STUDY PROTOCOL

A pharmacokinetic randomised interventional study to optimise dihydroartemisinin-piperaquine dosing for malaria preventive treatment in Malawian infants: A protocol for the OPTIMAL study [version 1; peer review: awaiting peer review]

Clifford G Banda[1-3], Mphatso S Kantonya[2], Steven Munharo[2], Marumbo E Chirwa[2], Mayamiko D Kapulula[2], Hellen D Chavula[2], Aubrey Chiyana[2], Vincent Katunga-Phiri[2], Diksha Patel[2], Vusumuzi Katangwe[2], Elizabeth Allen[1,4], Feiko ter Kuile[5], Victor Mwapasa[2,3], Dianne J Terlouw[2,5], Joel Tarning[6,7], Karen I Barnes[1,4]

[1]Department of Medicine, Division of Clinical Pharmacology, University of Cape Town, Rondebosch, Western Cape, 7925, South Africa
[2]Malawi-Liverpool-Wellcome Research Programme, Blantyre, Malawi
[3]Kamuzu University of Health Sciences, Blantyre, Malawi
[4]WorldWide Antimalarial Resistance Network (WWARN), Pharmacology Scientific Group, University of Cape Town, Cape Town, South Africa
[5]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK
[6]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, OX3 7LG, UK
[7]Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400, Thailand

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Abstract

Background

A newer malaria preventive treatment, dihydroartemisinin-piperaquine (DP), has been identified as an effective alternative to sulfadoxine-pyrimethamine, to which malaria parasites are increasingly becoming resistant. However, how best to dose DP to safely prevent malaria in infants when aligned with routine health facility visits remains unresolved. As infants are usually excluded from participating in early dose optimisation clinical trials, the present study seeks to shift the paradigm and develop optimised DP dosing strategies for malaria preventive treatment in infants.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

Any reports and responses or comments on the article can be found at the end of the article.

Methods

A randomised, single-blind, placebo-controlled, two-arm, interventional study will be conducted in southern Malawi. At 10 weeks (2.5 months) of age, 220 eligible infants will be randomised to receive DP (intervention group, n=110) or placebo (control group, n=110) with routine vaccines. They will be followed until 12 months of age and receive three further DP or placebo treatment courses at 14 weeks, six- and nine months. Infants in the intervention group will contribute capillary samples for piperaquine concentrations pre-dose and at three-, seven-, 14- and 28-days post-DP dosing as well as capillary samples pre-dose and on day 28 post-DP to quantify malaria parasitaemia using microscopy and quantitative PCR. In the control group, infants will contribute capillary blood samples for malaria parasitaemia at the same time points as the intervention group. Malaria incidence and adverse events will be compared between the two groups. Population pharmacokinetic-pharmacodynamic modelling techniques will be applied to derive feasible, optimised, efficacious, and safe DP dosing strategies for malaria preventive treatment in infancy.

Conclusions

The findings will provide the much-needed evidence to inform DP dosing for malaria preventive treatment in infants when administered with routine health facility visits. Additionally, they will help inform optimal DP dosing for malaria treatment in infants.

The trial was registered with the Pan African Clinical Trials Registry; (PACTR202211575727659) on 8 November 2022. Protocol version 3.1, dated 29 September 2022.

Keywords
Pharmacokinetics-pharmacodynamics, modelling, infants, malaria, antimalarials, dihydroartemisinin-piperaquine

Corresponding author: Karen I Barnes (karen.barnes@uct.ac.za)

Author roles: Banda CG: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Kantonya MS**: Investigation, Project Administration, Supervision, Writing – Review & Editing; **Munharo S**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Chirwa ME**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Kapulula MD**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Chavula HD**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Chiyana A**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Katunga-Phiri V**: Data Curation, Investigation, Methodology, Project Administration, Writing – Review & Editing; **Patel D**: Data Curation, Investigation, Methodology, Project Administration, Writing – Review & Editing; **Katangwe V**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Allen E**: Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **ter Kuile F**: Conceptualization, Funding Acquisition, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Mwapasa V**: Funding Acquisition, Investigation, Methodology, Project Administration, Writing – Review & Editing; **Terlouw DJ**: Conceptualization, Funding Acquisition, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Tarning J**: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing; **Barnes KI**: Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Writing – Review & Editing

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Introduction

Plasmodium falciparum malaria is estimated to affect up to 36% of infants in high-transmission settings[1]. These very young children are at an increased risk of rapid disease progression, severe malaria and death[2]. The World Health Organisation (WHO) has, since 2010, highlighted the need to protect infants from malaria with a treatment course of sulfadoxine-pyrimethamine (SP) [IPTi-SP], given with scheduled immunisation visits, in areas of moderate to high malaria transmission such as Malawi[3]. Recently, this recommendation has been extended beyond the first year of life and is now termed perennial malaria chemoprevention (PMC)[4]. However, there is increasing evidence of SP resistance and reduced protective efficacy[5]. Notably, IPTi with a different antimalarial medication, dihydroartemisinin-piperaquine (DP), which is an artemisinin-based combination therapy, has been shown to have a higher malaria protective effect than SP[6,7]. This is mostly due to significantly lower drug resistance and the longer post-treatment prophylactic effect of the partner drug, piperaquine. DP is, therefore, a potential candidate to replace SP for IPTi or PMC.

