Title:

Efficacy and safety of high-dose ivermectin for reducing malaria transmission (IVERMAL) - protocol summary for a double-blind, randomised, placebo-controlled, dose-finding trial in western Kenya

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Abstract:

Background:

Innovative approaches are needed to complement existing tools for malaria elimination. Ivermectin is a broad spectrum antiparasitic endectocide clinically used for onchocerciasis and lymphatic filariasis control at single doses of 150-200 mcg/kg. It also shortens the lifespan of mosquitoes that feed on individuals recently treated with ivermectin. However, the effect after a 150-200 mcg/kg oral dose is short-lived (6-11 days). Modelling suggests higher doses, that prolong the mosquitocidal effects, are needed to make a significant contribution to malaria elimination. Ivermectin has a wide therapeutic index and previous studies have shown doses up to 2,000 mcg/kg, i.e. 10x the US Food and Drug Administration approved dose, are well tolerated and safe; the highest dose used for onchocerciasis is single-dose 800 mcg/kg.

Objective:

To determine the safety, tolerability, and efficacy of ivermectin 0, 300, 600 mcg/kg/day for 3 days, when provided with a standard 3-day course of the antimalarial dihydroartemisinin-piperaquine, on mosquito survival.

Methods:

This is a double-blind, randomised, placebo-controlled, parallel-group, 3-arm, dose-finding trial in adults with uncomplicated malaria. Monte Carlo simulations based on pharmacokinetic modelling were performed to determine the optimum dosing regimens to be tested. Modelling showed that a 3-day regimen of 600 mcg/kg/day achieves similar median (5-95 percentiles) Cmax concentrations of ivermectin to single-dose of 800 mcg/kg, while increasing the median time above the LC50 (16 ng/mL) from 1.9 days (1.0-5.7) to 6.8 (3.8-13.4) days. The 300 mcg/kg/day dose was chosen at 50% of the higher dose to allow evaluation of the dose response. Mosquito survival will be assessed daily up to 28 days in laboratory-reared *Anopheles gambiae s.s.* populations fed on patients' blood taken at days 0, 2 (Cmax), 7 (primary outcome), 10, 14, 21, and 28 after the start of treatment. Safety outcomes include QT-prolongation and mydriasis. The trial will be conducted in 6 health facilities in

western Kenya and requires a sample size of 141 participants (47 per arm). Sub-studies include: (1) rich pharmacokinetics and (2) direct skin vs membrane feeding assays.

Results:

Recruitment started July 20th, 2015. Data collection was completed on July 2nd, 2016. Unblinding and analysis will commence once the database has been completed, cleaned and locked.

Discussion:

High-dose ivermectin, if found to be safe and well tolerated, might offer a promising new tool for malaria elimination.

Trial registration:

ClinicalTrials.gov: NCT02511353 (July 15, 2015).

Keywords:

Malaria, *Plasmodium falciparum*, ivermectin, dihydroartemisinin-piperaquine, *Anopheles gambiae s.s.*, insecticide, clinical trial, pharmacokinetics, Kenya, study protocol.

Background:

Ivermectin is a potential new tool that is being considered in malaria transmission reduction strategies [1]. Ivermectin is a broad spectrum antiparasitic endectocide active against a wide range of internal and external parasites. It was originally introduced as a veterinary drug, predominantly for use in domestic livestock, but since 1987 has been widely used in human medicine [2]. Ivermectin at a dose of 150 or 200 mcg/kg is the first-line treatment for *Onchocerca volvulus* (the cause of river blindness) [3], *Wuchereria bancrofti* (the cause of lymphatic filariasis) [4], and *Strongyloides stercoralis* (roundworm, an intestinal helminth) [5]. To date more than 2.7 billion treatments have been distributed as part of a mass drug administration (MDA) strategy [6].

Ivermectin has secondary effects on ectoparasites, such as head lice, mites, bedbugs and scabies, that feed on recently treated individuals [2, 7], and it is also active against *Anopheles spp*. at concentrations present in human blood after standard doses. It reduces the re-blood feeding capacity, female fecundity, hatch rate of their eggs, the survival of progeny larvae, and importantly, it reduces the vector's lifespan [1, 8-11]. It may also inhibit parasite sporogony [12]. Ivermectin has a different mode of action from other insecticides, and therefore may be effective against mosquito populations that are resistant to insecticides used on long-lasting insecticidal nets (LLINs) or indoor residual spraying (IRS). Furthermore, it is able to kill exophagic and exophillic vectors that can escape the indoor killing effects of LLINs and IRS [8].

However, several studies have shown that the effects after the standard 150-200 mcg/kg doses of ivermectin are generally short-lived. Three *in vivo* studies assessed the long-term effect of ivermectin on mosquito survival by conducting feeding at least 7 days after administration of ivermectin [10, 13, 14]. A single low dose of 200 mcg/kg showed a 1.33 fold increase in mosquito mortality when fed on blood taken from humans who had received ivermectin 1 day earlier, but there was no longer an effect when mosquitoes were fed on blood taken on day 14 post-treatment [10], while a repeated dose of 200 mcg/kg given on days 0 and 2 showed a modest effect on reduced survival 7-days post-treatment [14], and a dose of 250 mcg/kg in a single human volunteer showed a potent effect for at least 2 weeks post-treatment [13]. Population-based studies of the effect of MDA with ivermectin on malaria transmission or mosquito survival showed that MDA with a single dose of 150 mcg/kg for the control of onchocerciasis in Senegal affected survivorship of *An. gambiae s.s*. for up to 6 days, resulting in an estimated reduction of malaria transmission for at least 11 days as a result of a change in age-structure of *An. gambiae s.s*. [15-17]. Similarly, in three different west African transmission settings, this same dose reduced *An. gambiae* survivorship by 33.9% for one week, their parity rates for more than two weeks, and sporozoite rates by >77% for two weeks [18].

Modelling has also shown that adding 3 days of ivermectin 150 mcg/kg/day to MDA with dihydroartemisinin-piperaquine (DP) would potentially provide an important boost to the effect of MDAs with ACTs by allowing transmission to be interrupted faster and in areas with a higher malaria prevalence than MDA with ACTs alone [19]. However, the effects are modest, and higher doses, providing a longer effect are required for ivermectin to boost malaria transmission reduction activities [19].

Ivermectin 400 mcg/kg has been suggested as an improved treatment for head lice[20], and has been found to be safe and well tolerated [21]. No studies in humans have compared the effect of ivermectin doses above 400 mcg/kg on the ability of anopheline vectors to transmit malaria (henceforth referred to as infectivity), or evaluated the effect of any dose of ivermectin higher than 400 mcg/kg on mosquito survivorship.

Ivermectin has an excellent safety profile [1], and experience with higher doses show that it is remarkably well tolerated in humans [22-27], even at doses up to 2,000 mcg/kg, ten times the 200 mcg/kg dose currently approved by the US Food and Drug Administration [24] (Table 1). In invertebrates, ivermectin causes the opening of glutamate-gated chloride channels resulting in flaccid paralysis and death [28]. Glutamate-gated chloride channels do not exist in humans. Other weakly sensitive channels are found in the human central nervous system, but the blood-brain barrier limits drug access to these channels [29].

