allocated to the different study arms in a randomization ceremony involving community representatives as described in section 3.3.1.

Community mobilization teams will visit all villages and meet with leaders and community members to inform about them of the upcoming trials. The information methods will include door-to-door visits and focal group discussions (see stand-alone community mobilization plan).

All data will be collected in electronic formularies using tablets.

6.1.1. Treatment/safety cohort

6.1.1.1. Screening

Appropriately trained and qualified field workers will visit the selected households, explain the study objectives, methods and procedures to all household members who will be invited to participate in the study. The field workers will explain the inclusion and exclusion criteria and offer to answer questions as part of the informed consent process.

6.1.1.2. Enrollment

After answering questions, written informed consent will be obtained from all eligible household members above 18 years of age, written assent will be obtained from household members 12-17 and parents/guardians will provide written consent for participants under 18 years of age. Illiterate participants will be asked to nominate an independent witness to join them in the consent/assent process. The informed consent form will include specific opt-in sections for participants randomized to participate in the pharmacology, parasite immune response, and COVID-response outcomes.

During the consent procedure, women of child-bearing age will be informed of the potential hazards to an unborn child and will be offered referral services to family planning should they wish to avoid becoming pregnant during the trial. They will be informed that if they become pregnant, they will not receive any more doses of the study medication, they will be withdrawn from the study and that the team will monitor the outcome of the pregnancy.

In subsequent visits, household members not previously present and/or enrolled will be invited to participate.

Unique study ID numbers based on the demographic platform Ext-IDs will be assigned to participants upon enrollment.

6.1.1.3. First visit procedures

a. A site visit template containing visit related questions will be completed by the fieldworker as it is answered by each participant or parents/guardian in case of children.

b. A urine-based pregnancy test will be privately administered to all women 13-49. If results are positive the woman will receive a confidential referral to the antenatal clinic. Women breast-feeding babies younger than one week will be excluded from treatment during that visit.

c. The field worker will perform a simple anthropometric assessment (height, weight, and Mid-Upper Arm Circumference (MUAC) for nutritional status, and examine exposed skin areas for specific NTD lesions (caused by scabies, tunga, bed bugs and head lice). In addition, the field worker will ask about passing of intestinal worms, and presence of bed bugs in the house.

d. Vaccination cards for all children under 6 years of age will be reviewed for immunization status and mothers encouraged to have the child vaccinated regularly and in accordance with vaccination program requirements.

e. For participants consenting to participate in the pharmacology, COVID response, or parasite response outcome, the described samples will be obtained at visits detailed in Table 8, if more than one simultaneous sample is collected, all efforts will be done to use the same finger prick.

f. The IP will be administered following a weight-band table and the field worker will remain in the household for 30 minutes after the last household member has taken the drug to monitor immediate tolerance (directly observed treatment, DOT).

g. A pictorial side effect diary will be given to each participant with brief instructions for daily completion (days 1-6) and review with the fieldworker at the post dosing safety visit on day 6 +/- 1 day.

6.1.1.4. Subsequent visits procedures

a. Steps a-e will be repeated at treatment each visit.

b. The participants diaries will be reviewed by a fieldworker at the two post dosing safety visits and information transcribed to the tablet.

c. The study ID number of any women previously treated with a newly positive pregnancy test will be added to a list of post-treatment pregnancy follow-up.

d. Surveillance of pregnancies occurring within one month of dosing consists on monthly visits to assess pregnancy progress and outcome plus passive pregnancy outcome assessment at health facility.

6.1.2. Active pediatric cohort

6.1.2.1. Screening

Field workers will visit the selected households, explain the study objectives, methods and procedures to the parents/guardian of children eligible to participate in the study. The field workers will explain the inclusion and exclusion criteria and answer operational questions; should the potential participant have medical questions the fieldworker will phone the study clinician for assistance.

6.1.2.2. Enrollment

After answering questions, written informed consent will be obtained from parents/guardians.

Unique study ID numbers based on the demographic platform Ext_IDs will be assigned to participants upon enrollment.

6.1.2.3. Visit procedures

a. An electronic visit template will be completed by the fieldworker containing responses to visit-specific questions from parents/guardians

b. The field worker will perform a simple anthropometric assessment (height, weight, and MUAC), measure the axillary temperature and examine exposed skin areas for specific NTD lesions (i.e.; caused by scabies, tunga, bed bugs and head lice). In addition, the field worker will ask about passing of intestinal worms and will measure axillary temperature. If the field worker detects any of the NTDs, he/ she will refer the child to the health facility

c. Vaccination cards for all infants will be reviewed at visits detailed in table 9 for currency and mothers encouraged to have the infant vaccinated regularly and in accordance with vaccination program requirements.

d. Samples for RDTs (malaria and COVID serology) and samples requiring blood spots on filter paper will be collected with a single finger prick. RDT results will be read according to the manufacturer's instructions.

e. For participants consenting to participate in the PK, PCR quality control, COVID response or parasite response outcome, the described samples will be obtained at visits detailed in table 9, all efforts will be done to use a single finger prick for all samples.

f. Should any of the RDTs yield a positive result and in the absence of symptoms, the child will receive treatment with Coartem according to the national treatment guidelines. The first dose will be directly observed by the field worker and the rest will be administered by the parent/guardian. In the presence of symptoms, the child will additionally be referred to the nearest health facility.

6.1.3. Cross sectionals

6.1.3.1. Screening

Field workers will visit the randomly selected households, explain the study objectives, methods and procedures to all household members who will be invited to participate in the study. The field workers will explain the inclusion and exclusion criteria and offer to answer questions. Medical questions will be referred telephonically to the study clinician.