Unfortunately, there is a paucity of evidence to guide DP's optimal dosing in infants. In a previous effort to optimise DP dosing for IPTi, a randomised clinical trial in Uganda suggested that monthly IPTi-DP is better than three-monthly dosing for malaria protection in children under two years of age[8]. However, the programmatic feasibility of such a monthly dosing schedule would be challenging as mothers have historically found chemopreventive treatment administered at routine health facility visits to be more convenient, which increases adherence and coverage[9–11]. Optimising DP dosing in infants for administration during routine health facility visits as part of PMC is, therefore, a programmatic necessity.

This pharmacokinetic study aims to OPTImise the dosing of DP for MALaria preventive treatment in infants (OPTIMAL study). We will define how best to dose DP in this important subpopulation when the administration is aligned with routine health facility visits. Our hypothesis is that, due to physiological changes in infancy that are known to increase drug clearance, the currently recommended weight-based dosing regimen of DP results in decreasing piperaquine exposure with increasing age, which may not be sufficient to prevent malaria in older infants. Understanding the variation in piperaquine exposure within the first year of life is important to inform dose optimisation of DP for malaria preventive (and symptomatic) treatment in infants. This evidence is needed by the WHO and National Malaria Control Programs to inform preventive treatment guidelines in this complex subgroup that carries a disproportionately high malaria burden.

Protocol

Study setting and disease burden

The study will be conducted at the under-five/vaccination clinic at Chikwawa District Hospital in the southern Lower Shire Valley of Malawi. The Chikwawa site is part of a floodplain of the large Shire River, which fuels local transmission. *Plasmodium falciparum* malaria is endemic in the area, with a prevalence rate standardised to the age group of 2–10 years of 21–40%[12]. The catchment area of the Chikwawa District Hospital is approximately 20 square kilometres with a population of around 50,000 that is demarcated by natural borders, decreasing population migration, which should aid follow-up.

Study aim

The overall primary objective of the study is to define the optimal dose of DP for PMC when administered during health facility visits at the time of routine vaccinations in infancy.

Specific objectives

The specific objectives of the study are to:

1. To describe age-related changes in population pharmacokinetic properties of piperaquine following the administration of DP for PMC at four different routine health facility visits in infancy (i.e., at 10-, 14 weeks, six months, and nine months of age).

2. To evaluate the association between piperaquine exposure and the incidence of symptomatic and asymptomatic malaria detected on quantitative PCR in infants randomised to DP for PMC.

3. To compare the efficacy, safety and tolerability between infants receiving DP and those receiving DP-placebo for PMC together with routine immunisation.

4. To apply population pharmacokinetic-pharmacodynamic modelling and simulation techniques to optimise the dosage of piperaquine when administered as DP for PMC

Study outcomes

In line with the study objectives, the following are the outcomes of the study:

- Age- and body weight-related changes in primary pharmacokinetic properties of piperaquine (such as clearance and volume of distribution) in infants receiving DP for PMC at four different routine health facility visit time points in infancy (i.e., at 10-, 14 weeks, six months, and nine months of age).
- Incidence of malaria from 2.5 months to 12 months of age in DP-exposed compared with unexposed infants; and, in infants receiving DP, the association between piperaquine pharmacokinetic parameters and risk of developing malaria.
- Malaria incidence and adverse events as a proxy for safety and tolerability in infants receiving DP for PMC compared with those receiving placebo at routine health facility visits.
- Optimised dosing regimens of DP for PMC in infants.

Study design

A randomised, single-blind, placebo-controlled, two-arm, interventional trial will be conducted in southern Malawi (Figure 1). At 10 weeks (2.5 months) of age, 220 infants will