The only known severe adverse events have been in individuals with *Loa loa,* possibly due to rapid lysis of parasite biomass [30]. Assessment of *Loa loa* is recommended before ivermectin administration in areas endemic for *Loa loa* filariasis [31].

Table 1: Studies of safety and tolerability of ivermectin incorporating dosages ≥800 mcg/kg.

Reference	Highest single dose	Participants with single dose ≥800 mcg/kg	Total study population	Single doses in mcg/kg (participants)	Adverse events: increased vs control
Awadzi 1995, 1999 [22, 23]	800 mcg/kg	36	100 adult males with onchocerciasis in Ghana	- 150 (15) - 400[a] (25) - 600[a] (24) - 800[a] (24) - 800[b] (12)	No
Guzzo 2002 [24]	2,000 mcg/kg	36	68 healthy adults, non-pregnant, in USA	- 0 (17) - 500[c] (15) - 1,000[c] (12) - 1,500 (12) - 2,000 (12)	No
Kamgno 2004 [25-27]	800 mcg/kg	330	657 adult males with onchocerciasis in Cameroon	- 150[d] (327) - 800[d,e] (330)	Transitory mild visual side effects, without structural abnormalities upon ophthalmological exam

a) Preceded 3-days earlier by 150 mcg/kg or placebo.
b) Preceded 13-days earlier by 800 mcg/kg.
c) Repeated three times a week (days 1, 4, 7).
d) Repeated 3-monthly or 1-yearly.
e) Preceded 3 or 12 months earlier by 400 mcg/kg.

Dihydroartemisinin-piperaquine (DP) and ivermectin have, to the best of our knowledge, never been studied under simultaneous administration. Piperaquine, the long-acting component of DP, is metabolized by, and is an inhibitor of, cytochrome-P450 CYP3A4 [32]. There is a potential for an increase of piperaquine plasma concentrations when it is co-administered with other CYP3A4 substrates (due to competition) or CYP3A4 inhibitors [32]. Dihydroartemisinin (DHA), the short-acting component of DP, is not metabolized by cytochrome-P450, but is deactivated via glucuronidation catalysed by UDP-glucuronosyltransferases, in particular UGT1A9 and UGT2B7 [33]. DHA has been shown to induce CYP3A activity and also up-regulate CYP2C19 and CYP2B6 [33]. DHA is a known inhibitor of CYP1A2 [32].

Ivermectin is primarily metabolized by CYP3A4 [34]. *In vitro* studies using human liver microsomes suggest that ivermectin does not significantly inhibit the metabolizing activities of CYP3A4, CYP2D6, CYP2C9, CYP1A2, and CYP2E1 [34]. When DP and ivermectin are administered together, however, there may be some competition for CYP3A4. The CYP3A4-inhibitory properties of piperaquine may lead to an increased availability of ivermectin. As ivermectin is not a CYP3A4-inhibitor, the potential increase in the availability of piperaquine due to competition is expected to be low.

We will conduct a placebo-controlled dose finding study to determine the safety, tolerability and mosquitocidal effect of 3-day courses of ivermectin when given in combination with standard 3-day course of dihydroartemisinin-piperaquine (DP) to identify safe and practical regimens to boost the arsenal of available tools to reduce or interrupt malaria transmission. Pharmacokinetic data will be collected to facilitate the construction of a PK/PD model to guide future study design.

Study design & methods:

Design Overview

This is a double-blind, randomised, placebo-controlled, parallel-group, 3-arm, superiority trial to determine the safety, tolerability, and mosquitocidal effect of different doses of ivermectin (ClinicalTrials.gov: NCT02511353). The primary endpoint will be mosquito survival 14 days after a blood feed from a patient who started ivermectin 7 days earlier (i.e. 5 days after the last dose of ivermectin with a 3-day regimen administering ivermectin at 0, 24, and 48 hours [days 0, 1 and 2]). Because mosquito feeding involves approximately 100 mosquitoes per feed, the study will use a clustered design with the patient as the unit of randomisation and the mosquito as the unit of analysis. The study will have a nested rich pharmacokinetic component in the first 36 patients that give additional consent for rich/frequent sampling and a sparse sampling population pharmacokinetic component in the remaining patients. A second nested study will compare the effects of ivermectin when assessed by membrane feeding versus direct skin feeding in all patients who give additional consent for direct skin feeding.

Primary objective

To determine the safety, tolerability, and efficacy of ivermectin 0, 300, 600 mcg/kg/day for 3 days, when provided with a standard 3-day course of the antimalarial dihydroartemisinin-piperaquine, on mosquito survival.

Secondary objectives

1. To determine the effect of different doses of ivermectin on oocyst development
2. To determine the pharmacokinetic profile of the different ivermectin regimens
3. To determine if ivermectin interacts with the pharmacokinetics of piperaquine
4. To determine whether the addition of ivermectin to DP affects the clinical and parasitological response to DP treatment
5. To determine the role of genetic variants of CYP3A4 activity in metabolizing ivermectin