6.1.3.2. Enrollment

After answering questions, written informed consent will be obtained from parents/guardians.

Unique study ID numbers based on census Ext_IDs, will be assigned to participants upon enrollment.

6.1.3.3. Visit procedures

a. An electronic visit template will be completed by the fieldworker containing answers to visit specific questions from each participant or parents/guardian in case of children.

b. The field worker will perform a simple anthropometric assessment (height, weight, and MUAC), axillary temperature and examine exposed skin areas for specific NTD lesions.

c. RDTs (malaria and COVID serology) will be performed and the results read according to the manufacturer's instructions. Samples for PCR will be obtained.

d. Should any of the RDTs yield a positive result and in the absence of symptoms, the participant will receive treatment with Coartem according to the national treatment guidelines. The first dose will be directly observed by the field worker and the rest will be either self-administered or administered by the parent/guardian in case of children. In the presence of symptoms, the participant will additionally be referred to the nearest health facility.

6.2. Concomitant therapy

Participants in the treatment/safety cohort will receive IP from the study personnel. Participants in the incidence cohort will receive no treatment from the study personnel other than Coartem when indicated.

Exclusion from the trials based on concomitant drug therapy is described on section 7.2.1.

There will be no other restrictions based on concomitant medications. Concomitant medications taken by the participant for other indications will be recorded by field workers at every home visit and those administered during hospitalizations will be retrieved from hospital records.

6.3. Premature discontinuation from the study

6.3.1. Participants

Participants may voluntarily withdraw from the study for any reason at any time after informing the study team. Reasons for premature discontinuation may include but not be limited to:

a. Intending to permanently move out of the study district

b. Consent withdrawal or participant no longer willing to continue

c. Concerns over side effects

d. Intention to become pregnant

e. Pregnancy during participation

Every reasonable effort will be made to complete a final evaluation of participants who withdraw consent for the study. Study staff will record the reason(s) for all withdrawals in participants' records

6.3.2. Samples and future usage

In cases of voluntary discontinuation, it should be established whether consent remains for sample storage. Should the consent be withdrawn, the stored sample will be destroyed and the withdrawal noted in the source notes and CRF. The site shall send a Sample destruction letter to the laboratory for actioning. The laboratory shall return a copy of the completed destruction letter once the sample has been destroyed.

6.4. Participant withdrawal criteria

The study sponsor, national RAs, the DSMB or any of the RECs may request to terminate the study prior to its planned end date.

The Site PI may remove participants from the trial in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures and/or if the participant is deceased.

Children in the efficacy cohort that miss two consecutive visits will be considered lost to follow up.

6.4.1. Type and timing of the data to be collected for withdrawn participants

All data collected up to the time of withdrawal shall be included in the trials' analysis.

6.4.2. Replacement of withdrawn participants

Withdrawn participants shall not be replaced.

6.4.3. Follow-up for participants withdrawn from treatment

Female participants on the pregnancy log shall be followed until pregnancy outcome. All SAEs related to the IP and SUSARS shall be followed until resolution / return to baseline or for a period defined by the Sponsor on an individual case basis.

7. Laboratory requirements

7.1. Laboratory requirements and assessments

7.1.1. Tests performed in situ

- Urine-based pregnancy tests will be performed in all women of child-bearing age receiving IP (section 8.1.1).
- Malaria and COVID RDTs (serology) will be performed in children enrolled in the incidence cohort

7.1.2. Additional samples

- Pharmacology samples consisting of dry blood spots in filter paper will be shipped to Switzerland for determination of ivermectin levels and pharmacogenomics.
- Parasite immune response samples consisting of dry blood spots in filter paper and one micro-capillary will be shipped to Barcelona for assessment of response to parasite infection using transcriptomic and antibody response patterns.
- Samples for *Plasmodium* PCR consisting of two spots of 20 mcl on filter paper from a sub-sample of the incidence cohort and from all participants in the cross-sectionals will be shipped to KEMRI for processing.

All these samples will be stored in a monitored -20 °C freezer in Mopeia and Rufiji and transferred to the central processing laboratories.

7.2. Volume of blood to be drawn

7.2.1. Treatment/safety cohort

Group	Sample	Volume /visit [µL]	Visits	Total
Sub-sample participating in the pharmacology assessment	Capillary whole blood on filter paper	160 [a]	5	< 1,200 mcl
Sub-sample participating in the COVID response assessment	Serology RDT	20 mcl	12	240 mcl
Sub-sample participating in the parasite response assessment	Blood on filter paper and micro-capillary tube	90 [b]	3	270 mcl
Sub-sample participating in CNS AE pharmacogenetics assessment	Capillary whole blood on filter paper	< 100 [c]	1	< 100 mcl
Approximate total				1.8 ml

Table 6.Volume of blood to be drawn for the treatment/safety cohort

[a] One sample consists of 2 filter papers containing 4 spots of 20 µL each (160 µL per visit), there will be 5 sample time points, the total would be 800 µL, but assuming potential spills, we assume a maximum of 1200 µL; [b] one sample consists on one filter paper containing two spots of 20 µL plus one 50 µL capillary for RNA preservation (90 µL per visit); [c] one sample consists of 4 spots of 20 uL each (80 uL, assumed <100 uL approximately)