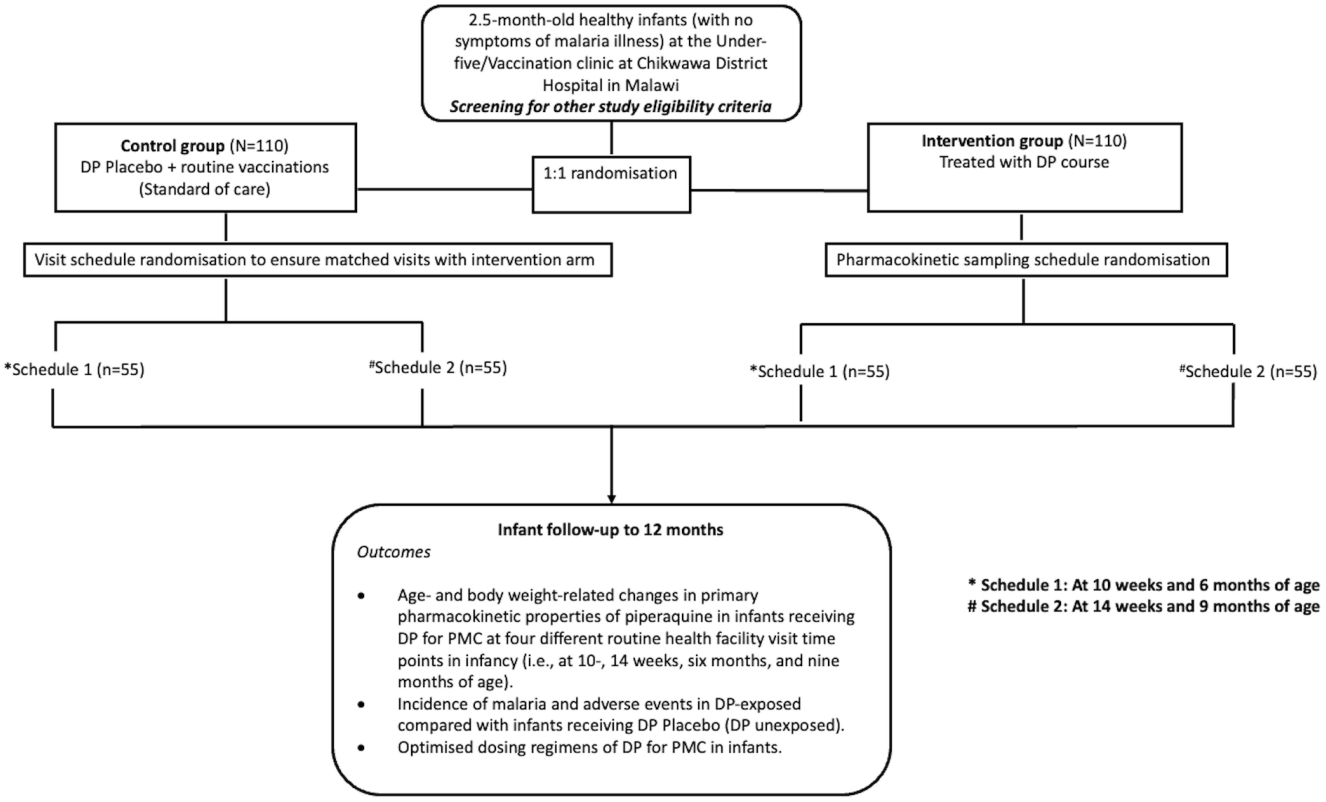

Figure 1. Outline of the study design; a single-blind, placebo-controlled, randomised interventional study.

be randomised to receive DP with routine vaccines (intervention group, n=110) or matched placebo (control group, n=110) with routine vaccines. They will be followed until 12 months of age and receive three other treatment courses at 14 weeks, six- and nine months.

Infants in the intervention group will contribute capillary samples for piperaquine concentrations pre-dose and at three-, seven-, 14- and 28-day post-DP dosing as well as capillary samples at pre-dose and day 28 post-dosing to quantify malaria parasitaemia using microscopy and quantitative PCR. In the control group, infants will contribute capillary blood samples for malaria parasitaemia at the same time points as the intervention group. In both groups, adverse event data will be collected. The inclusion of the placebo arm will enable the determination of the baseline incidence of malaria and adverse events among infants receiving routine vaccinations, and this will be compared with malaria incidence and adverse events in the DP group.

Study participants

Study team members will identify infants at the under-five clinic during the routine six- or 10-week health facility visit. Interested parents or guardians will be asked to provide written informed consent for the infant's participation in the study. For infants who are six weeks old, the consenting guardian/parent will be informed that their infant will only be enrolled into the study at the next health facility visit (10 weeks).

Inclusion and exclusion criteria

Inclusion criteria

- Infants from 2.5 months (10 weeks) whose parent/guardian has provided informed consent.
- No symptoms of malaria at the time of recruitment.
- Parent or guardian willing to adhere to study procedures including infant follow-up until 12 months.

Exclusion criteria

1. Known allergy or contraindication to any study drugs.
2. Known HIV exposure.
3. Pre-existing medical history of significant comorbidities that may influence drug exposure, e.g., renal, liver, gastrointestinal or cardiac diseases.
4. Severe anaemia (haemoglobin <7 g/dL).
5. Infant (or breastfeeding mother) on medications that are known to have clinically significant interactions with DP.
6. Participation in another clinical trial

Reason for excluding HIV-exposed infants. HIV-exposed infants receive cotrimoxazole prophylaxis for the prevention of opportunistic infections, and this continues up to 24 months of age. Fortunately, cotrimoxazole has antimalarial activity and is known to reduce the incidence of malaria. However, there is not yet robust evidence on whether combining DP with cotrimoxazole prophylaxis would further reduce the risk of malaria. The complexity of DP drug interactions with antiretrovirals and/or cotrimoxazole, as well as their effect modification in the prevention of malaria in infancy or in young children, requires a different study design which could not be included within the scope of the present OPTIMAL study.

Study procedures

Baseline procedures. Measurement of haemoglobin to ascertain that the infant does not have severe anaemia will be carried out using a point-of-care device (HemoCue Hb 301 analyser). Other baseline procedures will include the measurement of anthropometric indices (weight-for-height, weight-for-age, length/height-for-age) and the physical examination of the infant (Table 1).