6. To determine the effect of direct feeding versus membrane feeding on mosquito survival

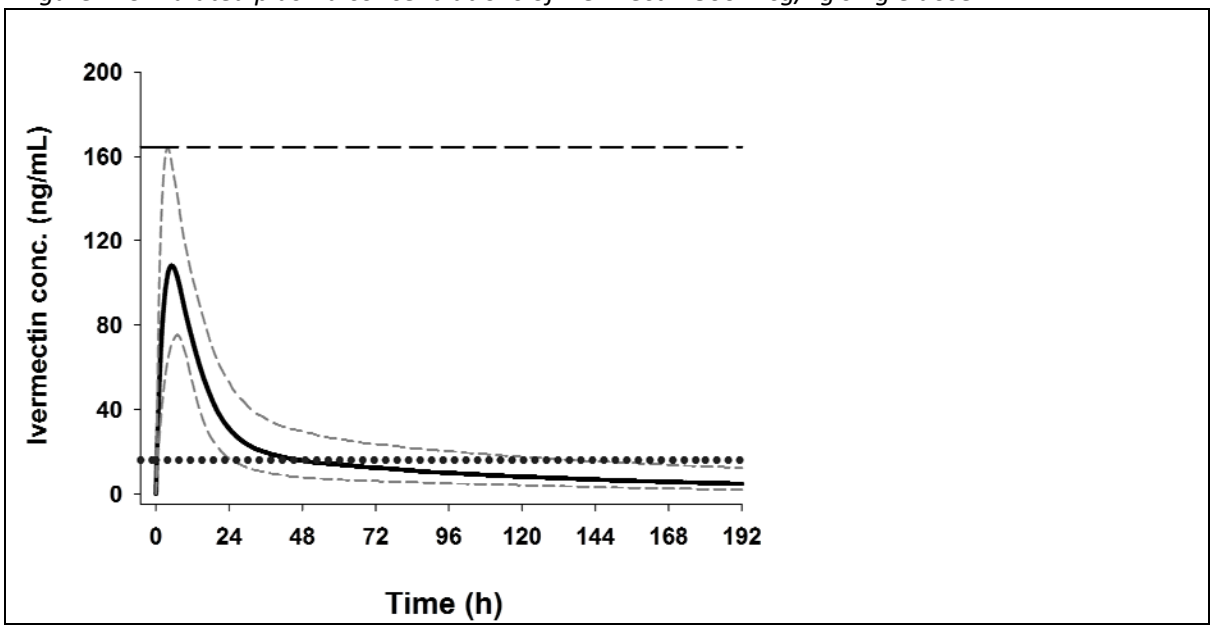
Design Considerations

Rationale for ivermectin dose of 300 and 600 mcg/kg/day

The goal was to design and evaluate a high-dose ivermectin regimen that could be given daily as adjunct therapy to a 3-day ACT regimen and that builds on the existing safety data available from previous studies. The highest dose of ivermectin used in studies for onchocerciasis is 800 mcg/kg given as a single dose (i.e. about 48 mg in an adult male weighing 60 kg). The pharmacokinetic profile of this 800 mcg/kg dose was used to design a 3-day regimen that would achieve a similar Cmax after the third dose. Since the highest dose of ivermectin used in humans that was tested and found to be well tolerated and safe is 2000 mcg/kg given as a single dose, this provides a large margin of safety allowing for inter-individual variation of pharmacokinetics. The middle group was chosen at 50% of the highest dose to allow for a dose response in terms of tolerance and efficacy.

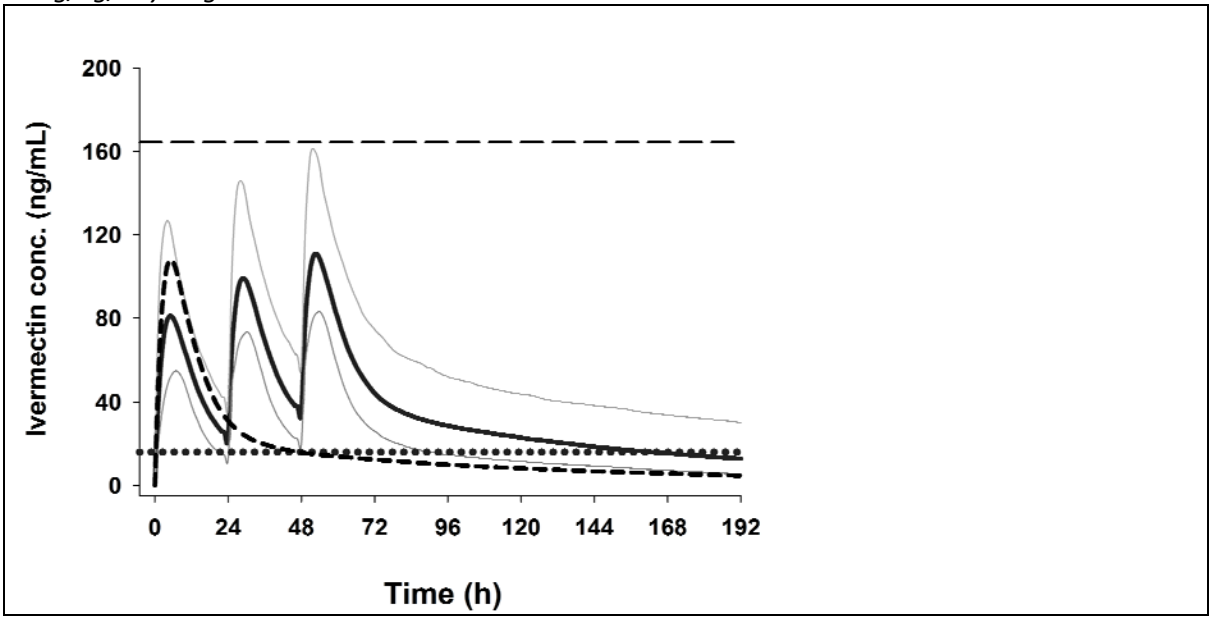
Using existing literature data [24, 35] we developed a pharmacokinetic model for ivermectin in humans. Using the parameter estimates from the model, Monte-Carlo simulations were performed for 1000 theoretical subjects assuming a 30% variability in parameter estimates (CL/F 11.8 L/h, Vc/F 195.0 L, Q 18.9 L/h, Vp 882 L, and Ka 0.24/h). The simulations showed that the Cmax associated with a single dose of 800 mcg/kg was estimated at 108 ng/ml and the 95% percentile as 164 ng/ml (*Figure 1*). A regimen of 600 mcg/kg/day for 3 days would give a similar Cmax (111 ng/mL) and corresponding 95% percentile (161 ng/mL) as the single dose 800 mcg/kg regimen (*Figure 2* and Table 2). A regimen of 300 mcg/kg/day for 3 days would give approximately half those values. The 3-day regimens were predicted to increase the time that ivermectin concentrations remain above the lethal concentration 50% (LC50) of 16 ng/ml [12] from 46 hours with the 800 mcg/kg single dose to 86 and 162 hours, respectively with the 300 and 600 mcg/kg/day regimens. The 16 ng/mL threshold was chosen as this was the median of three LC50 concentrations reported previously [12, 14, 15]. The simulated data were in excellent agreement with actual data observed in a dose finding study by Guzzo et al. 2002 [24] (which indicated proportional pharmacokinetics at doses ranging from 30-120 mg), thus giving confidence in the parameters used in the simulations.

Figure 1: Simulated plasma concentrations of ivermectin 800 mcg/kg single dose



Monte-Carlo simulation of 1000 theoretical subjects of ivermectin concentration with 800 mcg/kg single dose (median: solid line, and 5th and 95th percentiles: dashed lines). Cmax: 108.1 ng/mL (CI 75.3-164.4). Time above LC50 (16 ng/mL; dotted line): 1.9 days (CI: 1.0-5.7).

Figure 2: Simulated plasma concentrations of ivermectin 600 mcg/kg/day 3-day regimen and 800 mcg/kg/day single dose



Monte-Carlo simulation of 1000 theoretical subjects of ivermectin concentrations following 600 mcg/kg/day for 3 days (median: solid line, and 5th and 95th percentiles: grey lines), achieving similar Cmax concentrations compared to 800 mcg/kg single dose (median: dash curve, and 95th percentile of Cmax: dashed horizontal line). The median time above LC50 (16 ng/mL; dotted horizontal line) increases from 1.9 days with 800 mcg/kg single dose to 6.8 days with 600 mcg/kg/day for 3 days.

Table 2: *Summary of simulated Cmax and time above LC50*

Ivermectin Dosing Regimen	C_{max} (ng/mL) Median (5th-95th percentiles)	Days (d) above LC50 (16ng/mL) Median (5th-95th percentiles)
800 mcg/kg single dose	108.1 (75.3-164.4)	1.9 (1.0-5.7)
600 mcg/kg/day for 3 days	111.0 (83.2-161.2)	6.8 (3.8-13.4)
300 mcg/kg/day for 3 days	55.4 (41.6-80.6)	3.6 (2.8-7.5)

Parallel versus dose-escalation design

The proposed study uses a standard parallel design, comparing the 2 intervention arms with the placebo arm. This parallel design, instead of a dose-escalation design (when the lower dose group would be studied first prior to enrolling patients in the higher dose group), was considered appropriate because the Cmax levels and the 95th percentile concentrations in the proposed highest dose group of 600 mcg/kg/day will be equivalent to the Cmax found with single dose 800 mcg/kg, which has been administered to at least 402 patients before as treatment for onchocerciasis or as part of regulatory studies (see Table 1). Furthermore, with 30% variation assumed, the Cmax is estimated to remain well below the Cmax value obtained with 2,000 mcg/kg, the highest dose tested and which was well tolerated in a dose escalation study.