The maximum volume per visit will be 0.18 ml

The maximum volume throughout the trial will be: 1.3 ml

7.2.2. Incidence cohort

Group	Sample	Volume	Visits	Total
All in incidence cohort	HRP2-RDT	20 mcl	6	120 mcl
All in incidence cohort	pLDH-RDT	20 mcl	6	120 mcl
Sub-sample participating in the COVID response assessment	Serology RDT	20 mcl	6	120 mcl
Sub-sample participating in the RDT quality control assessment (PCR)	Blood on filter paper	80 mcl	6	480 mcl
Sub-sample participating in the parasite response assessment	Blood on filter paper and micro-capillary	90 mcl	6	540 mcl
Approximate total				1.1 ml

Table 7.Volume of blood to be drawn for the incidence cohort

The maximum volume per visit will be 0.13 ml

The maximum volume per year will be approximately 2.2 ml

7.2.3. Cross sectionals

Group	Sample	Volume
All	HRP2-RDT	20 mcl
All	pLDH-RDT	20 mcl
All	Serology COVID RDT	20 mcl
All	Blood on filter paper	80 mcl
Approximate total		0.14 ml

Table 8.Volume of blood to be drawn for cross sectionals

Total volume collected will be 0.14 ml

7.3. Shipping

All samples will be labeled and prepared for shipping by an International Air Transport Association (IATA) certified staff member at each trial site. Samples will be couriered from the trial sites to the laboratories in Switzerland (Basel) for PK analysis, Kenya (KEMRI) for PCR and parasite genomics and Spain (ISGlobal) for immune response to the parasite.

Details and templates for packaging, shipping and quality checks of samples prior to shipment will be detailed in the Study Operations Manual.

7.4. Storage and future use

Blood samples will be stored in the clinical trial sites in silica gel as desiccant in -20°C freezers and/or 4ºC fridges according to the specific requirements of the processing lab. Samples to be sent to Basel Hospital (Switzerland), KEMRI (Kenya) and ISGlobal (Spain) will be stored separately in different bags, each identified with the corresponding identification number.

Samples may be stored by ISGlobal or its contractor/s for a period of 5 years. The samples will be stored in an accredited, secure, access-controlled laboratory. The samples will be identified by participant ID and visit code until the end of the trials where after they will be delinked so that they cannot be linked to a specific participant. Additional research regarding the use of ivermectin to control malaria may be conducted to evaluate hypotheses generated during data analysis i.e., if a direct effect of ivermectin on the parasite is suspected based on epidemiological results. This includes: parasite genetics, serological and immune response to the malaria parasite and NTDs. Additional uses could include re-analyses with methods being developed during the course of the study, and to answer follow-up questions posed by stakeholders.

The positive RDTs from Mozambique will not be discarded. They will be stored at -20° for five years for the conduct of malaria transmission studies (parasite genetics) relevant to the study site (Mopeia). These studies are not part of the BOHEMIA clinical trial and will have independent protocols and approvals for their conduct.

8. Considerations contributing to efficacy assessment

8.1.1. Enhanced surveillance (passive case detection)

At each site, BOHEMIA personnel will be placed within the local health facilities. The study personnel will work with existing health practitioners or health workers (not replace them). They will support health workers in the completion of health registers/books thereby assuring that all visiting patients are recorded in the book/register (completeness) and that patient entries are complete (quality), including the village or origin and its study code [village codes allow for determination of incidence by treatment status]. This enhanced passive surveillance and reporting system will allow the assessment of not only efficacy (incidence) in all age groups but also safety (side effects). These personnel will also monitor RDT and drug supplies in facilities and provide alerts to relevant facility staff to avoid stock-outs.

8.1.2. Cross-sectional survey

Cross-sectionals surveys for malaria infection will be conducted one month after the last MDA round to capture the differential impact on malaria prevalence across the study arms, communities and all ages. In this case PCR does provide significant added value given the expected large proportion of adults with asymptomatic/sub-microscopic infection in high transmission sites. The relatively smaller sample required for this component of the assessment makes PCR a much more valuable asset. Assessing the parasite biomass via PCR at community level can help further explain any changes seen in malaria incidence in children. The multiplicity of infection will be determined in the cross-sectional samples using molecular techniques.

9. Considerations contributing to safety assessment

9.1. Adverse Events

Participants will be asked to record signs and symptoms associated with known ADRs in the diary for review by the field worker during the post-dosing safety visit occurring 2-6 +/- 1 days post dose.

The participant will be asked to provide information on any other events, physician visits or hospitalizations, and treatment taken during Day 0 through Day 6 during the post dosing safety visit.

Participants will be asked about any AEs occurring after the 6th day and up to the next dosing day. As the IP will no longer be present in the participant's system at detectable levels, these events will be deemed unrelated to the IP. These events will be recorded on adverse event logs, which form part of source data and will not be included in the data analysis. These unrelated events will be reported to IRBs and DSMB in 6 monthly line listings unless local regulations specify otherwise.

Participants shall be encouraged to contact the Investigator by telephone to report severe events in case the investigator deems an ad hoc visit by the participant to the site or local clinic is required.

The investigator and/or sub-investigator with assistance from delegated study staff will visit local hospitals in the district to review in- and out-patient records to ensure all adverse events for trial participants are captured. The investigator or appropriately qualified sub-investigator shall determine severity and causality for all events.

All AEs and SAEs occurring from the time a volunteer consents to participate in the study until 4 Weeks after he or she has completed or discontinued the IP must be recorded in the Patient's electronic records and trial database. BOHEMIA will use MedDRA coding and CIOMS forms to ensure standardization of terminology across the trial.

Importantly, SAEs will have to be reported, either by email or by Fax, by the site PI/Co-PI or sub - investigator to IntuVigilance Limited within 24 hours of awareness of an SAE.