Randomisation, allocation concealment and blinding. Infants will be individually randomised to either the DP- or placebo group (Figure 1). To minimise sampling events, infants will be further randomised to one of two sampling schedules at alternate routine visits (Figure 1). Pre-determined randomisation codes, concealed in opaque envelopes, will be used to allocate the infant into either study group. Study staff assigning participants will, therefore, not know the arm to which an enrolled infant will be allocated until the assignment is done. Throughout, the study period, guardians of the participants will be blinded as to whether their infant is in the intervention or control group. Furthermore, the number of visits for participants in the control and intervention groups will be matched. This will be done to minimise bias in the reporting of adverse events. However, blinding of clinic research staff will not be possible given that pharmacokinetic sampling will only be conducted in the intervention group to avoid unnecessary blood collection from these infants.

Administration of study drug. DP has been approved for uncomplicated *Plasmodium falciparum* malaria treatment, and several studies have repurposed it for malaria-preventive treatment in children[7,8,13]. The OPTIMAL study will use a WHO-prequalified dispersible fixed-dose combination DP tablet (D'Artepp®, Fosun Pharma, Shanghai, China), which is licenced for use in Malawi for oral administration. Two strengths and pack sizes will be used in this study:

- 160 mg/20 mg tablets of piperaquine/dihydroartemisinin – packs of three tablets
- 240 mg/30 mg tablets of piperaquine/dihydroartemisinin – packs of three tablets

Dosing recommendations by the manufacturer in line with the latest WHO recommended dosing regimen will be used, with children <5 kg receiving a dose similar to that administered in children between 5–<8kg as recommended by the revised WHO malaria treatment guidelines (Table 2)[14].

D'Artepp® will be administered with water and without food. The manufacturer recommends that food should not be taken at least three hours before and after administration. However, for purposes of this study, since participants are infants, we will not restrict breastfeeding but will encourage guardians not to provide any other food to the infant at least one hour before and after drug administration. D'Artepp® is a dispersible tablet and will be mixed with water and administered according to the manufacturer's instructions.

Placebo administration. Matched DP placebo tablets, also manufactured by Fosun Pharmaceuticals, will be administered to infants in the control group. These have been formulated in pack sizes of three tablets, matched to the 160 mg/20 mg active DP tablets. For each infant, the number of placebo tablets to be administered will be based on weight as with the active DP (Table 2). Since the placebo tablets are also dispersible, they will also be mixed with water and administered according to the manufacturer's instructions.

Blood sampling for the pharmacokinetic outcomes. Infants randomised to sampling visit schedule 1 will contribute capillary piperaquine blood samples after DP dosing at 10 weeks and six months. Infants randomised to schedule 2 will contribute blood samples after DP dosing at 14 weeks and nine months of age. In each sampling visit schedule, capillary (heel-prick) blood samples will be collected pre-dose and then at three, seven, 14 and 28 days after DP treatment to determine piperaquine concentrations. Each infant in the active DP arm will, therefore, contribute only 10 capillary pharmacokinetic blood samples (~200 µl for each pharmacokinetic sample, i.e., <2.5 mL throughout the study period), while receiving all four treatment courses of DP for PMC. In the remaining two treatment periods, the participant will be asked to visit the clinic on day one for observed DP treatment but no pharmacokinetic samples will be collected. This will result in a total of 14 scheduled study visits in the intervention group over the 9.5-month study period.

Piperaquine sample processing, storage, and analysis. The blood volume of 200 µL, collected using calibrated microcollection tubes, will be applied on four pre-labelled dry blood spot (DBS) filter papers (50 µL spots on Whatman 3MM) and left to dry. The DBS filter papers for each participant, and at each sampling time point, will be stored with desiccant in sealed zipper plastic bags to ensure that they do not pose any potential risk of biological hazard and to avoid the risk of cross-contamination between samples.

The filter papers will be stored at room temperature at the site laboratory before transportation (at room temperature) to the Malawi-Liverpool-Wellcome Programme (MLW)'s sample archive. Two papers per pharmacokinetic sample will be shipped to Mahidol Oxford Tropical Medicine Research Unit

Table 1. Study procedures.

	Recruitment	On the first day of each scheduled treatment course*	Treatment			Follow-up			Unscheduled	Study exit visit
Visit number	1	1	1	-	2	3	4	5	-	-
Visit description	Baseline	-	1st DOT**	Dose at home	2nd DOT**	Follow-up visit 1 week	Follow-up visit 2 weeks	Follow-up visit 4 weeks	Unscheduled visit	Exit visit
Study time[$]	Day 1	Day 1	Day 1	Day 2	Day 3	Day 7±1	Day 14±2	Day 28±5	Any other day	12 months of age
Informed consent discussion	x	x								
Medical history	x	x								
Review of eligibility criteria	x	x								
Randomisation into study arm (control or intervention arm)	x									
ID number assignment	x									
Visit schedule randomisation: schedule 1 or 2*	x									
Anthropometric measures	x	x								
Physical examination[#]	x	x	x		x	x	x	x	x	x
Recording of current and concomitant medication	x	x			x	x	x	x	x	x
Capillary haemoglobin measurement	x	x							x	
Study drug administration			x	x	x					
Pharmacokinetic sampling										
Capillary pharmacokinetic sample onto a filter paper		x			x	x	x	x	x	
Efficacy assessment in the intervention group[##]										
Malaria slide		x						x	x	