Why patients with malaria?

The study will enrol patients with symptomatic uncomplicated malaria, instead of asymptomatic patients with malaria parasites (carriers) or malaria negative individuals who are the predominant target population in MDA campaigns. However, it is unlikely that the mosquitocidal effect of ivermectin will differ much amongst these groups. Preference is given to symptomatic patients based on the rationale that this study is labour intensive, requiring very frequent patient follow-up and blood sampling and thus requires a major commitment from study participants. Symptomatic patients, as well as requiring antimalarial treatment, are more likely to favour hospital admission and frequent out-patient visits than asymptomatic patients or other volunteers. The frequent follow-up is potentially also more beneficial to the patients with symptomatic malaria than asymptomatic patients.

Justification for host genetic studies

The cytochromes P450s (CYPs) are the major enzymes involved in drug metabolism. To be able to interpret variations in the pharmacokinetic drug profiles of piperaquine and ivermectin and any drug interactions we need to determine the genotypes of the genes encoding CYP enzymes (see above).

Direct skin feeding vs membrane feeding

The primary endpoint is based on membrane feeding of mosquitoes using blood obtained by venepuncture from patients recently treated with ivermectin. However, a nested sub-study, in all those that give additional consent, will compare mosquito mortality rates between clusters fed using standard membrane feeding versus clusters fed directly (by allowing them to feed on the arm of the study participant). Ivermectin feeding studies with direct feeding on humans [13], and cattle [36], have shown a longer mosquitocidal effect (>2 weeks) in comparison with studies using membrane feeding (< 7days) [14].

We hypothesise that direct feeding could result in higher mosquito mortality due to potential differences between venous blood (used in membrane feeding) and blood in subdermal venuoles and arterioles (the main source of blood for mosquitoes during direct skin feeding) due to drug accumulation in subcutaneous fat, dermal, and fascial tissue (2-3-fold higher concentrations than in venous blood [37]), or increased exposure of the mosquito to ivermectin through other means like perspiration.

There have been no studies conducted directly comparing direct feeding versus membrane feeding on mosquito mortality following ivermectin administration. However, previous studies looking at infectivity (i.e. the ability of the vector to develop oocysts and sporozoites after ingesting gametocytes) showed significant differences in terms of infectivity in favour of direct feeding (odds ratio 2.39) [38]. Although the mechanisms involved in infectivity studies may differ from studies addressing the killing effect of ivermectin, this recent infectivity study [38] indicates the importance of addressing the potential that the feeding method to expose mosquitoes to ivermectin may be an important effect modifier and that studies using membrane feeding may potentially underestimate the true effect of ivermectin.

Membrane feeding will be used as the primary outcome because direct skin feeding is labour intensive, may be unpleasant to the study participants, and result in higher refusal rates.

Study setting

The study will be conducted in the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in Kisumu, western Kenya, a major tertiary care hospital. Almost 25,000 outpatients are treated for clinical malaria at JOOTRH annually, of which one-third are laboratory confirmed. Approximately 20% of these patients are 18-50 years old. Malaria positive individuals will also be pre-screened at 5

nearby health facilities; those that pass pre-screening and give consent will be brought to JOOTRH for screening and all further study procedures.

Eligibility criteria

Inclusion criteria

- Symptomatic, uncomplicated *P. falciparum* infection
- Positive malaria microscopy or malaria RDT (pLDH)
- Age: 18-50 years
- Provide written informed consent
- Agree to be able to travel to clinic on days: 1, 2, 7, 10, 14, 21, and 28

Exclusion criteria

- Signs or symptoms of severe malaria
- Unable to provide written informed consent
- For women: pregnancy or breast feeding
- Hypersensitivity to ivermectin or DP
- QTc > 460 ms on ECG
- Body Mass Index (BMI) below 16 or above 32 kg/m2
- Haemoglobin (Hb) concentration below 9 g/dL
- Taken ivermectin in the last month
- Taken DP in the last 12 weeks
- *Loa* as assessed by travel history to Angola, Cameroon, Chad, Central African Republic, Congo, DR Congo, Equatorial Guinea, Ethiopia, Gabon, Nigeria and Sudan
- History and/or symptoms indicating chronic illness
- Current use of tuberculosis or anti-retroviral medication
- Previously enrolled in the same study

Trial Medications and interventions

Participants will be randomised to one of 3 arms:

1. "0 mcg/kg" (placebo) arm: Dihydroartemisinin-piperaquine (DP) plus ivermectin-placebo 600 mcg/kg/day for 3 days.
2. "300 mcg/kg" arm: DP plus ivermectin 300 mcg/kg/day and ivermectin-placebo 300 mcg/kg/day for 3 days.
3. "600 mcg/kg" arm: DP plus ivermectin 600 mcg/kg/day for 3 days.

Patients will receive their weight-based doses of DP and ivermectin/placebo. Each dose will be given as directly observed therapy by study staff, after which participants will be monitored for 30 minutes for any vomiting and adverse reactions. If vomiting occurs within 30 minutes, then the participant will be withdrawn from the study, DP will be re-administered, and no further ivermectin will be given.

Dihydroartemisinin-piperaquine (DP)

DP was selected as the drug of choice as it is the most likely candidate to be used in future MDA campaigns because of the longer prophylactic effect against malaria (4–6 weeks) compared with 2-3 weeks with artemether-lumefantrine (AL). Each participant will receive a weight-based dose of DP 320/40mg (Eurartesim®, Sigma Tau, Italy) as per the product insert (36-75kg: 3 tablets, ≥75kg: 4 tablets) once a day for 3 days.

Ivermectin and placebo (IVM)

Ivermectin and/or placebo 6mg tablets (Iver P®, Laboratorio Elea, Argentina) will be administered per bodyweight. The 600 mcg/kg/day arm will receive only ivermectin tablets, the 300 mcg/kg/day arm will receive half the number of ivermectin tablets and an equal number of placebo tablets, and

the 0 mcg/kg/day arm will receive only placebo tablets. All participants will receive the same total number of tablets once a day for 3 days based on their bodyweight: 45-55kg: 5 tablets, 55-65kg: 6 tablets, 65-75kg: 7 tablets, 75-85kg: 8 tablets, 85-95kg: 9 tablets, 95-105kg: 10 tablets.

Endpoints / Outcome measures

Primary efficacy outcome (see Table 3):

Mosquito survival: Survival of mosquitoes at 14 days after feeding on blood taken from study participants who started the 3-day ivermectin and DP regimen 7 days earlier.

Secondary outcomes (see Table 3):

- Mosquito survival: Survival of mosquitoes at each day, up to day 21 or 28, after each feeding experiments performed at 0, 2 days+4h, 7, 10, 14, 21, 28 days after start of treatment
- Oocyst prevalence: Occurrence of oocysts from day 10 onwards after each feeding as determined by PCR
- Malaria clinical and parasitological treatment response by day 28
- Plasma concentration profiles of piperaquine and ivermectin as described by standard pharmacokinetic metrics (e.g. $AUC_{0-\infty}$, $AUC_{0-tlast}$, C_{max}, $t_{½}$, $t_{max,}$ etc).