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ICH guidance requires the investigator to ensure that any related SAEs are reported after the patient signs informed consent to participate in the study.

The following points must be recorded for each event:

- A description of the event in medical terms using MedDRA terminology, not as reported by the patient;

- Date of onset (start date);
- Date of resolution (stop date);
- The time of onset with respect to administering the IP;
- The severity of the sign/symptom or clinically significant abnormal laboratory value according to CTC-AE Classification (Most recent version);
- The causal relationship between the IP and the occurrence of each AE. This will be assessed by each investigator using clinical judgement. Alternative causes, such as natural history of the underlying diseases, concomitant medications, other risk factors and the temporal relationship of the event to the IP will have to be considered. The causality of all AEs should be assessed by the investigator with the following question: Is there a reasonable possibility that the AE may have been caused by the IP? And answered "NO" (if not related) and "YES" (if related);
- Action taken regarding the IP:
  - No action;
  - Temporary discontinuation;
  - Permanent discontinuation;
  - Patient's outcome:
  - Recovered without sequelae / resolved without sequelae;
  - Recovered with sequelae / resolved with sequelae;
  - Recovering/Resolving;
  - On-going;
  - Fatal (for SAEs only).

If in any one patient, the same AE occurs on several occasions, the AE in question must be documented and assessed a new each time.

In cases of death, the DSMB will need to receive a report of the death within 7 days. The DSMB will be entitled to temporarily halt the study according to pre-established criteria. Safety reports will form part of the standard reports provided to the DSMB.

9.2. Diaries and questionnaires

Participants will be provided with a pictorial diary for the documentation of solicited AEs, concomitant medications and procedures starting on dosing day and for five days following dose administration (i.e., from Day0 to Day 5, inclusive, or 6 days total).

ADRs that are reasonably likely to occur will be solicited daily in the diary with standardized severity grades. Visit specific questions will form part of the electronic source document per visit.

9.3. Serious Adverse Events

A SAE includes any event that: (a) is fatal or (b) life-threatening, (c) results in persistent or significant disability/incapacity, (d) requires or prolongs hospitalization, (e) is a congenital anomaly.

All participants treated will be actively monitored for SAEs for 30 days after each MDA round. This period has been chosen to match six half-lives when ivermectin is expected to be present in plasma at concentrations below 5% of the peak achieved.

Throughout the study, the reporting of SAEs to the Sponsor or its designee will be done through the SAE forms.

It is the investigator's responsibility to ensure that the SAE report is submitted to IntuVigilance Limited **within 24 hours after knowledge of the event(s).**

Any SAE must be reported (in writing using the SAE Report Form) to Intuvigilance Limited within 24 hours of the investigator's first knowledge of the event, regardless of the presumed relationship to the IP. The investigator or qualified designee must complete the SAE Report Form, sign, and transmit the completed form to the Intuvigilance team. As much information as possible must be provided, but inability to provide a comprehensive account should not delay the report. The report will contain at least the participant identifier, the investigative site identifier, a characterization of the event, an assessment of the relatedness of the event to IP, the criteria for considering the event serious, and the name and dated signature of the investigator. If possible, the report will also contain the date of onset of the SAE.

Additional or follow-up information relating to the initial SAE report, will be requested, if necessary. This information is to be completed and submitted using the SAE forms within 24 hours of receipt of the information by the local study MD.

A follow-up report shall be sent as soon as additional information becomes available. The follow-up report will provide additional information on the SAE in the form of a narrative, and by adding information to the SAE Report Form. All available information that may assist in further characterizing and elucidating the nature of the event should be provided.

The investigator is responsible for obtaining detailed information to support all SAE reports, including records of inpatient and outpatient care, laboratory reports, and autopsy or medical examiner reports (where available).

ISGlobal has a legal responsibility to notify, as appropriate, both the local RAs and other RAs about the safety of the investigational medicinal product. It is therefore important that the investigator promptly (within 24 hours) notifies the ISGlobal Safety Team (Intuvigilance) of any SAEs, in order for legal obligations and ethical responsibilities towards other patients to be met.

ISGlobal as the sponsor will be responsible for notifications of unexpected related SAEs and other qualifying events that are considered to be unexpected and related to study agent as expedited (e.g., 7- or 15-Day) reports to the relevant RAs and to all participating investigators. In the absence of existing local and national requirements, unrelated and/or possibly related SAEs will be reported in a 6 monthly line listing report.

For BOHEMIA, ISGlobal has contractually delegated the reporting responsibility to the trials´ sites. Sites will be provided with the relevant contact information for the Intuvigilance Safety team during site training and it is specified in the Study Operations Manual.

Each investigator must comply with the applicable local and national regulatory requirements related to the reporting of SAEs to the IRBs/ Institutional Ethics Committees (IECs) responsible for reviewing the trial at their site, as well as the RAs (where applicable).

9.4. Events of Special Interest

9.4.1. Pregnancy

Female participants of child-bearing age 13-49, shall be informed of the risk of becoming pregnant whilst on the clinical trials. Female participants will be advised to avoid pregnancy during the 3 active months and for one month after the final dose due to the unknown risk to the fetus. Pregnancy testing will be performed at all dosing visits prior to administration of IP as well as one month after the last dose. A positive pregnancy test will result in discontinuation from treatment. The participant's details will be entered on a pregnancy log and the pregnancy followed until outcome. The study team shall refer pregnant participants for further obstetric care and management in accordance with local guidelines.