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	Recruitment	On the first day of each scheduled treatment course*	Treatment			Follow-up			Unscheduled	Study exit visit
Quantitative PCR filter paper sample for malaria parasitaemia		x						x	x	
Malaria incidence assessment in the control group##										
Malaria slide		x						x	x	
Quantitative PCR filter paper sample for malaria parasitaemia		x						x	x	
Safety assessment										
Adverse event monitoring		x			x	x	x	x	x	x
Study exit###										
Checking completion of all forms (adverse event and concomitant medications) and logs										x

$ Samples will be collected at the earliest time if a sampling window is missed

* Infants in both groups will be randomised to a blood sampling schedule in both arms at 10 weeks and 6 months (schedule 1) or 14 weeks and 9 months (schedule 2).

** DOT: Directly observed therapy at study clinic

Vital signs on every visit and symptom directed examination after recruitment

Assessment time points in both control and intervention groups are similar, and translate to the following time points: at 14 weeks, 15 weeks, 6 months, 7 months, 9 months and 10 months of age

If an adverse event is on-going at the time of study exit, an addition unscheduled visit should be arranged for follow up on outcome of event

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Table 2. Recommended DP dosing by WHO.

Body weight (kg)	Daily dose (mg)		Table strength and number of tablets per dose
	Piperaquine	Dihydroartemisinin	
5 – <8	160	20	1 x 160mg / 20mg tablet
8 – <11	240	30	1 x 240mg / 30mg tablet
11 – <17	320	40	2 x 160mg / 20mg tablet
17 – <25	480	60	2 x 240mg / 30mg tablet

(MORU) in Bangkok, Thailand, and the rest will be retained at MLW's sample archive as a backup. At MORU, piperaquine concentrations will be determined using liquid chromatography coupled to a tandem triple-stage mass spectrometry (LC-MS/MS) assay which is fully validated[15].

Blood sampling for the efficacy outcomes. Although light microscopy is the current gold standard for ascertaining malaria parasitaemia and will be used to guide the clinical management of participants in the study, it may not be sufficiently sensitive to detect low parasitaemia. The study will use high-throughput ultrasensitive quantitative PCR (qPCR)[16] to overcome the microscopy limitation. However, qPCR results will only be available at the end of the study to assess efficacy and can not guide the clinical management of the participants during the study.

Infants randomised to both groups will contribute capillary blood samples for a malaria blood smear, using microscopy, and qPCR to allow active detection and quantification of malaria parasitaemia. These samples will be collected before DP/placebo treatment and 28 days post-DP treatment at routine health facility visits at 10 weeks, 14 weeks, six months and nine months. This will result in qPCR sampling during visits at 10 weeks, 14 weeks, 18 weeks, six-, seven-, nine- and 10 months, as well as any unscheduled visits (Table 1).

Each malaria parasitaemia blood sample will be 200 µL of capillary blood for quantitative PCR and 100 µL for a malaria blood smear. Each infant in both study arms will thus contribute a total of 2.1 mL of blood for the efficacy endpoint.

Quantitative PCR, microscopy sample processing and storage. The 200 µL capillary blood sample collected will be applied on a different set of four pre-labelled filter papers (50 µL spots on Whatman 3MM) and left to dry. These will later be stored at room temperature, with a desiccant, in sealed zipper plastic bags at the study site before transportation (at room temperature) to MLW's sample archive. Later, two of the four filter papers will be shipped to the Blantyre Malaria Project laboratory in Malawi for the detection of malaria parasites using absolute quantitative real-time PCR (qPCR). The remaining two filter papers will remain at MLW's sample archive for backup.

An additional 100 µL of blood will be used to prepare thin and thick peripheral blood smears for malaria microscopy with standard Giemsa staining for parasite identification and count at the study site. Each blood smear will be read by two microscopists in a blinded fashion (blinded to the reading of other microscopists, randomisation arm and infant age). In the event of a discrepancy, the slide will be read by a third microscopist and parasite density will be calculated by averaging the two most concordant counts. All the smears will be preserved at the site laboratory for possible third-party confirmation (if needed).

Safety assessment. In both the intervention and control arms, safety and tolerability will include any adverse events (AEs) following active-DP or DP-placebo administration. All participant's guardians will be asked routinely, using standardised wording at each visit, about any symptoms since the previous follow-up visit. This will be followed by symptom-directed physical examination and investigations as needed. Two physician investigators will independently review all AEs detected at scheduled or unscheduled visits to assess their relationship to the study drug as well as recommend any action(s) to be taken.

Treatment during breakthrough malaria infections. A breakthrough infection will be defined as any symptomatic or asymptomatic malaria parasitaemia, ascertained using microscopy, at any time after receiving a DP or placebo. At any such episode of malaria parasitaemia, a blood sample for molecular quantification of malaria parasitaemia (qPCR) will be collected. In the intervention arm, a blood sample for piperaquine concentrations will also be collected to determine the association between piperaquine concentrations and efficacy.