Tolerability and Safety endpoints

Tolerability

- Any adverse events assessed in general toxicity questionnaires asked at each study visit

Safety

- Primary: Mydriasis quantitated by pupillometry [24]
- Secondary:
    - CNS effects
    - General toxicity
    - Serious adverse events
    - Haemoglobin concentrations
    - QTc interval (see below “ECG monitoring”)

Participants’ timeline

Overview Study Phases

The study plan and schedule of assessment is provided in Table 3. The participant’s timeline will consist of a pre-screening visit (visit 1), consent, screening and enrolment visit (visit 2), two subsequent treatment visits (3 and 4) on days 1 and 2, and six follow-up visits for assessment of efficacy parameters (visits 5 to 10). For those enrolled in the pharmacokinetic study additional visits for drug level sample are required as outlined in “Appendix 1: Full study protocol”.

Visits 1 and 2: Pre-screening, Consent, Screening, and Enrolment

Patients presenting to the outpatient departments of the study clinics will be pre-screened to determine if they meet readily apparent study eligibility criteria 1) age: 18-50 years, 2) uncomplicated malaria, 3) in Kisumu next 4 weeks, 4) Hb ≥9g/dL [if already performed], 5) not pregnant or breast feeding, 6) no known chronic illness, 7) not previously enrolled in IVERMAL. Patients passing pre-screening will be approached to obtain consent. For those consenting, study specific screening procedures will take place, including: demographics, full history, past medication use, travel history (*Loa* endemic countries), physical examination, ECG, pupillometry, and laboratory tests (to confirm malaria, Hb and pregnancy). Those fulfilling all enrolment inclusion criteria and not meeting any exclusion criteria will be enrolled into the study, randomized and treated with the appropriate tablets according to study arm. Estimated duration: 1.5-2.0 hours.

Visits 3 and 4: Treatment visits

They will return to the out-patient clinic on day 1 and 2 for the 2nd and 3rd dose of study drugs. In exceptional cases a participant will be permitted to take the study medication at home or the participant will be visited at home by study staff to administer the medication. A follow-up ECG will be taken just prior to and 4-6 hours after the last dose of DP+ivermectin on day 2.

Visits 5 to 10: Scheduled follow-up visits

Participants will return to the out-patient clinic for follow-up as specified (see Table 3). A questionnaire will assess the presence of signs and symptoms, including any adverse effects. A brief clinical examination will be performed and a venous blood sample will be taken for malaria diagnosis, Hb, and drug levels. On visits 5 (Day 2+4h) and 6 (Day 7), drug levels will also be determined in a finger prick sample. A final follow-up ECG will be taken on the day 28 visit. Participants will be asked to provide telephone numbers so that study staff may make every effort to follow-up participants who have missed scheduled visits as outlined in "Appendix 1: Full study protocol, section 8.5.5, page 31".

Unscheduled visits

At any time, participants displaying signs or symptoms of severe malaria will be admitted to the inpatient ward for further evaluation and treatment free of charge. Blood samples for malaria smears, parasite genetics (filter paper dried blood spots) and haemoglobin will be taken if clinically indicated (e.g. documented fever ≥ 37.5 °C axillary, or a history of fever in the last 24 hours).

Table 3: Summary of Study Design and Schedule of Assessment (SPIRIT Flow Diagram)

Phase	Recruitment Phase	Enrolment	Treatment Phase			Post-treatment Follow-up phase					
Location	OPD	OPD	OPD	OPD[b]	OPD[b]	OPD visits					
Visit number	#1	#2	#2	#3	#4	#5	#6	#7	#8	#9	#10
Study Time Hour	-1h [a]	-0.5h	0h	24h	48h	52h	168h	240h	336h	504h	672h
Day	D00	D00	D00	D01	D02	D02+4h ±2h[c]	D07 ±3d[c]	D10 ±3d[c]	D14 ±3d[c]	D21 ±3d[c]	D28 ±3d[c]
Recruitment											
Pre-screening	X										
Enrolment											
Eligibility screen		X									
Informed Consent		X									
Study code issued		X									
Allocation		X									
Interventions											
IVM-0 arm			X[i]	X[i]	X[i]						
IVM-300 arm			X[i]	X[i]	X[i]						
IVM-600 arm			X[i]	X[i]	X[i]						
Clinical assessments		D00			D02	D02+4h	D07	D10	D14	D21	D28
Copy Clinic/Lab data from hospital records											
Physical Exam.		X				X	X	X	X	X	X
Pupillometry		X				X	X	X	X	X	X
ECG		X			X[j]	X					X
Questionnaire AE		X				X	X	X	X	X	X
Blood sample[d,e,h]		V				V+C	V+C	V	V	V	V

	5.4ml	5.9ml	5.9ml	5.4ml	5.4ml	5.4ml	5.4ml
Unscheduled sick-patient clinic visits		Passive surveillance for 28 days (clinical malaria and other acute illnesses)[k]					
Entomological assessments	D00	D02+4h	D07	D10	D14	D21	D28
Membrane feeding[f]	X	X	X	X	X	X	X
Direct feeding			X[g]				

Visit 1: Pre-Screening interview
Visit 2: Consent, Screening, & Enrolment. First treatment dose given under direct observation.
Visits 3 and 4: Treatment visits. 2nd and 3rd treatment doses given under direct observation. In exceptional cases doses of day 1 and 2 can be taken at home.
Visits 5 to 10: Scheduled follow-up visits for assessment of efficacy parameters

a. Patients can be pre-study screened any time from visiting the OPD. The figure of -1 hour is provided for illustration purposes only.
b. The day of enrolment is always considered as Day-0. Doses given under direct observation. In exceptional cases doses of day 1 and 2 can be taken at home.
c. Visit window= number of days an actual subject visit may fall outside of the planned protocol schedule visit to still meet protocol requirements. It is preferential to stick to the scheduled days of visit. However, if this is not feasible (e.g. due to other commitments of the patient) then it preferable to allow flexibility in the schedule. The date of actual visit will always need to be recorded in the CRF.
d. Enrolment & baseline blood sample (~5.4 mL) by venepuncture: haemoglobin (0.01 mL), malaria smear / RDT (0.01 mL), dried blood spots (DBS) for PCR (0.3 mL) will be stored for parasite genetics to differentiate reinfection from recrudescence in case of treatment failure, membrane feeding (~1 mL), pharmacology (~4 mL: ~2mL plasma for drug levels, 2mL pellet for host metabolism genetics).
e. Follow-up blood sample (~5.4 mL) by venepuncture: haemoglobin (0.01 mL), malaria smear (0.01 mL), malaria RDT (0.005 mL), dried blood spots for PCR (0.3 mL), membrane feeding (~1 mL), pharmacology (~4mL: ~2mL plasma for drug levels).
f. Membrane feeding will be used to assess: Mosquito survival (daily up to 21 to 28 days after feed; Oocyst prevalence at day 10 after feeding).
g. Direct skin feeding in a sub-sample only.
h. Finger prick (capillary) blood sample on Day 2+4h and Day 7: drug levels (~0.5ml to obtain ~0.25ml plasma).
i. Each treatment visit: IVM-0 (DP, placebo 600 mcg/kg), IVM-300 (DP, ivermectin 300 mcg/kg/day, placebo 300 mcg/kg/day), IVM-600 (DP, ivermectin 600 mcg/kg/day)
j. Before 3rd dose.
k. RDT/smear, Hb, dried blood spots for parasite genetics.