9.5. Severity

Non-visible AEs (e.g., pain), solicited AEs and unsolicited AEs will be assigned a severity based primarily on interference with daily activities:

- Mild - Mild symptoms or diagnostic observations; Intervention not indicated; No interference with normal activity
- Moderate – Moderate symptoms or diagnostic observations; Some interference with normal activity, may require treatment or medical intervention e.g. painkiller taken for a headache
- Severe - Severe symptoms, significantly disrupts or prevents normal daily activities; Generally, requires medical attention/intervention

Medical care-seeking is typically absent for grade 1 (mild) and often present for grade 3 (severe) events, but is not the primary determinant, since individuals behave differently in this regard.

9.6. Causality

The relationship of AEs to the IP must be assessed and documented by the investigator or a qualified sub-investigator, and classified accordingly. The study specific SAE form should be completed as thoroughly as possible, with all the available details of the event and signed by the investigator or designee. The investigator will assess causality between the administered drug and the (AE) / (SAE) according to the table outlined below **(Table 13)**

Relationship	Relationship Description
Unrelated	• **May or may not follow a reasonable temporal sequence from administration of the test article;** • **No plausible mechanism based on known or suspected actions of the test article or product class;** • **Readily explained by known characteristics of the participant's clinical state, common intercurrent illnesses, or other treatments administered to the participant.**

Relationship	Relationship Description
Unlikely	• **May or may not follow a reasonable temporal sequence from administration of the test article;** • **No plausible mechanism based on known or suspected actions of the test article or product class;** • **Readily explained by known characteristics of the participant's clinical state, common intercurrent illnesses, or other treatments administered to the participant.**
Possibly	• **Follows a reasonable temporal sequence from administration of the test article;** • **Based on known or suspected actions of the test article or product class, a plausible mechanism could exist;** • **May be reasonably explained by known characteristics of the participant's clinical state, common intercurrent illnesses, or other treatments administered to the participant; but the Investigator deems this less likely than test article effect.**
Probably	• **Follows a reasonable temporal sequence from administration of the test article;** • **Based on known or suspected actions of the test article or product class, a plausible mechanism could exist;** • **Cannot be reasonably explained by known characteristics of the participant's clinical state, common intercurrent illnesses, or other treatments administered to the participant**
Definitely	• **Follows a reasonable temporal sequence from administration of the test article;** • **Consistent with known actions of the test article or product class;** • **Cannot be reasonably explained by known characteristics of the participant's clinical state, common intercurrent illnesses, or other treatments administered to the participant.**

Table 9.Causality assessment

The investigator should use his/her best medical judgment to consider whether the temporal sequencing of treatment and event, the existence (or lack thereof) of a biologically plausible mechanism (which is consistent with the temporal sequence), and the existence (or lack thereof) of likely alternative explanations support the causality assessment given.

An assessment of causality should always be provided at the time of the initial report. If the investigator or designee does not have all information regarding the SAE, he/she should not wait to receive additional information before completing the form and notifying IntuVigilance Limited. The sponsor will assign a causality to each SAE. Where the Investigator's assessment differs, both causality assessments will be included in the narrative section of the final CIOMS 1 report.

9.7. Follow-up after occurrence of safety events during and at end of study

Female participants on the pregnancy log shall be followed until pregnancy outcome. All SAEs related to the IP and SUSARs shall be followed until resolution / return to baseline or for a period defined by the Sponsor on an individual case basis

9.8. Data and Safety Monitoring Board (DSMB)

An independent DSMB has been assembled for this study. The details for the operation and responsibilities of the DSMB is defined in a DSMB Charter. The Charter delineates the composition, duties, responsibilities and procedures of the DSMB, data required at each meeting as well as any analyses that will be conducted.

After each DSMB meeting, the Chairperson will issue a written report describing all recommendations.

10. Data management

10.1. Recording and collection of Data

Data will be collected through a comprehensive electronic data capture (EDC) system with digital data entry forms deployed and stored on the broad OpenHDS (Health and Demographic System). Specifically, the study questionnaires will be designed and deployed on the Open Data Kit (ODK) platform running on the open-source Android operating system. Data transfer from mobile devices (tablets) to servers will be executed using ODK-Collect and Mirth. Data validation will take place both at (a) point of entry via restricted and confirmatory steps, and (b) on the server, via aggregate-level quality checks and alerts. Backups of both the raw input data and the processed data will be stored on the main project server as well as at each site. An audit trail/log of all data modifications, both in terms of inputs and edits, will be generated automatically and available to RAs or authorized study team members upon request. **Figure 12** shows a general overview of the data system:

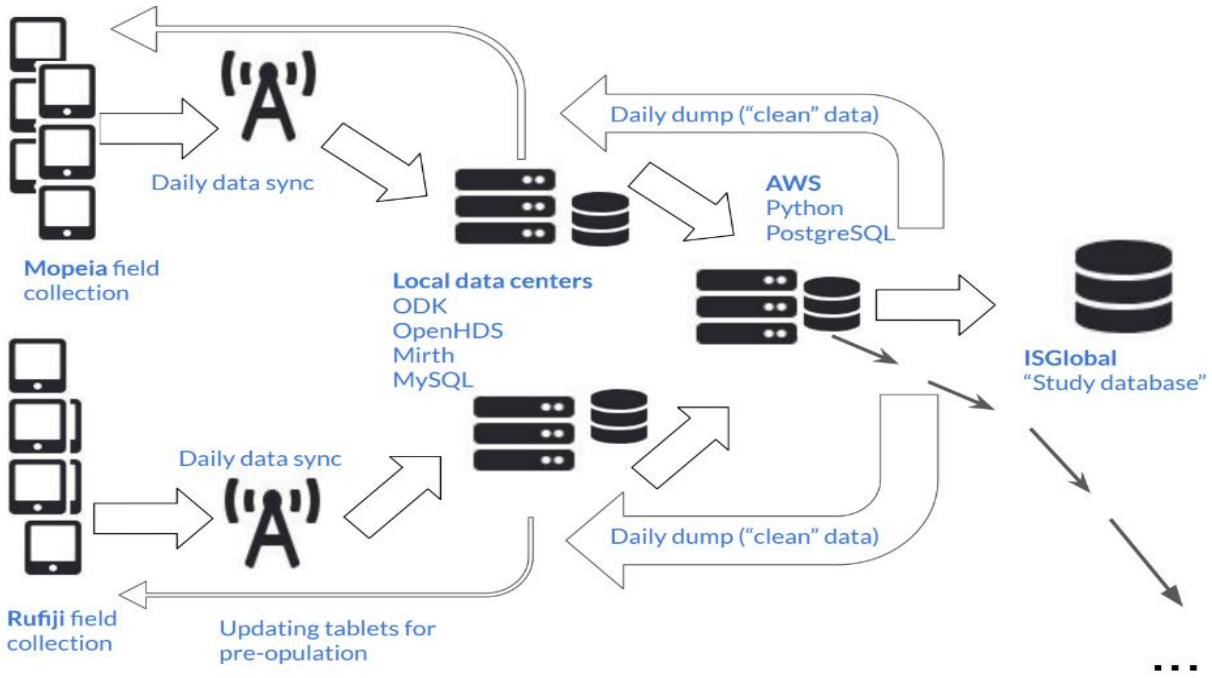

Figure 12.Data system overview

Every effort will be made to ensure the protection of participant data. In general terms, this means that no identifying data will be transferred to any server unless both (a) its transfer falls within the scope of the project's protocol, and (b) it is appropriately encrypted and secure.

Paper-based source (e.g. hospital records) will be scanned into the safety database locally and entered into the trials´ databases. Paper-based records will redact all personal identifiers of the participant (e.g.; name of the participant, address or telephone number, hospital file number) and will be stored securely at the site.

Clinical data management shall be performed in accordance with applicable ISGlobal standards and data cleaning procedures.

While completed eCRFs are reviewed by ISGlobal Clinical Research Associates (CRAs) both remotely and at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions and inconsistencies with documentation and approval by the investigator or an appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a physical data drive (in form of CD-ROM, USB, or HDD) of the final version of the data generated by the project once the database is archived and the study report is complete and approved by all parties. The physical data drive will be compliant with international requirements for data storage and retrieval.

10.2. Data Quality Assurance

The trials will use electronic source documents for each visit as well as additional log pages for aspects such as AEs and concomitant medications. The source documents will have the option for limited free text to be entered by the fieldworker and/or study clinician and/or investigator prior to signing off the visit. Logic checks will be programmed to reduce the error ratio and ensure all required information is completed before sign off or the next page becomes available. Post-ingestion alerts will be automatically generated.

The investigator is responsible for ensuring that all sections of source notes are completed correctly, and that entries can be verified against paper source data where applicable (e.g. hospital notes for SAEs). If certain data are not available or not applicable this will be indicated as such on the appropriate space of the source notes and eCRF. The CRA will review the eCRFs against source and check them for completeness. Information from the source notes will be automatically transferred into the eCRF and data queries issued where data is non-conformant. The investigator shall be responsible for signing off the eCRF at the end of the trials.

10.3. Monitoring

Risk based monitoring of the trials shall be conducted by qualified and experienced CRAs and shall be in accordance with a Monitoring Plan that clearly describes the strategy, methods, responsibilities, and requirements for monitoring the trials.

In accordance with applicable regulations, GCP, and ISGlobal procedures, CRAs shall meet with the site prior to the start of the study to review with the site staff the protocol and their responsibilities to satisfy regulatory, ethical, and ISGlobal requirements. When reviewing data collection procedures, the discussion shall also include training on the electronic source requirements, entry into the clinical trials' database, as well as access and security procedures.

The investigator and the head of the institution (where applicable) agrees to allow the monitor direct access to all relevant documents. The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

As the trials make use of electronic source reporting, most data will be monitored remotely, and queries will be resolved electronically to reduce monitoring time on site.

Upon completion or premature discontinuation of the study, the CRA will conduct site closure activities with the investigator and site staff in accordance with applicable regulations, GCP, and ISGlobal SOPs.

10.4. Audits and Inspection

To ensure compliance with GCP, all applicable regulatory requirements, as well as the protocol, ISGlobal, Unitaid (the funder), RAs and/or ethics committees may conduct a routine quality assurance audit or regulatory inspection of this study. Additionally, in instances where there is a high occurrence of non-compliances at a trial site, or misconduct is suspected, any one of the parties may institute a for-cause audit/inspection.

Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and the research institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to discuss findings and any relevant issues.

10.5. Retention of Records

ISGlobal, the investigators and site staff are responsible for ensuring maintenance of a comprehensive and centralized filing system of all trial-related essential documentation, readily available for monitoring, audits, or inspections at any time.