Both symptomatic and asymptomatic breakthrough episodes of malaria will be treated with the WHO-recommended weight-based dosage regimen of artemether-lumefantrine (AL), after excluding any features of severe malaria. A child with severe malaria will be managed appropriately according to standard treatment guidelines through routine hospital service.

Any subsequent DP treatment courses, due at / shortly after the time a breakthrough infection occurs, e.g. at 14 weeks or six months, will still be administered at least 14 days after

starting the rescue treatment (i.e. 11 days from completion of the rescue medication), and re-aligned with the vaccination schedule as soon as possible thereafter. As artemether-lumefantrine has a relatively short half-life (two hours and four to six days, respectively[20]), the 14-day window would provide an adequate washout period. Additionally, retaining such an infant in the study would allow for a more accurate assessment of the efficacy of DP in preventing malaria in the first year of life. It would avoid bias introduced by withdrawing infants at higher risk of malaria and would enable the estimation of cumulative malaria incidence in both the DP and placebo arms.

Treatment of asymptomatic parasitaemia at enrolment. Asymptomatic malaria diagnosed at enrolment (10 weeks of age) using microscopy will not be an exclusion criterion as the OPTIMAL study seeks to include vulnerable infants at higher risk of malaria. Instead, following enrolment, AL will be prescribed for malaria treatment in line with standard guidelines. However, the administration of the 10-week dose of DP or DP-placebo will be withheld. The infant will be followed up on days three, seven, 14 and 28, with repeat microscopy on days seven and 14. If parasitaemia is persistent on either of these days, national second-line treatment will be prescribed. On day 28, before the scheduled administration of DP or DP placebo, microscopy will be conducted, in line with study procedures, to ensure there is no parasitaemia.

Retreatment after vomiting. If a child vomits within 30 minutes of taking DP or placebo on the days of observed treatment at the clinic (days one and three), the whole dose will be re-administered. If vomiting occurs within 30 to 60 minutes of drug administration, half the dose will be administered. No further treatment will be administered if post-dose vomiting is repeated. All episodes of vomiting, including vomiting on day two when treatment is administered at home, will be recorded as an adverse event and followed up until resolution and/or study exit.

Treatment for concomitant conditions. All concomitant medications taken during the study will be recorded with indication, dose information, and dates of administration. Previous, current, or new medications not identified as prohibited may be given as needed based on the investigator's judgement and the participant's medical needs. A list of prohibited medications will be created and updated every quarter. Patients who are found to have illnesses other than malaria will receive ambulant standard-of-care treatment or will be referred to the paediatric ward for further management. Where a potentially interacting treatment is essential, and it is not possible to provide alternative therapy, a note to file will be made regarding the reason for prohibited concomitant medication being used. This will be accounted for during data analysis.

Concurrent administration of the malaria vaccine. At present, the study site area is not in the vaccinating cluster of the malaria vaccine implementation pilot study commissioned by WHO. In the lifespan of the study, we do not expect enrolled infants to be offered a malaria vaccine. However, should a national malaria vaccine roll-out happen while the study is underway, this could potentially result in an underestimation of the true efficacy of DP for PMC in infancy, especially in relation to preventing severe illness. On the other hand, this would mimic a real-world, programmatic setting in which DP for PMC and malaria vaccination would be implemented concurrently to control the burden of malaria in infancy. To provide some evidence on the combined effect of DP for PMC and malaria vaccination in infancy, secondary analyses will explore the impact of the malaria vaccine on the efficacy, safety and tolerability of DP and the pharmacokinetic parameters of piperaquine.

Sample size calculation

To describe age-related changes in population pharmacokinetic properties of piperaquine following the administration of DP for PMC, a stimulation re-estimation approach was employed using NONMEM software (ICON Development Solutions, Hanover, MD, USA) to estimate the precision of the impact of increasing age through infancy on piperaquine clearance. With this approach, and the currently proposed study design and sampling schedule (Figure 1 and Table 2), a sample size of 100 infants was shown to provide at least 28% precision. After adjusting for a 10% loss to follow-up, a total of 110 participants have been planned for recruitment in both the intervention arm and placebo arm.

Furthermore, 98 infants will provide >80% power to detect an absolute difference of at least 20% in malaria incidence between the control and intervention groups. After adjusting for a 10% loss to follow-up, a total of 108 infants would need to be recruited per arm. The assumption of a 20% difference was based on detecting a clinically relevant difference between the intervention and control groups as previously suggested by a study in Tororo, Uganda which has a similar malaria transmission intensity and pattern as Chikwawa in Malawi[18,19].

No formal sample size calculation has been done for safety and tolerability as this will only describe adverse events as they occur with a post-hoc assessment of any correlation with piperaquine exposure. A total sample size of 110 infants in each arm will also provide adequate precision and power for population pharmacokinetic-pharmacodynamic modelling to optimise the dosing of DP in infancy.