V=venepuncture. C=capillary, DP=dihydroartemisinin-piperaquine, IVM=ivermectin, Hb=haemoglobin, MS=malaria smear, Pf=Plasmodium falciparum

Sample Size

The study requires a total of 141 participants (47 participants in the 0, 300 and 600 mcg/kg/day groups each). This is powered at 80% to detect a relative increase of 30% (RR 1.300) in the 14-day post-feeding mortality rate (primary outcome) from 24% in the control group (0 mcg/kg ivermectin) to 31.2% in the 300 mcg/kg/day group, and a 25% (RR 1.246) increase from 31.2% with 300 mcg/kg/day to 38.9% in 600 mcg/kg/day recipients, measured from blood taken 7 days after the start of intake of ivermectin and using clusters of 100 anopheline mosquitoes allowing for 10% non-feeders (α=0.05). The same sample size would give 90% power to detect a 35% [RR 1.348] increase from 24% (0 mcg/kg/day) to 32.4% (300 mcg/kg/day), and 27.7% increase [RR 1.285] from 32.4% (300 mcg/kg/day) to 41.3% (600 mcg/kg/day). The calculations assume an intracluster correlation coefficient (ICC) of 0.0622 and allow for 6.5% loss-to follow-up of participants by day 7 (i.e. 44 of the 47 patients per arm contribute to the primary analysis) [14]. The 10% non-feeding rate is based on current data from the same laboratories at KEMRI, Kisian, Kenya. The 24% mortality rate estimate by day 14 post-feeding in the control arm is average of observation at KEMRI (18.3%) and in a recent study in Burkina Faso, which showed a 21.2% mortality by day 10 [14], which when extrapolated with 4 additional days predicted a mortality of 29.7% by day 14. The ICC value of 0.0622 was

calculated using data from the recent study in Burkina Faso (Bousema, personal communications) [14].

Assignment of interventions

Allocation

The study will use stratified randomisation by BMI and sex (4 strata) as these are important determinants of the pharmacokinetics of ivermectin [14]. The high/low BMI thresholds are: females 23 kg/m^2, and males 21 kg/m^2. Participants will be randomly assigned to 1 of the 3 study arms. The study statistician will computer-generate a randomisation sequence using permuted block randomisation with fixed block sizes.

Blinding

The study will be double-blinded to participants and study staff. Allocation concealment will be achieved by use of sealed opaque envelopes. All study participants in all 3 arms will receive standard dose DP, and also active (600 mcg/kg/day arm), placebo (0 mcg/kg/day arm), or a combination of active and placebo ivermectin tablets (300 mcg/kg/day arm), such that each arm receives the same number of tablets in each weight strata.

Pharmacokinetic (PK) studies

Overview

The first 36 patients to give additional consent for rich pharmacokinetics (~12 per arm), will be enrolled in a rich pharmacokinetic study using frequent sampling per individual (26 samples per patient, See "Appendix 1: Full study protocol, Table 2, page 15") to determine the detailed pharmacokinetic profile of the two regimens and assess whether any drug interaction occurs with piperaquine that is of clinical relevance. The remaining patients (~35 per arm) will contribute to a population PK study consisting of sparse PK sampling (maximum 13 samples per patient including baseline [1 venous sample], six scheduled visits as part of the main trial [6 venous and 2 finger prick samples] and two extra visits for population PK sampling [2 venous and 2 finger prick samples]).

The rich and population PK studies combined will allow us to determine the main sources and correlates of variability in drug concentrations (for both ivermectin and piperaquine), including demographic, pathophysiological, such as body mass index and gender, and other factors that might alter dose-concentration relationships. As this is a placebo controlled trial, the sampling methodology for the 47 patients in the ivermectin-placebo arm will be identical to that used for the 300 and 600 mcg/kg arms. The patients in the placebo-ivermectin arm will allow us to determine the piperaquine kinetic profile in the absence of ivermectin.

Finger prick blood draws will be performed at a maximum of 4 time points in addition to the venous blood draws. The aim is to compare the capillary and venous drug concentration levels as it has been hypothesized that these might differ for ivermectin, similar to other drugs including piperaquine. A difference between capillary and venous drug concentrations could help further explain any observed difference in mosquito mortality between membrane and direct skin feeding (see also above "Direct skin feeding vs membrane feeding").

Standard pharmacokinetic study (rich sampling)

All of the rich PK participants (~12 per arm) will have venous blood sampled (4 ml whole blood to obtain 2 ml plasma, or 5.2 ml of whole blood if coinciding with a scheduled follow-up visit for the main trial) at baseline and each of 21 follow-up time points listed in "Appendix 1: Full study protocol, Table 2, page 15". Additionally, 4 finger pricks (0.5ml whole blood) will be taken at Days 2+4h, 3, 4 and 7. The total blood volume to be drawn from these patients is 98.4 mL whole blood over 28 days, 82.8 mL of which is taken during the first 10 days. If more than 2 patients withdraw from the study

without giving more than 12 samples, the withdrawing patients will be replaced. Outpatients who consent to the standard pharmacokinetic study will admitted in the hospital for the first 3 days.

Population pharmacokinetics (sparse sampling)

Each of remaining patients (~35 per arm), not enrolled in the rich pharmacokinetic sub-study, contribute to the population pharmacokinetic study, which consists of 13 sampling points (See "Appendix 1: Full study protocol, Table 2, page 15"), 7 of which coincide with the timing of sample for the membrane feeding (including the baseline sample), thus not requiring an extra venepuncture (i.e. days 0, 2 [52 hours; 4 hrs after last dose of ivermectin], days 7, 10, 14, 21 and 28), and 6 of which are specific for the population PK study and will require an extra venepuncture (50, 54, 60, 72, 96 and 120 hours, i.e. 2, 6, 12, 24, 48, and 120 hours after the third and last dose of ivermectin). To ensure an equal distribution of samples across the different sampling time points for the extra 2 visits, participants will be divided into 4 extra sampling groups; each of which will contribute 2 extra time points, with the exception of group B which will contribute 1 extra time point (Table 4). Additionally, a maximum of 4 finger pricks (0.5ml whole blood) will be taken at Days 2+4h, 7, and at each of the two population pharmacokinetic visits. Thus the total number of samples per participant will be 13 and involve a total of 46.4 mL of whole blood (including the 7 samples for the main trial). The sampling times will be noted in the CRF, and the patient given a reminder card to return to clinic at their allocated time.