Following closure of the study, the investigator must maintain all site trial records in a safe and secure location approved by ISGlobal. The records must be maintained to allow easy and timely retrieval, when needed (e.g. audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. scanned, electronic for studies with an eCRF). The investigator must assure that all reproductions are legible, that are a true and accurate copy of the original, and that meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is a back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

No study document should be destroyed without prior written agreement between ISGlobal and the investigator. The minimum retention time of the data will meet the strictest standard applicable to any site for the trial (as dictated by ICH-GCP), any institutional requirements or applicable laws or regulations, or ISGlobal SOPs. Should the investigator wish to assign the study records to another party or move them to another location, they must notify ISGlobal in writing of the new responsible person and the new location.

11. Statistical considerations

This section describes the final study analyses, unblinded with respect to treatment intervention assignment within study sites (Tanzania and Mozambique). The statistical aspects of the study are summarized here with details fully described in a Data Analysis Plan (SAP) that will be available before completion of the follow up. The SAP will be fully reviewed by the DSMB and finalized before any analysis takes place.

11.1 Statistical analyses

Baseline characteristics in the intervention groups will be compared and assessed for similarity.

The primary outcome of these trials should be the incidence across 6 months of follow up.

This study comprises three data collection strategies: at community level with a pediatric active cohort and cross-sectional surveys, and at health facility level with a passive surveillance system. The main

analysis will be conducted at community level and some secondary and exploratory outcomes will be assessed at health facility level.

11.1.1 Analysis variables

Key analysis variables consist of baseline socio-demographic characteristics, malaria infection incidence (efficacy) and AEs and SAEs data (safety).

11.1.2 Efficacy analysis

The primary analysis will be the differential malaria incidence after the 6-month follow up period between the intervention and control groups. Incidence will be based on monthly RDT results from the active cohort. Each RDT performed will be taken to represent one visit, which is equivalent to one child-month at risk. In the per-protocol correction, 14 days of time at risk for each treatment received (based on the post treatment prophylaxis provided by AL).

Differential incidence between intervention and control clusters will be assessed using a count data regression model accounting for the cluster design effect. Primary analysis will be assessed using generalized estimating equations.

Analysis in the multivariate model will be adjusted for potential confounders identified in the univariate analysis. Based on previous vector-control trials, the variables to be considered can be classified in the following categories: demographics (age and gender), socio-economics (head of household formal education, head of household occupied as a subsistence farmer, electricity in the household, livestock-human ratio, number of nets owned and distance to the nearest health facility), clinical (having experienced fever in the previous 48h, having slept under a bed net the previous night and infection of a sibling in the same household). A step-wise approach will be applied for building the final multivariate model, considering those variables with p-values lower than 0.20 in the univariate analysis. For further details on model building see the SAP.

To assess the bias due to LTFU, sensitivity analyses limited to participants who receive all treatments may be performed. Additional support will be obtained from a comparison of the baseline characteristics of individuals who remain vs. those that are LTFU.

Assessment of the direct benefit to the population that receives treatment will be done through passive surveillance at health facilities and cross-sectional studies to allow for a better risk-benefit analysis in this population.

11.1.3 Safety analysis

For safety co-primary objective, AEs will be analyzed in total and by body system. Tables will show the number of AEs observed and the number and percentage of participants experiencing them within a system organ class or within preferred term category by severity or by relationship to study product. Participants with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Differences between AE rates in the intervention groups will be assessed by means of generalized estimating equations to account for the cluster design.

A listing of SAEs reported to the DSMB will provide details of the events including severity, relationship to study product, time between onset and last treatment, and number of treatments administered and received by study participant.

The number and percentage of study participants who discontinue treatment and who terminate from the study early will be tabulated by reason and treatment arm.

11.2 The number of participants planned to be enrolled.

Please refer to section 3.4 for sample size calculations and to sub-sections 3.4.1 and 3.4.2 for the numbers expected to enroll in each country.

There will be an approximate total of 49,000 - 54,000 volunteers enrolled per country distributed as follows:

a. Treatment/Safety group
- Approximately 45,000 – 48,000 randomized to human ivermectin, human + livestock ivermectin, or control.

b. Pediatric active cohort
- Approximately 4,000 – 6,000 from clusters randomized to human ivermectin, human + livestock ivermectin, or control.

11.3 Level of significance

A significance level of α=0.05 (two-sided test) will be used throughout all analyses.

11.5 Procedure for accounting for missing, unused, and spurious data

Missing data frequency and patterns will be studied for the main variables and, if appropriate, multiple imputation techniques will be considered. Unused and spurious data will be excluded from data analysis as soon as it is recognized

11.6 Procedures for reporting any deviation(s) from the original statistical plan

Any deviations from the statistical methods explained in the protocol will be described and justified in the final report.

The SAP will be reviewed by the DSMB and updated where necessary as a result of interim analyses of the data. A formal record will be kept detailing when the SAP was finalized. It will suffice to update the statistical analysis plan with the considerations suggested from the reviews as well as any protocol amendment.

A detailed review of the SAP will also be carried out prior to study close out to ensure any protocol amendments have been incorporated. Statistical changes such as rationale for newer or improved analytic methods for the data will be documented.

11.7 Selection of participants to be included in the analyses

The analysis will be conducted in an intention-to-treat (ITT) manner. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of number of follow-up visits completed.

12. Legal and ethical requirements

12.1. Compliance with Regulatory Requirements

These trials shall be conducted in accordance with the protocol, current Declaration of Helsinki, current ICH-GCP Guidelines, and all applicable regulatory requirements in the region(s) the study is conducted including the relevant national or local regulatory body having jurisdiction and relevant international regulations.

12.2. Compliance with Ethics Committee Requirements

These trials shall be conducted under the auspices of properly constituted Ethics Committees as defined by ICH-GCP E6 (R2) Guidelines. These committees shall review and approve all aspects of the study and subsequent amendments as stipulated by the ICH-GCP Guidelines prior to and during the conduct of the study.