Data collection tools

The demographic and follow-up data will be collected using case report forms adapted from the Malaria Case Record Form (CRF) template[20] developed by the WorldWide Antimalarial Resistance Network through the Malaria Clinical Trials Toolkit and validated to ensure the collection of quality data compliant with international data standards such as those of the Clinical Data Interchange Standards Consortium (CDISC)[21]. The data will be electronically recorded in a data management system (DMS) on a tablet, using the ODK platform. All steps of data curation and generation of meta-data will be recorded for audit purposes.

Statistical analysis plan

Baseline data will include participant characteristics such as age (in months), anthropometric indices and any medical conditions. Follow-up data will include concomitant medications

(including traditional, alternative, and complementary medicines), adverse events and dosing of the study drug. Pharmacokinetic and efficacy data will be generated by assaying the collected pharmacokinetic and qPCR samples described above. Table 3 summarises the statistical analysis plan according to each of the four study objectives.

Ethics related to the study

Ethics approval. The study received ethics approval from the local ethics committee in Malawi, the College of Medicine Research Ethics Committee (P.06/22/3663) on 8 September 2022, the University of Cape Town Human Research Ethics Committee (361/2022) on 11 October 2022, and the Liverpool

Table 3. Outline of data analysis and outcomes according to study objectives.

Objective	Outcomes
1. To describe age-related changes in population pharmacokinetic properties of piperaquine following the administration of DP for PMC at four different routine health facility visits in infancy (i.e., at 10-, 14 weeks, 6 months, and 9 months of age).	• A population pharmacokinetic model will be used to describe changes in primary pharmacokinetic parameters (e.g., clearance and volume of distribution) over the first year of life. These will be allometrically scaled for body weight. The impact of covariates such as age, weight-for-height, weight-for-age, sex, maternal social economic status, will be assessed. • The model building will be guided by likelihood ratio tests to determine statistical significance, diagnostic plots, and validation techniques, including visual predictive checks. Due to the sparseness of collected data, a frequentist prior approach might be needed to stabilise the structural model parameters in the commonly used 3-compartment distribution model following a transit-absorption profile[22].
2. To evaluate the association between piperaquine exposure and the incidence of symptomatic and asymptomatic malaria detected on quantitative PCR in infants randomised to DP for PMC	• Incidence of malaria from 2.5 months to 12 months of age will be evaluated by comparing 1) DP exposed and unexposed infants and 2) pharmacokinetic parameters of piperaquine such as trough concentrations between infants who develop an episode of malaria and those who do not. This step will first use cox proportional hazard models for cumulative malaria hazard and parametric survival models, adjusted for repeated malaria events to estimate overall symptomatic and asymptomatic malaria incidence. • Covariates related to malaria incidence such as piperaquine concentration, malaria transmission period, age, sex, weight-for-age, height-for-age, weight-for-height, the maternal socioeconomic status, will be assessed. • Piperaquine concentrations associated with protection from malaria will be defined as the median piperaquine concentration predicted to provide a 95% reduction in the hazard of malaria in the intervention arm compared with the control arm.
3. To compare the efficacy, safety and tolerability between infants receiving DP and those receiving DP-placebo as PMC together with routine immunisation.	• Safety and tolerability will be assessed by comparing the frequency and severity of adverse events in infants receiving DP for PMC and in those receiving routine immunisation without DP for PMC. • In an exploratory analysis, frequently occurring adverse events, or those assessed to be related to DP administration will be correlated with derived secondary pharmacokinetic parameters such as peak concentration (possibly linked to acute toxicity) and overall exposure (possibly linked to potential systematic toxicity).
4. To apply population pharmacokinetic-pharmacodynamic modelling and simulation techniques to optimise the dosage of piperaquine when administered as DP for PMC in infancy	• Data from objectives 1 and 2 will be used to develop population pharmacokinetic-pharmacodynamic models: (i) to understand the impact of different determinants, including age, body weight, sex and nutritional status on pharmacokinetic parameters of piperaquine and the associated efficacy of DP when administered for PMC; and (ii) to propose as well as simulate optimised dosing regimens of DP for PMC in infancy. • Monte-Carlo simulation will be conducted to predict malaria incidence and time above protective piperaquine concentration under: a. Varying malaria transmission intensities b. Novel optimised dosing regimens based on age and weight band (i.e., dosing regimens that incorporate additional or fewer doses other than those tested in the present study) • All modelling and simulation will be conducted using a nonlinear mixed-effects modelling approach in NONMEM and/or R as previously described[23].

School of Tropical Medicine Research Ethics Committee (22-038) on 2 November 2022. Additionally, regulatory approval was granted in Malawi from the Pharmacy Medicines Regulatory Authority (PMRA/CTRC/IV/22112022140) on 15 December 2022. The trial was registered with the Pan African Clinical Trials Registry (PACTR202211575727659) on 8 November 2022.

Risks of side effects of drugs not stated in the drug label. This study will be conducted in a well-controlled environment with a detailed safety assessment and oversight by an independent data safety and monitoring board (DSMB) to assure the safety of study participants. We do not anticipate any DP-related adverse effects other than those already described on the drug label and as previously studied[6,7,24]. Nevertheless, the DSMB will review any such new information, and appropriate recommendations made. Participants will be insured against any research-related harm.