Table 4: Schedule of extra sampling points for Population PK study by 4 sampling groups

Subject Group	Sample Day * (+hours after 3rd ivermectin dose)	Sample Absolute time (hrs)*	Number per sampling strata
A	2.08 (+2h) 2.25 (+6h)	50 54	9
B	2.25 (+6h)	54	8
C	2.50 (+12h) 3 (+24h)	60 72	9
D	4 (+48h) 5 (+72h)	96 120	9
Total			35

* Extra visits that need to be made specifically for the population PK samples. The other 7 visits contributing to the population pharmacokinetic analysis (Days 0, 2, 7, 10, 14, 21, 28) coincide with the scheduled visits in the main trial. The first day is day=0; day 1 starts 24 hours after the first dose. The allocation to the sampling strata will be at random. However, if a participant indicates he/she is not able to attend a certain follow-up day, the strata can be replaced by another sampling schedule (within the same allocation strata e.g. for BMI, gender etc) until all 15 or 16 allocations per sampling group have been used.

In anticipation of a 40% refusal rate or loss to follow-up, we estimate that the combined rich and population PK sub-studies will contribute 361 samples including 47 baseline samples (100%) and 314 (60%) follow-up samples out of a potential 524 follow-up samples across 22 sampling time points after baseline, 20 of which overlap, with a total of 12 to 47 observations per time point (See "Appendix 1: Full study protocol, Table 2, page 15").

Laboratory Procedure

Mosquito colonies

See also above "Procedures for Assessing Efficacy and Safety Parameters" for use of mosquito colonies and procedures to assess the primary (mosquito survival) and secondary entomological endpoints (sporogony). This section below describes the maintenance of the mosquito colonies.

The mosquito colony used in this study will be *An. gambiae s.s.* Kisumu strain, originally from Kisumu, Kenya. The strain is maintained at the KEMRI/CGHR insectaries and is susceptible to all insecticides approved by WHO. When performing the membrane feeds on infected human blood, mosquitoes will be kept, and fed in cages or paper cups. The cages or paper cups will be kept in a temperature and humidity controlled insectary. The feeding and the storage of live infected mosquitoes will occur in sealed rooms with at least two doors/barriers separating the inner rooms from the outside. Mosquitoes will not be removed from their enclosures, with exception of the cage for oocyst determination. During transportation, live infected mosquitoes will be transported within paper cups that are covered with a moist towel and enclosed within locked cool-boxes to remove any chances of escape. The cool-boxes will only be opened within the confines of a double door insectary.

Ivermectin plasma concentration

The lethal concentration of ivermectin able to kill 50% of exposed mosquitoes (LC50) has been estimated using spiked blood (blood to which known concentrations of ivermectin are added) in membrane feeding assays [12, 15]. We will test the concentration of ivermectin in human plasma in order to provide data for a pharmacokinetic/dynamic analysis to obtain estimates of the 10-day-LC50 and time post-treatment that the transmission blocking effects (on mosquito survival and oocyst rates) lasts.

Haemoglobin testing

Haemoglobin will be tested using HemoCue® (Angelholm, Sweden) photometers.

Thick and thin blood smears for malaria

Thick and thin blood films for parasite counts will be obtained and examined. Malaria parasites will be counted against 200 high power fields before a slide is declared negative [39].

Processing of pharmacokinetic samples

Plasma will be stored locally at site at –20° C or in liquid nitrogen and shipped to a central laboratory for storage at –70° C prior to batch analysis at the Liverpool School of Tropical Medicine / University of Liverpool. Samples will be shipped in dry ice to the laboratories in Liverpool, UK where the plasma concentrations of ivermectin and piperaquine will be determined using assays validated to international FDA standards. Plasma concentration-time data will be used to evaluate pharmacokinetic parameters including: CL/F (oral clearance), V/F (oral volume of distribution), Ka (absorption rate constant) using population pharmacokinetic methods. Area under the curve and half-life will also be calculated.

Statistical methods

A study statistical analytical plan for the final analysis, that supersedes the study protocol, has been drawn up during the course of the study before the unblinding of data at database lock (See "Appendix 2: Statistical Analytical Plan").

Procedures for Assessing Efficacy and Safety Parameters

Membrane Feeding [MF] procedure

The following procedures will be conducted in accordance with a standard membrane feeding protocol [40]. 1 mL of the participant's blood will be drawn into a sodium heparinised tube pre-heated to 37.5° C. Within 2 minutes the blood will be placed in a glass bell membrane feeding

system and cups of mosquitoes will commence feeding. For each feeding three new cups (2 cups for mosquito survival, and 1 cup for oocysts) of 50, 3-5 day old female, insectary-reared *An. gambiae s.s.* mosquitoes will be presented to the membrane feeder for 20 minutes. The number of mosquitoes with an engorged abdomen will be counted and those with lean abdomens discarded. Each day up to day 28 (mosquito survival cups) or day 10 (oocyst cup), the number of dead mosquitoes will be counted and removed. After the initial feeding on human blood, the mosquitoes will be kept in an incubator and maintained on sugar feeds. Insectary staff assessing mosquito survival and oocyst development will be blinded to all characteristics of the cups (including: participant ID, study arm, duration between treatment and feeding, and feeding method).

Primary efficacy outcome

Mosquito survival (at day 14; from D07 feed)

The primary outcome will be the survival of mosquitoes (from the 2 mosquito survival cups) at 14 days after feeding on blood taken from study participants who started the 3-day ivermectin and DP regimen 7 days earlier.

Secondary efficacy outcomes

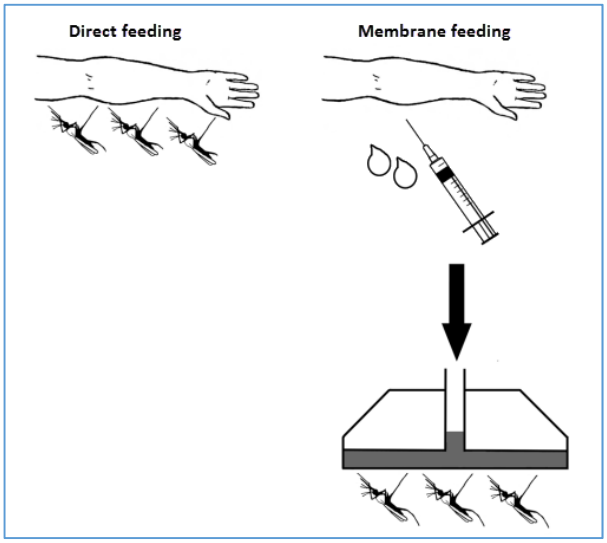
Mosquito survival (daily; from all feeds)

Although the primary endpoint is assessed at day 14, the study will collect survival data of mosquitoes at each day up to day 21 or 28 for the mosquito survival cups and day 10 in the case of oocyst cups, after each feeding experiments performed at 0, 2 day+4h, 7, 10, 14, 21, 28 days after start of treatment. The methods will be identical to that described for the primary outcome above where each day beyond day 14 the number of dead mosquitoes will be counted and removed until day 28 inclusive. The exact number of follow-up days (21 or 28 days) will be subject to logistical constraints of the laboratory, and mortality rates in the mosquito populations which will be further determined prior to the start of the study. The aim is to be able to determine the median time to mortality, which requires that at least half of the mosquito population has died in each arm. It is anticipated that 21 days will be sufficient.