Additionally, the protocol shall be submitted by ISGlobal to the Ethics Committee overseeing clinical research at ISGlobal and the WHO Ethics Review Committee.

All Ethics Committee approvals shall be in place prior to enrolment of the first participant.

The PIs at each site shall be responsible for obtaining annual reapproval from their respective Ethics Committee, and ISGlobal shall be responsible for obtaining annual reapproval from the WHO Ethics Review Committee and the Ethics Committee overseeing clinical research at ISGlobal.

All changes to the protocol or Informed Consent Form must be reviewed and approved prior to implementation, except where necessary to eliminate apparent immediate hazards to human participants.

12.3. Informed consent

The informed consent document shall contain all aspects stipulated in the ICH-GCP Guidelines.

Translations and back-translations shall be provided as required by the trial sites. If required by the local IRB, back-translations shall be reviewed by the BOHEMIA Clinical Trial Manager to ensure that the document has been accurately translated prior to submission for approval. If not, back-translations will be reviewed prior to the enrolment of the first participant and submitted to the Ethics Committees for notification purposes.

The investigators or appropriately delegated site trial staff shall be responsible for obtaining written informed consent from each trial participant prior to any trial procedures, including screening procedures being undertaken.

Assent to participate shall be sought from children aged 12 to 17, consent from participants aged 18 and above, and consent from the parent / legal guardian for minors less than 12 years of age meeting inclusion/exclusion criteria for the trials. Assent will be sought after the parent or legal guardian has signed consent. In order to maintain participant confidentiality, assent will be sought in a private space.

The parent/legal guardian consent for a minor to participate in the clinical trials will state that pregnancy results will not be provided to the parent or legal guardian.

Informed consent shall be obtained from each participant after a full explanation including but not limited to, the purpose of the trials, that participation is voluntary, the right to withdraw at any time, the risks and discomforts involved, potential benefits, etc., have been provided by the investigators or appropriately delegated site staff member, both verbally and in writing. Female participants will be advised to avoid pregnancy during the 3 active months and for one month after the final dose due to the unknown risk to the fetus.

In accordance with ICH-GCP Guidelines, the participant shall write their own name and date before signing the document. Illiterate participants and minors may provide a thumbprint and an independent, literate witness shall sign to attest to the consenting process as stipulated in ICH-GCP Guidelines.

The original individual informed consent documents shall be filed and maintained as part of the Investigator Site records and a copy shall be provided to the participant.

12.4. Participant confidentiality

Documented evidence that a potential investigator is aware and agrees to the confidential nature of the information related to the study must be obtained by means of a confidentiality agreement.

Every effort shall be made to protect participant privacy and confidentiality to the extent permitted by law. All trial-related information shall be stored securely at the trial site.

All paper-based participant information shall be stored in lockable file cabinets in areas with access limited to trial staff. Data collection, process, and administrative forms, laboratory specimens, and other reports shall be identified by a coded number only, to maintain participant confidentiality. Participant identifiers (PID) shall contain the number of the trial site and the individual participant number allocated at randomization.

All records that contain names or other personal identifiers, such as locator forms, participant identification code list and informed consent forms, shall be stored separately from study records identified by code number. Only the trial health-care personnel directly responsible for the participant's care and one designated data management member of staff shall have access to these records.

All databases shall be secured with password-protected access systems.

If the participant's name appears on any other document (e.g., pathologist report), it shall be obliterated before a copy of the document is supplied to ISGlobal.

Participants' study data, as identified by PID number only, shall not be released without the participant's written permission, except as necessary for review and monitoring by:

- Authorized study representatives
- Local and international RAs
- IECs/ IRBs
- Sponsor Monitors
- Sponsor and third-party Auditors

When the results of the study are published, the participant's identity shall remain confidential.

12.5. Dissemination of results

A publication policy and publication Has been completed and signed off by all consortium members. Dissemination of study products in conferences or publications will be conducted following international guidelines. Findings from this study will not be disseminated without adequate leadership and representation of local researchers.

12.6. Direct access to source data

ISGlobal is responsible for securing agreement from all involved parties to ensure direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by ISGlobal and third-party auditors, and inspection by domestic and foreign RAs.

Permission to examine, analyze, verify, and reproduce any records and reports that are important to the evaluation of a clinical trial shall be granted in writing by the investigator (s) / institution(s).

Any party with direct access should take all reasonable precautions within the constraints of the applicable regulatory requirement(s) to maintain the confidentiality of participant's identities and ISGlobal's proprietary information (e.g. coding of the data system, internal documentation not part of the trial record as well as SOPs).

12.7. Insurance

The study will contract a sponsor liability insurance coverage by ISGlobal, which is in line with applicable laws and/or regulations.

12.8. Posting of information on clinical trial registries

Trial information from this protocol shall be posted by ISGlobal on www.clinicaltrials.gov, www.pactr.org and other applicable trial registries before participant enrolment commences.

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14. Supplementary material

Supplementary material contains two files, the first covers the following sections

1. Preclinical safety experience with ivermectin
2. Clinical safety experience with ivermectin*
3. Modelled predicted impact of the intervention
4. An analysis of the potential of ivermectin MDA for malaria to fuel resistance of human parasites
5. A description of how the ivermectin LD_{50} for Anopheles mosquitoes was determined

6. A description of ivermectin pharmacokinetics in different species of cattle and swine

* A separate file is containing a table with studies using the 400 mcg/kg dose