Capillary blood sampling and mitigation of potential injury. Participants will undergo repeated heel or finger pricks for routine malaria diagnosis and assessments for anaemia. The risks of these procedures include pain, transient bleeding, and soft-tissue infection. A small bruise or mild pain on the site from where the blood is taken may develop. Only well-trained study staff will be hired for the project to mitigate the risk. Additionally, new disposable needles and lancets will be used for the blood collection procedures and safely discarded immediately after use. The pharmacokinetic and efficacy sample collection points have been spaced out adequately and the total blood volumes for each infant are well within safe sampling limits of no more than 3% of the total blood volume during a period of four weeks and ≤1% at any single time[25–27].

Quality control and study oversight

The study will be conducted according to the Declaration of Helsinki[28]. It will be sponsored by the Liverpool School of Tropical Medicine (LSTM). Additionally, an independent DSMB will be established comprising at least three members, including an experienced malaria researcher or clinical pharmacologist, a paediatric infectious diseases/tropical medicine specialist, and a statistician with African clinical trials experience. The DSMB will meet prior to the trial start, and subsequently after the recruitment of each additional 55 participants (when at least 25 infants are expected in each study arm). The DSMB will advise the Investigators on the different aspects of the study, focusing on reviewing safety data and advising them on trial continuation, amendment, or termination. No interim analyses are planned. Pharmacokinetic and qPCR efficacy endpoint data will only be available after study completion.

Dissemination plan and data sharing

Community sensitisation. Community sensitisation meetings will be conducted by the research team at the beginning of the study and periodically during the study. The sensitisation meetings will provide a platform for local community leaders and the community members residing in the catchment area of the study hospital to ask questions regarding the OPTIMAL study. Activities for this community sensitisation program will include talks in the local language and a community radio program. At the end of the research project, the site study team will disseminate the research findings, in the local language, to local communities and study participants.

Dissemination of findings to inform policy. Dissemination of research findings from this study will include a policy brief and engagement with policymakers in Malawi, at the regional level (within sub-Saharan Africa) and with the WHO Global Malaria Programme. Scientific conferences and publications in open-access journals will inform the scientific community and provide room for appropriate further debate. The inclusion of these data in future related analyses including individual participant data meta-analyses will be made possible through the secure WorldWide Antimalarial Resistance Network repository and its independent Data Access Committee[29].

Study status

The study started recruitment in February 2023 and enrolment is currently ongoing.

Discussion

The WHO has acknowledged the potential for ACTs as alternatives for SP in malaria chemoprevention in young children, including infants. However, it has called for more evidence to inform its preventive treatment guidelines. To the best of our knowledge, the OPTIMAL Study is the first to investigate aligning DP for chemopreventive treatment in infancy with routine health facility visits. By understanding the variation of piperaquine exposure in the first year of life, we will be able to correlate such variation with the incidence of symptomatic and asymptomatic malaria as well as its safety and tolerability. We will then apply population pharmacokinetic-pharmacodynamic modelling techniques to simulate and derive optimised regimens of DP for malaria chemoprevention when administered during routine health facility visits.

The present study further provides an opportunity to implement a proposed quantitative PCR (qPCR) method to estimate parasitaemia more accurately in malaria chemopreventive clinical trials[30]. In this method, malaria qPCR is ascertained at specific time points to better quantify sub-microscopic malaria parasitaemia, which is relevant in defining the success of chemoprevention (Table 1). This method has the potential to be scaled up in future clinical studies and routine practice to support precise estimation of chemopreventive treatment efficacy in programmatic settings.

In addition, the study will explore how best to combine sparsely sampled pharmacokinetic data with malaria quantitative PCR results to understand the protective efficacy of an antimalarial medication for malaria preventive treatment in infants. This will be carried out as part of a suggested novel pharmacometric antimalarial resistance monitoring (PARM) methodology for evaluating slowly eliminated antimalarial

drugs in areas of high transmission[31]. In PARM, antimalarial drug concentrations at the time of recurrent parasitaemia are measured to identify outliers (i.e., recurrent parasitaemia in the presence of normally suppressive drug concentrations). Within the OPTIMAL study, drug concentrations will be measured routinely pre-dose, on day 28 after each DP/placebo dose, and whenever an unscheduled visit occurs (Table 1). With preventive treatment, as is the case with the OPTIMAL study, the focus would be to determine the antimalarial concentration at the time that any breakthrough infection occurs.

Conclusions

Infants bear a significant burden of malaria disease but are usually excluded from participating in early clinical trials that inform antimalarial dose optimisation. Our present study aims to shift the paradigm by conducting this dose optimisation clinical trial in this complex subgroup to inform appropriate dosing of the antimalarial, DP, for malaria chemopreventive treatment during routine health facility visits. Furthermore, such a paradigm shift sets the precedence for including infants in early dose optimisation studies for various antimicrobial agents. It also highlights the role of quantitative pharmacology in supporting clinical trials that address key public health challenges to inform treatment dosage regimens.

Data availability

No data are associated with this article. Data collected will be available through the WorldWide Antimalarial Resistance Network repository and its independent Data Access Committee[29].

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