Direct Skin Feeding [DF] and mosquito survival (daily; from D07 feed)

A sub-study will determine the effect of direct feeding versus membrane feeding on mosquito survival, after feeding experiments performed at 7 days after the start of treatment. In direct skin feeding assays, one cup of 50 mosquitoes is placed directly on the skin of the human host and allowed to feed for 15 minutes (see Figure 3). Further procedures after direct feeding are identical to those after membrane feeding.

*Figure 3: Difference between membrane feeding and direct feeding (*Adapted from Bousema et al 2012 [38])



Infectivity to mosquitoes (oocyst PCR)

On day 10 post membrane feeding, when residual DNA from the blood meal is highly unlikely [14, 41, 42], all surviving mosquitoes in the oocyst cup will be preserved to determine oocyst prevalence by polymerase chain reaction (PCR). Mosquitoes will be homogenized and processed, in two pooled batches per cup.

Asexual treatment response and parasite clearance

Standard methods will be used to assess the *in vivo* treatment response to DP using the microscopy and RDT data collected at each scheduled follow-up visit and criteria described by WWARN [43].

Safety outcomes

Pupillometry

In animal studies, mydriasis has been shown to be a first sign of ivermectin toxicity. To monitor for possible toxicity, pupil diameter size will be measured at baseline and each scheduled visit using a portable, single-button activation, battery operated hand-held pupillometry device which very accurately measures pupil size requiring no calibration (NeurOptics VIP™-200 Variable Pupillometer, which measures the pupil 30 times per second over a five-second period and provides the average pupil diameter and standard deviation (+/- 0.1 mm)). The measurements will be taken in a dark room with standardized lighting conditions.

ECG monitoring

Piperaquine can potentially lead to QTc interval prolongation. To exclude a possible interaction between ivermectin and piperaquine leading to an increased QTc interval, 12-lead ECGs will be performed to measure the QTc interval at baseline, Day 2 pre-last dose, Day 2 at 4-6h post-last dose and again at Day 28. The day 28 sample is included as a true baseline is difficult to assess in patients with acute malaria, as malaria and fever are known to increase the heart rate and decrease the QTc interval. On day 28 most, if not all, patients, will be malaria free and residual piperaquine levels low enough not to affect QTc intervals. A portable ECG machine (MAC 600®, General Electric, United States) will be used with automated ECG interpretation. Patients with a QTc value of 480 ms or greater prior to the last dose of DP will not receive the last dose of DP, but receive a full course of artemether lumefantrine instead. Fridericia's correction will be used to calculate the QTc values for final data analysis ($QTc = QT/RR^{0.33}$).

Adverse events

Adverse events (AE's) and serious adverse events (SAE's) will be monitored, managed and recorded during the course of the study. They will be recorded and tabulated for each treatment arm, overall and per body system. See also "Appendix 1: Full study protocol, Section 9.6, Safety Monitoring and Reporting".

Ethics approval and consent to participate

This protocol, the informed consent documents, and patient information sheets have been reviewed and approved by the Research Ethics Committees at the Kenya Medical Research Institute, Nairobi, Kenya (KEMRI protocol #2775), the Liverpool School of Tropical Medicine, Liverpool (LSTM protocol #14.002), and the Jaramogi Oginga Odinga Teaching & Referral Hospital (JOOTRH). The Centers for Disease Control and Prevention (CDC protocol #6720) gave approval for reliance on the KEMRI IRB. See Appendix 3: Ethics approvals KEMRI, CDC, LSTM and JOOTRH.

Results:

Recruitment started July 20th, 2015. Enrolment was completed May, 2016 and clinical follow-up were completed 4 weeks later in June, 2016. Mosquito follow-up was completed in July, 2016, 4 weeks after completion of the clinical follow-up. Unblinding and analysis will commence once the database has been completed, cleaned and locked.

Discussion:

New strategies for malaria control, and eventually for elimination are critically needed. This study will seek to answer the question as to whether higher doses of ivermectin (300 and 600 mcg/kg/day for 3 days) are well tolerated, safe and result in longer durations of mosquitocidal effects than

standard 150-200 mcg/kg single dose treatments. This study requires major infrastructure and collaboration, as it brings together the disciplines of clinical medicine, entomology, parasitology, pharmacokinetics, and pharmacogenetics in a clinical trial. 141 patients and 150,000 mosquitoes will each be followed for 28 days. For this reason, this trial has been placed at the KEMRI, CDC, and LSTM collaboration in western Kenya, a research site, which in collaboration with its partners, has been conducting research for over 35 years and has the capacity to undertake such a trial. An important possible limitation of this study is that it will enrol participants with symptomatic malaria, whereas possible future applications of high-dose ivermectin may involve MDA with ACT's targeting asymptomatic carriers and uninfected individuals in addition to symptomatic patients. Should this study show promising results, then the next step will be to evaluate safety, tolerability, and efficacy in younger age groups with the ultimate goal of testing its effect on malaria transmission when applied at the population level through MDA. High-dose ivermectin, if found to be safe and well tolerated, could potentially complement existing tools for malaria elimination.

Declarations:

List of abbreviations

95% CI	95 percent Confidence Interval
ACT	Artemisinin-based combination therapy
AE	Adverse event
AL	Artemether-Lumefantrine
AUC	Area Under the Curve
CDC	Centers for Disease Control and Prevention
Cmax	Maximum drug concentration
CRF	Case Record Form
DHA	Dihydroartemisinin
DP	Dihydroartemisinin-piperaquine
DMEC	Data Monitoring and Ethics Committee
ERC	Ethics Research Committee
FDA	Food and Drug Administration
GCP	Good Clinical Practice
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
IRB	Institutional Review Board
IVM	Ivermectin
JOOTRH	Jaramogi Oginga Odinga Teaching and Referral Hospital
KEMRI	Kenya Medical Research Institute
LC50	Lethal Concentration 50%
LLINS	Long-lasting Insecticide Treated Nets
LSTM	Liverpool School of Tropical Medicine
MDA	Mass drug administration
PCR	Polymerase Chain Reaction
RDT	Rapid diagnostic test
REC	Research Ethics Committee
SAE	Serious adverse event
T1/2	plasma half-life
QTc	QT corrected time interval between Q and T on electrocardiogram
QTcF	QT corrected time interval using Fridericia's correction on ECG
Tmax	time to maximum plasma concentration
TSC	Trial Steering Committee

WHO World Health Organization

Appendixes

Appendix 1: Full study protocol (incl. SPIRIT checklist), v4.1, dated 14-Jan-2016.
Appendix 2: Statistical Analytical Plan (SAP), v1.0, dated 19-Feb-2016.
Appendix 3: Ethics approvals KEMRI, CDC, LSTM and JOOTRH.

Conflicts of interest

None.

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Authors' contributions

Feiko ter Kuile (FtK) and Menno Smit (MS) conceived the study. MS, Penelope Phillips-Howard (PPH) and FtK wrote the grant. MS, Eric Ochomo (EO), and FtK drafted the protocol. Duolao Wang (DW) provided statistical expertise and verified the sample size calculation. Ghaith Aljayyoussi (GA) and Steve Ward (SW) conducted the Monte Carlo simulations to define the dosing regimen and further developed the pharmacokinetic sub studies. All investigators contributed to the refinement of the study protocol and approved the final version. MS and FtK drafted the manuscript. All authors read and approved the final manuscript prior to submission. The findings and conclusions in this paper are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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